FACTORES RELACIONADOS CON LA FUERZA MUSCULAR Y LA DENSIDAD MINERAL ÓSEA EN MUJERES POSTMENOPÁUSICAS

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CERTIFICAN que Pascual García Alfaro ha realizado bajo su dirección el trabajo de Tesis Doctoral titulado "Factores relacionados con la fuerza muscular y la densidad mineral ósea en mujeres postmenopáusicas".

El presente trabajo se ha estructurado siguiendo la normativa para Tesis Doctoral como compendio de publicaciones y ha sido realizado en el Servicio de Ginecología del Hospital Universitario Dexeus.

Barcelona, Febrero 2024

Dr. Francesc Tresserra Casas

Dr. Ramón Carreras Collado

Dr. Antonio Gil Moreno

A mis padres, por el esfuerzo que realizaron para que pudiera realizar mis estudios. De estar aquí, sé que se sentirían orgullosos.

A mi mujer, María Ángeles y a mi hija Natalia, por su paciencia, comprensión y cariño.

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1. INTRODUCCIÓN

El músculo y el hueso están estrechamente unidos tanto anatómica, funcional como genéticamente. El vínculo más aceptado y reconocible, es la relación mecánica entre ambos tejidos, ya que los músculos se adhieren al hueso y generan movimiento a través de la contracción activa. El músculo y el hueso comparten el mismo origen mesodérmico y, por tanto, es razonable suponer que los dos tejidos comparten determinantes genéticos.

En los últimos años existe un interés creciente por las interacciones bioquímicas, autocrinas, paracrinas y endocrinas entre el músculo y el hueso al ser considerados como órganos con propiedades endocrinas. Estas interacciones adquieren una particular importancia debido a que el músculo secreta factores reguladores del hueso y el propio tejido óseo puede modular el metabolismo del músculo esquelético (Cianferotti et al., 2014; Avin et al., 2015).

El envejecimiento está asociado con un incremento de la prevalencia de alteraciones musculoesqueléticas. Primero apareciendo una pérdida progresiva de la fuerza muscular, definida como dinapenia y posteriormente esta pérdida de fuerza muscular se acompaña de una disminución de la masa muscular, definiendo el término sarcopenia (Pérez-López., 2017). Así mismo, el envejecimiento también se asocia con un deterioro en la cantidad y calidad de la masa ósea, característico de la osteoporosis. Debido al aumento en la esperanza de vida de la población, se ha producido un incremento significativo de estas patologías. Este hecho ocasiona un gran problema de salud pública, no solo en el

estado de salud y calidad de vida de los pacientes, sino por el coste económico y social que conlleva su tratamiento y sus secuelas (Bohannon., 2008; Clynes et al., 2020).

Como la etiopatogenia de estas patologías es multifactorial, un abordaje sistemático de los factores de riesgo relacionados con el deterioro muscular y de la masa ósea es esencial para la prevención.

1.1 Fuerza muscular

La fuerza muscular es una función esencial del cuerpo humano, siendo un componente relevante de la salud y la forma física. También se refiere a la capacidad de un músculo o grupo de músculos para ejercer fuerza contra una resistencia. La fuerza muscular está involucrada en muchas actividades, tareas diarias, mantenimiento de la independencia funcional y autonomía. Así, la actividad física regular adaptada a la edad es una recomendación preventiva para un envejecimiento saludable (Warburton et al., 2017; Haider et al., 2019).

El término dinapenia define la pérdida de fuerza muscular relacionada con la edad, no causada por enfermedades musculares o neurológicas (Clark et al., 2008). La dinapenia está considerada como el indicador primario de la sarcopenia (Manini et al., 2012).

Los mecanismos fisiopatológicos de la debilidad muscular se pueden compartimentar en dos factores. En primer lugar, el deterioro del sistema muscular que causa un déficit en la producción de fuerza del músculo esquelético y en segundo lugar,

el deterioro del sistema nervioso que disminuye la capacidad de activación muscular voluntaria. Posteriormente, se producen limitaciones funcionales y riesgo de discapacidad física (Clark et al., 2012).

Tenemos que tener en cuenta, que la pérdida de fuerza muscular relacionada con la edad se explica solo parcialmente por los cambios en la composición muscular y la reducción de la masa muscular, por lo tanto, otros factores fisiológicos estarían involucrados en la explicación de la debilidad muscular en adultos mayores (Rizzoli et al., 2014). Entre los factores implicados se podrían incluir: el nivel de actividad física, el estado nutricional, el envejecimiento gonadal con la reducción de la producción de esteroides gonadales y la pérdida o disfunción de motoneuronas (Cipriani et al., 2012; Larsson et al., 2019; Borges et al., 2020).

1.1.2 Implicaciones clínicas de la fuerza muscular

La fuerza muscular de agarre es considerada como un biomarcador de fragilidad, en el cual hay un aumento de la vulnerabilidad del individuo para desarrollar dependencia y/o mortalidad (Sayer et al., 2015; Bohannon., 2019).

La medición de la fuerza muscular de agarre está incluida en los criterios validados para determinar la fragilidad y se ha propuesto como un componente clave de los fenotipos de fragilidad (Martín-Ruiz et al., 2014). La debilidad muscular, medida por la fuerza muscular de agarre, está asociada a un peor estado de salud y predice futuros eventos adversos para la salud (Bohannon., 2008). Por lo tanto, una fuerza muscular de agarre baja está asociada con

una mayor morbi-mortalidad por todas las causas, así como una mayor discapacidad (McGrath et al., 2020). La fuerza muscular sería un mejor indicador que la masa muscular respecto a la capacidad funcional, riesgo de hospitalización y mortalidad (Newman et al., 2006; Cawthon et al., 2009).

La enfermedad cardiovascular es una de las principales causas de muerte en todo el mundo y se ha identificado que las mujeres dinapénicas tienen una función cardiorrespiratoria más baja que las mujeres no dinapénicas, incluso si tienen la misma masa muscular (Barbat-Artigas et al., 2011). Presentar una fuerza muscular de agarre disminuida está asociada con el síndrome metabólico, considerado como un precursor de enfermedad cardiovascular (Wilson et al., 2005).

Diversos estudios han asociado una baja fuerza muscular de agarre con el deterioro cognitivo y se ha sugerido que la medición de la fuerza muscular de agarre puede ayudar a identificar la aceleración del deterioro cognitivo (Leong et al., 2015). Se ha observado que por cada disminución de 1 libra (0.454 kg) en la fuerza muscular de agarre basal se asocia con un aumento del 1.5 % en el riesgo de padecer enfermedad de Alzheimer (Buchman et al., 2007).

La fuerza muscular de agarre también es un indicador de discapacidad funcional y mayor riesgo de caídas. Se ha observado que una mayor fuerza muscular de agarre está asociada con un menor riesgo de discapacidad (Gopinath et al., 2017).

Además, la baja fuerza muscular de agarre se correlaciona con baja

densidad mineral ósea (DMO), mayor riesgo para la osteoporosis y mayor riesgo de futuras fracturas en mujeres posmenopáusicas (Li et al., 2018).

1.2 Densidad mineral ósea (DMO)

La osteoporosis se define como una enfermedad ósea sistémica caracterizada por una disminución de la masa ósea y un deterioro de la microarquitectura del tejido óseo, provocando un aumento de la fragilidad ósea y como consecuencia un aumento del riesgo de fractura. En la Conferencia de consenso del National Institutes of Health (NHI) de Estados Unidos, se simplificó la definición donde se destacaba la disminución de la resistencia ósea predisponiendo a un mayor riesgo de fractura. El concepto de resistencia integra cantidad y calidad del hueso (NIH., 2001). Existe una definición densitométrica de la osteoporosis realizada por el Comité de Expertos de la OMS, de fácil uso en la práctica clínica que permite homogeneizar poblaciones a la hora de realizar estudios clínicos o terapéuticos (WHO., 1994). La osteoporosis es la enfermedad metabólica ósea más frecuente.

La masa ósea aumenta durante las primeras décadas de la vida hasta alcanzar un máximo entre los 25 y los 30 años, denominado “pico de masa ósea”. Posteriormente se produce una fase de meseta con una duración entre 10 a 20 años y finalmente en la mujer, se produce una disminución brusca de la DMO coincidiendo con la menopausia y se prolonga con una disminución persistente, pero de forma más lenta durante la etapa del envejecimiento (Mora et al., 2003). **Figura 3.**

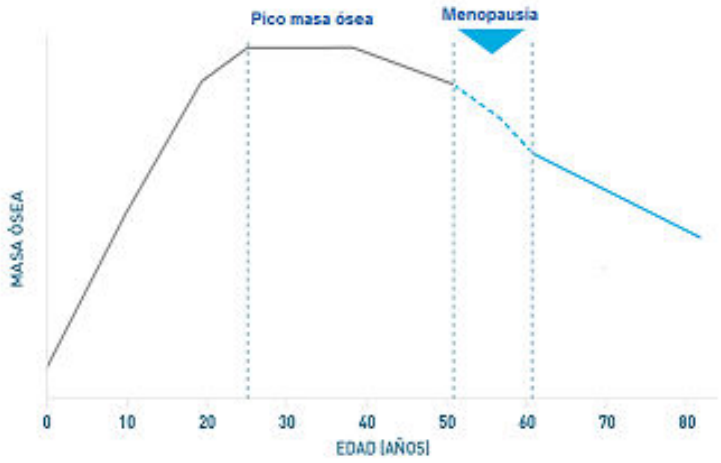


Figura 3. Evolución de la masa ósea en la mujer

La microarquitectura es uno de los componentes no directamente relacionados con la masa ósea. Cuando disminuyen el espesor y el número de trabéculas se produce un aumento de la fragilidad ósea, siendo especialmente importante para la resistencia del hueso trabecular (Brandi et., 2009).

La baja masa ósea es consecuencia de dos variables: el pico de masa ósea alcanzado en la juventud y la pérdida ósea en etapas más tardías. Por lo tanto, es importante conocer los factores que influyen en una baja masa ósea final, existiendo unos factores no modificables y otros modificables (Del Pino., 2010). Entre los factores no modificables encontramos: la edad, el sexo, la genética, la menopausia y otros factores hormonales, así como algunas enfermedades que pueden causar osteoporosis, como la artritis reumatoide, hepatopatías crónicas, síndromes de malabsorción y el mieloma. Con respecto a los factores de riesgo modificables estarían: el sedentarismo, una dieta pobre en calcio o hiperproteica,

el tabaco, el consumo de alcohol, la delgadez y el uso prolongado de algunos fármacos (González-Macías et al., 2004).

La edad, es uno de los factores más relacionados con la osteoporosis debido a la pérdida de masa ósea que se produce por el propio proceso de envejecimiento. De forma habitual, la osteoporosis debuta en el último tercio de la vida.
El sexo femenino tiene una mayor predisposición a la osteoporosis, en parte porque generalmente la mujer alcanza un pico de masa ósea inferior al del varón y sobre todo, por el déficit estrogénico que se produce durante la transición de la menopausia.

La osteoporosis es una enfermedad poligénica de la que empiezan a conocerse algunos genes implicados, como el gen del receptor de la vitamina D (VDR), el gen del colágeno tipo I alfa I (COLIAI) y el gen del receptor de estrógenos alfa (ESR1). Los factores genéticos son considerados como uno de los factores más importantes del pico de masa ósea del adulto, pudiendo contribuir entre un 50 - 80% en la variabilidad interindividual de la masa ósea. (Ralston et al., 2006).

En la mujer, todas aquellas situaciones que se asocian con un menor tiempo de exposición a las hormonas sexuales, como la menarquía tardía, la insuficiencia ovárica primaria y los hipogonadismos están asociadas a una mayor incidencia de osteoporosis. Además, existen otros factores hormonales distintos a la menopausia, entre los que incluiríamos al hipertiroidismo, el hiperparatiroidismo y la diabetes mellitus tipo I que también estarían incluidos como factores de riesgo de osteoporosis. Durante la

transición de la menopausia acontece una disminución progresiva de la producción de hormonas ováricas dando lugar a un descenso de la masa ósea. Cuanto más precoz es la menopausia, mayor es el riesgo y la pérdida es más intensa cuando la privación hormonal se produce bruscamente (Presa et al., 2022).

La actividad física desempeña un importante papel en el desarrollo y el mantenimiento de la masa ósea debido a que actúa aumentando la formación y disminuyendo la resorción ósea. El sedentarismo, las estancias prolongadas en cama y las enfermedades que producen discapacidad del aparato locomotor, se acompañan de una pérdida de masa ósea, en cambio las personas que realizan ejercicio regularmente presentan una mayor densidad ósea (Guinot et al., 2005). Se debe recomendar a toda la población realizar ejercicio físico regular, pero adaptándolo a la edad y a las características físicas.

Una dieta con baja ingesta de calcio y la insuficiencia de vitamina D, están asociadas a un mayor riesgo de osteoporosis por un aumento del remodelado óseo y una disminución de la masa ósea. Por lo tanto, una dieta adecuada debería aportar suficientes requerimientos de calcio y vitamina D para mantener una densidad mineral óptima.

El tabaco afecta negativamente a la masa ósea acelerando la pérdida de hueso, observándose que las mujeres fumadoras tienen menor masa ósea que las no fumadoras cuando llegan a la menopausia. Fumar tiene un impacto significativo en la DMO y se

ha mostrado como un factor independiente de riesgo de osteoporosis en mujeres postmenopáusicas (Bijelic et al., 2017).

Otro hábito tóxico relacionado con el hueso es el alcohol. El consumo elevado de alcohol está asociado con un mayor riesgo de fracturas osteoporóticas. Sin embargo, el efecto del consumo de bajas dosis de alcohol en el hueso no está tan claro, puesto que se ha observado una mayor DMO en los consumidores moderados que en los abstemios (Godos et al., 2022).

Las mujeres delgadas que presentan un IMC < 19 kg/m^2, tienen un mayor riesgo de osteoporosis y también un mayor riesgo para la fractura. En parte por la menor producción de estrógenos en el tejido graso periférico, así como un menor estímulo mecánico sobre el esqueleto que ejerce un mayor peso. Como paradoja, se ha observado que la obesidad puede ser factor de riesgo para algunas fracturas periféricas, en concreto de húmero y tercio distal de radio (Riancho et al., 2022).

Diversos tratamientos favorecen la aparición de osteoporosis, entre ellos los corticoesteroides (>7,5 mg/día de Prednisona o su equivalente, aumenta x 5 el riesgo de fractura vertebral y x 2 el riesgo de fractura de cadera), los inhibidores de la aromatasa, la heparina, los inhibidores selectivos de la recaptación de serotonina, los inhibidores de la bomba de protones y los citostáticos, al acelerar la pérdida de masa ósea (Riancho et al., 2022).

Conocer e identificar los distintos factores de riesgo y realizar una adecuada asistencia a la mujer en las distintas etapas de su vida constituyen los pilares básicos en la prevención de la osteoporosis.

1.2.1 Implicaciones clínicas de la densidad mineral ósea

La osteoporosis se reconoce como "una enfermedad silenciosa" porque se produce una pérdida lenta de la DMO a lo largo de los años sin ningún síntoma, hasta que de forma repentina se produce una fractura debido a la fragilidad ósea (Cosman et al., 2014).

La osteoporosis afecta globalmente a más de 200 millones de personas, con un elevado costo para los sistemas de salud (WHO., 2007). Estudios epidemiológicos estiman que una de cada dos mujeres mayores de 50 años, sufrirá una fractura osteoporótica a lo largo de su vida. Se calcula que en Estados Unidos, la osteoporosis es responsable de 1.5 millones de fracturas con un costo asociado de 17.9 billones de dólares por año (Clynes et al., 2020).

Las fracturas osteoporóticas se producen por un traumatismo menor, como una simple caída estando en bipedestación. Suelen aparecer después de los 50 años (Compston., 2009). Las localizaciones más frecuentes son la columna vertebral, muñeca y cadera. Las fracturas de los dedos, cráneo y las de tobillo no suelen incluirse como osteoporóticas (Seeman., 2002).

La fractura más prevalente es la vertebral, de localización preferente a nivel dorsal y presentándose como un dolor agudo en la columna que se exacerba con el movimiento y disminuye con el reposo, si bien dos tercios de las fracturas vertebrales son asintomáticas.

La fractura del extremo distal de radio (fractura de Colles) suele afectar típicamente a mujeres. Constituye un marcador de osteoporosis y se considera como un factor de riesgo para la presentación de futuras fracturas vertebrales o de cadera (Johnell et al., 2006).

La fractura de cadera es la más grave, precisando una intervención quirúrgica en la mayoría de los casos y ocasionando un gran deterioro en la calidad de vida. La fractura de cadera presenta la mayor tasa de mortalidad asociada a la osteoporosis, estimándose entre un 20-30% durante el primer año (Johnell et al., 2005). La mayoría de fracturas de cadera produce discapacidad y sólo el 20% de los pacientes son capaces de realizar una vida independiente al año postfractura (Johnell et al., 2006).

También habría que tener en cuenta el impacto psicológico y social que pueden acarrear las fracturas osteoporóticas. Tras una fractura, se ha asociado con frecuencia la aparición de depresión, ansiedad, miedo a nuevas fracturas y otros trastornos emocionales. Además, los pacientes con fractura de cadera suelen presentar dependencia física ocasionando grandes repercusiones familiares y sociales (Papaioannou et al., 2009).

2. HIPOTESIS

La valoración de los factores que influyen en la fuerza muscular de agarre y la DMO (como son la edad, edad de menopausia, hábito tabáquico, índice de masa corporal (IMC), adiposidad, actividad física, hormonas endógenas y la homocisteína), permitiría identificar la vulnerabilidad del estado de salud en la mujer postmenopáusica.

La homocisteina se ha propuesto como un biomarcador de salud ósea y fuerza muscular. La incorporación de la determinación de la homocisteina en la mujer postmenopáusica, podría ser una herramienta recomendable y útil en la consulta diaria.

3. OBJETIVOS

Objetivo 1.

Analizar la correlación entre la fuerza muscular de agarre y la DMO con factores asociados (edad, edad de menopausia, hábito tabáquico, IMC, adiposidad, actividad física y hormonas endógenas: variables independientes) en mujeres potmenopáusicas.

Objetivo 2.

Analizar la correlación entre los niveles séricos de homocisteina con la fuerza muscular de agarre y la DMO en mujeres postmenopáusicas.

4. MATERIAL y METODOS

4.1 Evaluación de la fuerza muscular

La fuerza muscular de agarre, medida con un dinamómetro de mano es una técnica simple y precisa para evaluar la fuerza muscular, estando relacionada con la fuerza muscular en otras partes del cuerpo. Por lo tanto, se considera una medida sustituta de enfoques más complicados, como la fuerza de brazos y piernas (Cruz-Jentoft et al., 2019).

La medida de la fuerza muscular de agarre se evalúa en la mano dominante con un dinamómetro digital Camry EH101 (Camry Industries Co. Ltd, Kowloon, Hong Kong) calibrado en kg. **Figura 1**.

Figura 1. Dinamómetro digital Camry EH101

Siguiendo el protocolo estandarizado propuesto por la American Society of Hand Therapists (Fess.,1992), las mediciones se realizan estando la señora sentada con el brazo contra el costado, el codo

doblado en un ángulo de 90° y el antebrazo en una posición neutral, como se muestra en la **Figura 2.**

Figura 2. Postura para la medida de la fuerza de agarre de la mano con dinamómetro.

Después de demostrar cómo utilizar el dinamómetro manual, se invita a la señora a apretar lo más fuerte posible. Se realiza el registro de dos mediciones y dado que la fuerza de prensión puede disminuir gradualmente cuando se repite, se usa el valor máximo como indicador de la fuerza muscular de agarre.

Para nuestro trabajo, la dinapenia se definió cuando la fuerza muscular de agarre de la mano dominante era <20 kg, según el punto de corte recomendado por el Consenso del Grupo de Trabajo Europeo sobre Sarcopenia en Personas Mayores (EWGSOP) (Cruz-Jentoft et al., 2010).

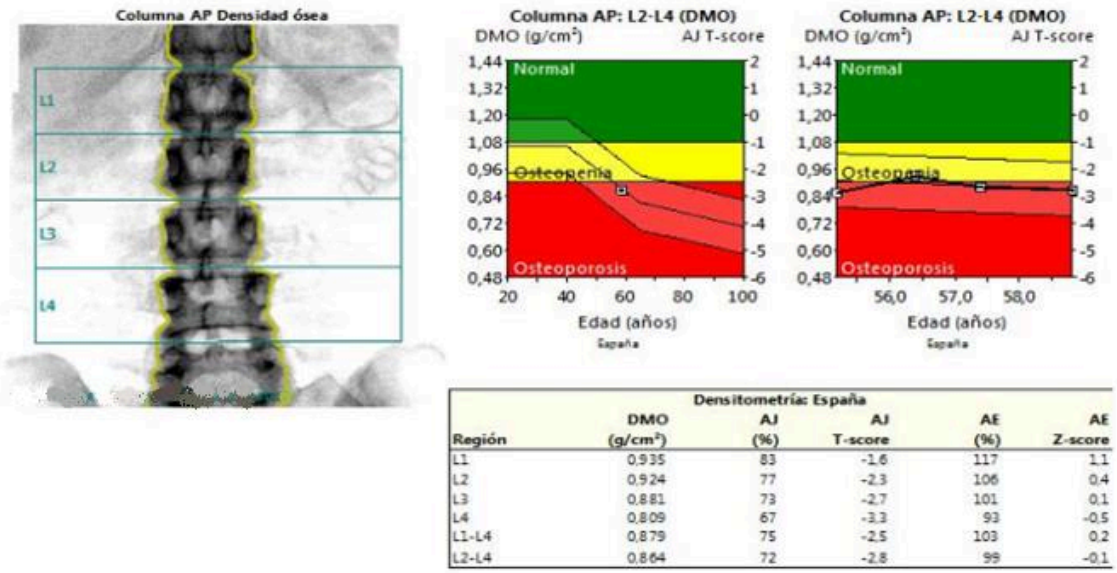
4.2 Evaluación de la densidad mineral ósea

La determinación de la DMO mediante densitometría se usa comúnmente para diagnosticar la osteoporosis y para predecir el riesgo de fractura individual.

La evaluación de la DMO en nuestro centro de trabajo se realiza mediante absorciometría de rayos X de energía dual utilizando el densitómetro Lunar iDXA (GE healthcare. Chicago, IL. EE. UU.). **Figura 4.**

Figura 4. Densitómetro Lunar iDXA

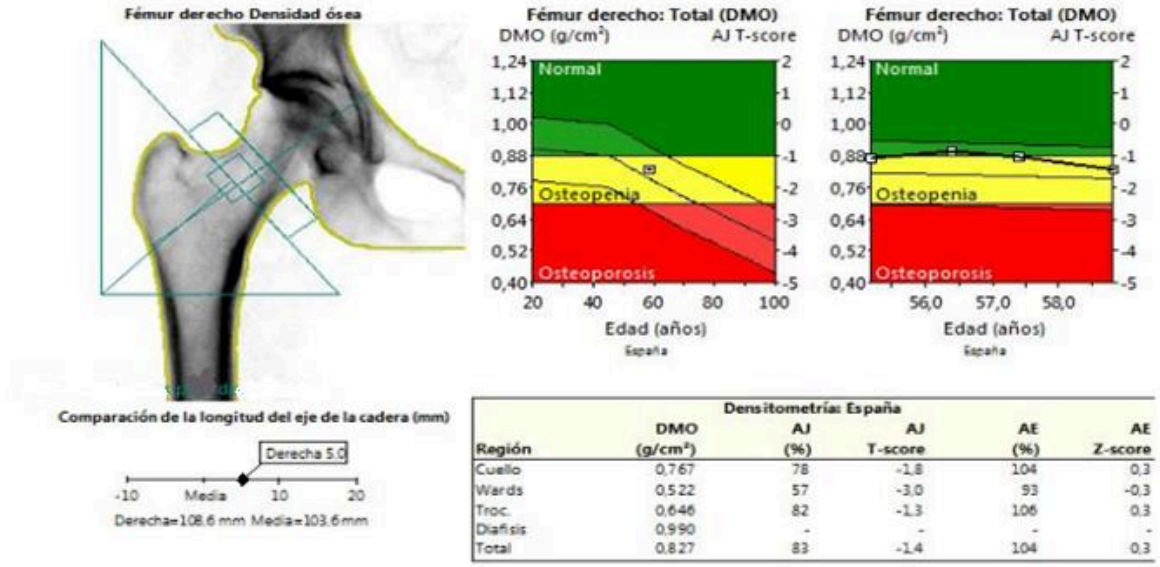
La DMO de la región de interés en columna lumbar se realiza analizando la DMO de L1 a L4, siendo evaluables todas las vértebras, excluyendo del análisis solo aquellas vértebras que están afectadas por cambios estructurales locales o artefactos (**Figura 5)**. En la región de interés de cadera, se evalúa el cuello femoral y cadera total **(Figura 6)** (Zysset et al., 2015).



Densitometría: España

Región	DMO (g/cm²)	AJ (%)	AJ T-score	AE (%)	AE Z-score
L1	0,935	83	-1,6	117	1,1
L2	0,924	77	-2,3	106	0,4
L3	0,881	73	-2,7	101	0,1
L4	0,809	67	-3,3	93	-0,5
L1-L4	0,879	75	-2,5	103	0,2
L2-L4	0,864	72	-2,8	99	-0,1

Figura 5. DMO en la región de interés en columna lumbar



Densitometría: España

Región	DMO (g/cm²)	AJ (%)	AJ T-score	AE (%)	AE Z-score
Cuello	0,767	78	-1,8	104	0,3
Wards	0,522	57	-3,0	93	-0,3
Troc.	0,646	82	-1,3	106	0,3
Diafisis	0,990	-	-	-	-
Total	0,827	83	-1,4	104	0,3

Figura 6. DMO en la región de interés en cadera.

En la evaluación de la DMO, la mujer debe estar con una bata, sin objetos metálicos y tumbarse en la mesa acolchada del densitómetro permaneciendo inmóvil. Para evaluar la columna, las

piernas se apoyan en una caja acolchada para aplanar la pelvis y la columna lumbar. Para evaluar la cadera, el pie se coloca en una abrazadera que produce una rotación interna de la cadera.

El control de calidad del densitómetro se realiza mediante la calibración diaria de un estándar de referencia o fantoma de densidad conocida que simula las partes anatómicas medidas en clínica (Shuhart et al., 2019). Las mediciones del fantoma mostraron resultados estables durante el transcurso del estudio. Los coeficientes de variación de las mediciones densitométricas oscilaron entre 0,23 % y 0,38 %. La evaluación de la densidad mineral ósea se realizó utilizando únicamente los datos crudos generados por el densitómetro. Los valores obtenidos se expresaron en valores absolutos como gramos de contenido mineral por centímetro cuadrado de área ósea (g/cm^2) y T-score.

La clasificación operativa para el diagnóstico de la osteoporosis propuesta por la Organización Mundial de la Salud (OMS) se basa en el número de desviaciones estándar en que la DMO en columna lumbar o cadera, varía con respecto a la media correspondiente a la población adulta joven de referencia (T-score).

De acuerdo con el sistema de clasificación de la DMO de la OMS, las mujeres se clasificaron sobre la base de la T-score más baja de la siguiente manera: DMO normal con una puntuación T > -1 desviación estándar (DE), osteopenia con puntuaciones T que oscilan entre -1 DE a -2,5 DE, y osteoporosis con un T-score ≤ -2,5 DE (WHO., 1994). **Figura 7.**

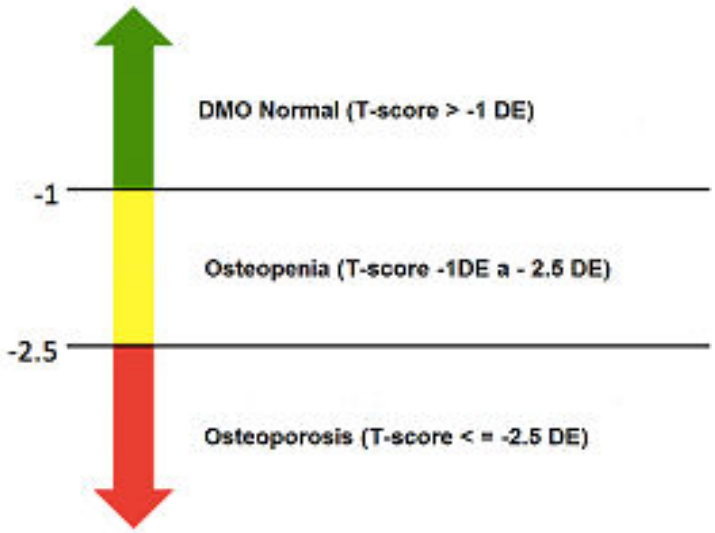


Figura 7. Clasificación de la DMO de la OMS. 1994

4.3 Factores analizados

4.3.1 Edad

La máxima fuerza muscular se alcanza entre la 2ª y 3ª décadas de la vida. En las mujeres se observa que la pérdida de fuerza muscular comienza alrededor de los 50 - 60 años. A partir de esta edad se estima que se produce una pérdida de fuerza muscular del 1,5 - 5% por año (Goodpaster et al., 2006; Manini et al., 2012). Como hemos comentado anteriormente el "pico de masa ósea" se alcanza, al igual que la máxima fuerza muscular, entre los 25 y los 30 años, seguido de un periodo de estabilidad en la DMO y en la mujer, posteriormente se produce una disminución brusca durante la menopausia y una pérdida lenta pero progresiva durante el resto de años de la vida (Seeman., 2002; Mora et al., 2003).

4.3.2 Edad de menopausia

La disminución de estrógenos que ocurre durante la menopausia se ha asociado con una peor función física. Estudios recientes muestran que una menopausia temprana se asocia con peor fuerza muscular de agarre, velocidad de la marcha y limitación funcional autoinformada (de Souza Macêdo et al., 2021). Además, una edad más temprana en el inicio de la menopausia se ha asociado con un mayor riesgo de presentar dinapenia (García-Alfaro et al., 2019).

Respecto a la densidad mineral ósea, se ha observado que cuanto más temprano en la vida ocurra la menopausia, menor será la densidad ósea más adelante en la vida. Asociándose la baja densidad ósea con una mayor tasa de fracturas (Gallagher., 2007).

4.3.3 Hábito tabáquico

Fumar se ha asociado con un aumento del catabolismo de proteínas, induce a la pérdida de masa muscular, reduce el suministro de oxígeno y altera la función mitocondrial. Como consecuencia, aumenta la fatiga muscular al comprometer la capacidad del sistema muscular para obtener energía (Degens et al., 2015).

El hábito tabáquico actúa inhibiendo el eje vitamina D - hormona paratiroidea, acelerando la metabolización de los estrógenos endógenos y disminuyendo la producción periférica de estrógenos en el tejido adiposo, comportando un aumento del riesgo de fracturas osteoporóticas (Trevisan et al., 2020).

4.3.4 Índice de masa corporal (IMC)

Aunque se sugiere que el sobrepeso o la obesidad pueden aumentar el riesgo de deterioro funcional y discapacidad de movilidad en adultos mayores (Mankowski et al., 2015), todavía existen controversias a la hora de evaluar la relación entre el IMC y la fuerza muscular de agarre. Estudios previos han encontrado una débil correlación entre el IMC y la fuerza de prensión (Lopes et al., 2018), contrastando con el resultado de otros estudios donde informan que la fuerza muscular de agarre no está relacionada con el IMC (Kamarul et al., 2006).

Se ha estimado que el IMC podría explicar del 2 al 16% de la variabilidad de la fuerza muscular (Leblanc et al., 2015) y un índice de masa corporal < 19 kg/m^2, representa un mayor riesgo de osteoporosis y riesgo de fractura. Pero, como hemos comentado anteriormente, la obesidad puede aumentar el riesgo de algunas fracturas periféricas (Riancho et al., 2022).

En nuestro protocolo, el IMC se ha calculado como el peso en kilogramos dividido por el cuadrado de la altura en metros (kg/m^2), después de pesar y medir a las participantes descalzas y vestidas con una bata desechable. Según los criterios de la OMS, el IMC se clasificó como bajo (<18.50 kg/m2), normal (18.50-24.99 kg/m2) y alto, donde se incluía sobrepeso (25.0-29.99 kg/m2) y obesidad (30.0 kg/m2) (WHO., 2002).

4.3.5 Adiposidad

Después de la menopausia, hay una disminución en el porcentaje de grasa total en las piernas y un aumento en las medidas de grasa central por lo que sería indicativo de un cambio en la distribución de la masa grasa (Ambikairajah et al., 2019).

El exceso de tejido adiposo induce un estado proinflamatorio por la acción de las citoquinas, entre ellas, el factor de necrosis tumoral alfa y la interleucina-6. La elevación de los niveles plasmáticos de estas citoquinas se asocia con una menor fuerza y masa muscular y con un impacto negativo en la calidad ósea (Bian et al., 2017; Gkastaris et al., 2020).

La aplicación de técnicas o medidas para determinar la composición corporal y la estimación del porcentaje de grasa corporal están adquiriendo a nivel clínico, una importancia cada vez más creciente. Las principales técnicas de valoración de la composición corporal serían, la absorciometría de rayos X de energía dual, la resonancia magnética nuclear y el análisis de impedancia bioeléctrica.

Para evaluar los cambios en la composición corporal el análisis de impedancia bioeléctrica (BIA) ha sido validado y es considerado como una buena alternativa portátil a la densitometría (Gibson et al., 2000). Además, está ampliamente disponible, es rápido, económico, fácil de realizar, fácilmente reproducible y requiere un entrenamiento mínimo del operador, siendo apropiado para fines epidemiológicos y clínicos (Marra et al., 2019).

La adiposidad se ha evaluado mediante BIA usando el monitor Omron BF 306 (Omrom Healthcare Co Ltd, Kioto, Japón) **Figura 8**.

Figura 8. Monitor Omron BF 306

El monitor mide la impedancia de brazo a brazo a lo largo de la cintura escapular y el resultado obtenido es expresado como porcentaje de grasa.

Tras introducir los datos de la edad, peso y altura en el monitor, el protocolo utilizado para la evaluación de la medición se realizó en posición de pie, utilizando una bata desechable, con las piernas separadas entre 30º a 45º y los brazos extendidos hacia adelante en un ángulo de 90º con respecto al tronco, sujetando el monitor con ambas manos. **Figura 9.**

Figura 9. Posición para la medición de la adiposidad con BIA.

4.3.6 Actividad física

La actividad física es otro factor que influye en la fuerza muscular y en la masa ósea. Se ha observado que practicar una actividad física de forma regular, explicaría del 1 al 3% de la variabilidad de la fuerza muscular (Leblanc et al., 2015).

La actividad física estimula la síntesis de proteínas musculares y tiene efectos beneficiosos sobre la masa muscular, la fuerza muscular y el rendimiento físico. También se ha demostrado que el ejercicio físico tiene efectos beneficiosos sobre la DMO, así como

para retrasar la aparición de la osteoporosis (Agostini et al., 2018). Mantener el hábito del ejercicio físico regular durante la mediana edad, está asociado con una baja prevalencia de sarcopenia, siendo eficaz para mantener la fuerza muscular y el rendimiento físico en la vejez (Akune et al., 2014).

Teniendo en cuenta estos hallazgos, la actividad física es una variable de estilo de vida modificable que podría ser interesante en la prevención y el manejo de la disminución de la fuerza muscular y de la DMO, por lo que debería fomentarse (Barbat-Artigas et al., 2014).

Para unificar criterios empleados en la valoración de la actividad física se han elaborado una serie de estándares. Uno de los instrumentos creados ha sido el Cuestionario Internacional de Actividad Física IPAQ (International Physical Activity Questionnaire). Se trata de un cuestionario estandarizado para estudios poblacionales a nivel mundial, del cual se ha evaluado su validez y fiabilidad (Craig et al., 2003).

La actividad física se ha evaluado utilizando la versión española del formato corto del IPAQ (**Anexo 1**). El cuestionario incluye siete ítems que aportan información sobre el número de días a la semana y el tiempo que se pasa en actividades moderadas y actividades vigorosas, caminando y sentado. Las categorías del nivel de actividad física se clasifican en: bajo, moderado y alto (The IPAQ group., 2016).

Anexo 1. CUESTIONARIO INTERNACIONAL DE ACTIVIDAD FÍSICA

IPAQ: FORMATO CORTO

*Piense en todas las actividades **intensas** que usted realizó en los **últimos 7 días.** Las actividades físicas **intensas** se refieren a aquellas que implican un esfuerzo físico intenso y que lo hacen respirar mucho más intensamente que lo normal. Piense solo en aquellas actividades físicas que realizó durante por lo menos **10 minutos** seguidos.*

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

   _____ **días por semana**

   ☐ Ninguna actividad física intensa → ***Vaya a la pregunta 3***

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

   _____ **horas por día**

   _____ **minutos por día**

   ☐ No sabe/No está seguro

   ☐

*Piense en todas las actividades **moderadas** que usted realizó en los **últimos 7 días.** Las actividades **moderadas** son aquellas que requieren un esfuerzo físico moderado que lo hace respirar algo más intensamente que lo normal. Piense solo en aquellas actividades físicas que realizó durante por lo menos **10 minutos** seguidos.*

3. Durante los **últimos 7 días**, ¿en cuántos días hizo actividades físicas **moderadas** como transportar pesos livianos, andar en bicicleta a velocidad regular o jugar dobles de tenis? **No** incluya caminar.

   _____ **días por semana**

   ☐ Ninguna actividad física moderada → ***Vaya a la pregunta 5***

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

   _____ **horas por día**

   _____ **minutos por día**

   ☐ No sabe/No está seguro

*Piense en el tiempo que usted dedicó a **caminar** en los **últimos 7 días**. Esto incluye caminar en el trabajo o en la casa, para trasladarse de un lugar a otro, o cualquier otra caminata que usted podría hacer solamente para la recreación, el deporte, el ejercicio o el ocio.*

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

   _____ **días por semana**

   ☐ Ninguna caminata → ***Vaya a la pregunta 7***

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

   _____ **horas por día**

   _____ **minutos por día**

   ☐ No sabe/No está seguro

*La última pregunta es acerca del tiempo que pasó usted **sentado** durante los días hábiles de los **últimos 7 días**. Esto incluye el tiempo dedicado al trabajo, en la casa, en una clase, y durante el tiempo libre. Puede incluir el tiempo que pasó sentado ante un escritorio, visitando amigos, leyendo, viajando en ómnibus, o sentado o recostado mirando la televisión.*

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

   _____ **horas por día**

   _____ **minutos por día**

   ☐ No sabe/No está seguro

4.3.7 Hormonas endógenas

Además de la disminución de la fuerza muscular y la DMO relacionada con la edad, el estado hormonal está involucrado en el mantenimiento del sistema músculo esquelético.

La disminución de la fuerza muscular ocurre a una edad más temprana en las mujeres en comparación con los hombres y se asocia en parte con la disminución progresiva de los niveles de estrógenos durante la transición menopáusica (Rolland et al., 2007; Cipriani et al., 2012). Sin embargo, existen estudios con resultados divergentes en cuanto a la relación entre los efectos de los estrógenos sobre la fuerza muscular (Schapp et al., 2005; Maddalozzo et al. 2004).

El efecto de los andrógenos sobre la fuerza muscular en mujeres postmenopáusicas no está bien aclarado. Al igual que el estradiol, los niveles de testosterona disminuyen con la edad. Después de la menopausia, la dehidroepiandrosterona (DHEA) se convierte en la única fuente para la síntesis de estrógenos y andrógenos. La DHEA y su sulfato (DHEAS) son en sí mismas inactivas, pero en los tejidos periféricos se transforman en el interior de las células en pequeñas cantidades de estrógenos y andrógenos (Labrie., 2019).

Con el envejecimiento, hay algunos cambios en el ritmo circadiano del cortisol. Se producen aumentos en los niveles de cortisol al final del día y al anochecer, con el pico de cortisol por la mañana temprano, una amplitud circadiana más baja y patrones de secreción de cortisol más irregulares (Veldhuis et al., 2013). Los

niveles elevados de cortisol están asociados con atrofia y debilidad muscular.

El factor de crecimiento insulínico 1(IGF-1) es una hormona polipeptídica con efectos autocrinos, paracrinos y endocrinos que juega un papel importante en la miogénesis esquelética y está asociada con el desarrollo de la masa y la fuerza muscular (Ahmad et al., 2020).

La vitamina D es un secosteroide sintetizado en la piel a partir del 7-deshidrocolesterol por efecto de los rayos ultravioleta de la luz solar. La vitamina D está implicada con la fuerza muscular y la DMO, pero sus relaciones presentan resultados ambiguos. Los diferentes resultados podrían estar relacionados con el umbral del nivel plasmático de vitamina D a partir del cual afecta la fuerza muscular o la DMO (López-Baena et al., 2020; Institute of Medicine., 2011).

Finalmente, comentar los efectos que la hormona paratiroidea (PTH) puede ejercer a nivel muscular y óseo. Los niveles circulantes de PTH aumentan con la edad independientemente de la función renal, los niveles plasmáticos de vitamina D, calcio y fósforo. La PTH influye en el metabolismo de las proteínas del músculo esquelético en modelos animales y aumenta la concentración de calcio intracelular alterando la función muscular (Visser et al., 2003). A nivel óseo, una PTH elevada provoca una hiperestimulación de los osteoclastos, con la consecuente reducción de la DMO y un aumento del riesgo de fracturas (Bruce et al., 1999).

4.3.8 Homocisteína

La homocisteína es un aminoácido sulfurado producido en el hígado por la transmetilación de la metionina. Se degrada en cisteína a través de la vía irreversible de transulfuración y en circunstancias normales, alrededor del 50 % sigue la vía de remetilación para regenerar metionina. Estas vías de reacción requieren la presencia de vitaminas B6, B12 y folato como cofactores. La deficiencia de actividad enzimática o de sus cofactores puede producir una elevación de los niveles plasmáticos de homocisteína (Kaye et al., 2020). La homocisteína plasmática aumenta significativamente con la edad, existiendo diferencias de género al observarse que el aumento es mayor en hombres que en mujeres (Chen et al., 2017).

La hiperhomocisteinemia se ha relacionado con un mayor riesgo de desarrollar síndrome coronario agudo, accidente cerebrovascular y mortalidad cardiovascular (Chrysant et al., 2018). Además, se ha asociado con deterioro cognitivo, demencia y enfermedad de Alzheimer (Ma et al., 2017).

Estudios recientes han mostrado una relación entre los niveles de homocisteína plasmática con la fuerza muscular y la sarcopenia, aunque con resultados controvertidos (van Schoor et al., 2012; Swart et al., 2013; Vidoni et al., 2018; De Giuseppe et al., 2021).

También se ha observado que los niveles séricos de homocisteína afectan la formación de tejido óseo a través de varios mecanismos. La homocisteína reduce el flujo sanguíneo óseo, aumenta la actividad de los osteoclastos y activa las metaloproteinasas de la

matriz ósea (Tyagi et al., 2011; Koh et al., 2006; Vacek et al., 2013), causado daño oxidativo e interfiriendo con la formación de enlaces cruzados en el colágeno (Saito et al., 2010). En consecuencia, la homocisteína puede disminuir la mineralización ósea y reducir la resistencia ósea, aumentando el riesgo de fracturas (Fratoni et al., 2015).

Dada la implicación que tiene la homocisteína en diversas enfermedades, se ha propuesto como un marcador del estado de salud y se sugiere que la homocisteína es más que un biomarcador de enfermedades, es una guía para la prevención de enfermedades (Smith et al., 2021).

5. RESULTADOS

Artículo 1: Objetivo 1

Handgrip strength, dynapenia, and related factors in postmenopausal women

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ORIGINAL STUDY

Handgrip strength, dynapenia, and related factors in postmenopausal women

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Abstract

Objective: This study aimed to evaluate the prevalence of dynapenia and factors related to low dominant handgrip strength (HGS) in postmenopausal women.

Methods: A cross-sectional study was performed on 249 postmenopausal women aged 50 to 84 years. The following variables were recorded: age, age at menopause, smoking status, and the HGS measured with a digital dynamometer, body mass index, and adiposity assessed by bioelectric impedance. The physical activity level was evaluated by using the International Physical Activity Questionnaire. Bone mineral density was reported as T-scores, and blood biochemical parameters (calcium, phosphorus, vitamin D, and parathormone levels) were measured.

Results: 31.3% of women had dynapenia, and those aged $\geq$65 years had lower HGS ($P < 0.001$). Age at menopause was also associated with HGS, with those with menopause < 51 showing lower HGS ($P = 0.005$). Likewise, fat content $\geq$ 40%, and osteopenia/osteoporosis were also related to lower strength ($P < 0.001$). There was no statistically significant difference among HGS with respect to body mass index, smoking status, and plasma levels of vitamin D. A logistic regression model with lower Akaine Information Criterion showed that for every year in age and for each 1% of adiposity, women were more likely to have dynapenia with odd ratio (OR): 1.09; 95% and confidence interval (CI): 1.04 to 1.14 and OR: 1.06; 95% CI: 1.00 to 1.13, respectively. Conversely, women with higher femoral neck T-score were less likely to have dynapenia (OR: 0.53; 95% CI: 0.35-0.78).

Conclusions: HGS was associated with age at menopause, bone mineral density, and adiposity adjusted by age. The age and adiposity were significantly associated with a higher risk of dynapenia, whereas women with higher femoral neck T-score were less likely to have dynapenia.

Key Words: Adiposity – Age at menopause – Body mass index – Bone mineral density – Dynapenia – Handgrip strength – Physical activity – Vitamin D.

Muscle strength is an essential function of the human body, being a relevant component of health and physical fitness. It also refers to the ability of a muscle or muscle group to exert force against a resistance. Muscle strength is involved in many activities, daily tasks, maintenance of functional independence, and autonomy. Thus, regular physical activity adapted according to age is a preventive recommendation for healthy aging.[1-3] Dynapenia defines the age-related loss of muscle strength, is the primary indicator of sarcopenia, and is a prognostic indicator of functional impairments in older adults.[4] Thus, it is very important to maintain muscle strength to reduce functional limitations with age. The grip strength is considered a biomarker of frailty, in which there is an increase in an individual's vulnerability for developing dependency and/or mortality.[5-7] In this context, it is convenient to dissociate the muscle mass reduction

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(sarcopenia) from the concept of muscle strength reduction (dynapenia).[8]

The pathophysiological mechanisms of muscle weakness can be compartmentalized into two factors. First the impairment of muscle system causes a deficit in the skeletal muscle force production and second the nervous system impairment that decreases the ability of voluntary muscle activation. Subsequently, it produces functional limitations and risk of physical disability.[9] Muscle-skeletal changes are associated with aging, although other factors are involved in maintaining muscle strength and mass, such as hormonal profile, physical activity level, and nutritional status. So that old age, postmenopausal women, low body mass index (BMI), and low physical activity have been highly associated with dynapenia.[8-10] Handgrip strength (HGS) measured with a hand dynamometer is a simple and accurate technique to assess muscle strength which is moderately related to muscular strength in other body sites. Therefore, it is considered a surrogate measure of more complicated approaches, such as arm and leg strength.[11] The purpose of the current study was to investigate the prevalence of dynapenia and investigate factors associated with low HGS in postmenopausal women.

METHODS

Study design

This cross-sectional study was approved by the Center Institutional Review Board and performed from March 2019 to March 2020, in women who had their periodic gynecological check-up at the Department of Obstetrics and Gynecology of the Dexeus Women's University Hospital, Barcelona, Spain. Women were excluded if (1) they had early menopause ($<$ 45 y) or surgical menopause; (2) were using menopausal hormonal treatment; or (3) had cardiovascular, liver, or renal diseases, a history of cancer, or physical disability. Finally, a group of 249 postmenopausal women aged 50 to 84 years, with at least 1 year of amenorrhea, participated in the study. We assessed their HGS, BMI, adiposity, bone mineral density (BMD), and physical activity. Smoking status and age at menopause were collected in the visit interview. The following blood biochemical laboratory parameters were also collected: calcium, phosphorus, vitamin D, and parathormone levels.

Muscle function assessment, body mass index, and adiposity

Physical activity was studied with the Spanish version of the short International Physical Activity Questionnaire. This tool includes seven-items that provide information about the number of days per week and the time spent in moderate and vigorous activities, walking and sitting. Physical activity categories were classified as low, moderate, and high levels.[12] Muscle strength was assessed by HGS measured with a digital dynamometer Camry EH101 (Camry Industries Co. Ltd, Kowloon, Hong Kong) calibrated in kg. The measurement was carried out with the arm against the side and the elbow bent to a 90° angle. The dominant hand was assessed twice and the maximum value was recorded. For the current study, dynapenia was diagnosed when the dominant hand force was $<$20 kg, according to the cut-off recommended by the European Working Group on Sarcopenia in Older People Consensus.[13]

BMI was calculated as the weight in kilograms divided by the square of the height in meters after weighing and measuring participants without shoes and wearing a disposable gown. According to WHO criteria,[14] BMI categories were established as low ($<$18.50 kg/m^2), normal (18.50-24.99 kg/m^2), and high that included both overweight ($\geq$25.0-29.99 kg/m^2) and obesity ($\geq$30.0 kg/m^2).

Adiposity was assessed by bioelectrical impedance analysis and expressed as a percentage of fat, using the Omron BF 306 monitor (Omrom healthcare Co Ltd, Kyoto, Japan) that measures arm-to-arm impedance along the shoulder girdle. The assessment was performed two times in the standing position, with the legs 35° to 45° apart and the arms extended forwards at a 90° angle with the trunk, using a disposable gown. Using the mean of the whole sample two categories were established: $<$40% fat and $\geq$40% fat.

Bone mineral density and biochemical parameters

BMD was measured by dual-energy x-ray absorptiometry (DXA) using the Lunar iDXA system (GE Healthcare, Chicago, IL). The measurements were carried out at the lumbar spine (L1-L4), femoral neck, and total hip, and the values were expressed as T-score. Following the WHO classification system,[15] based on their lowest T-score the women were classified as normal BMD with a T-score > -1 standard deviation (SD), osteopenia with a T-score between -1 SD and -2.5 SD and osteoporosis with a T-score ≤ -2.5 SD.

Blood samples were collected after an overnight fast. The plasma levels of calcium, phosphorus, vitamin D, and parathormone were measured by an automated COBAS 8000 modular analyzer System (Roche Diagnostics, Pleasanton, CA). The laboratory performs daily quality controls in accordance with the International Organization for Standardization, rule ISO 9001:2015 of each parameter. The results must be less than $\pm$2 standard deviations with respect to the daily control value. Vitamin D levels were categorized in: $\geq$30 ng/mL (sufficient) and $<$30 ng/mL (hypovitaminosis D).[16]

Statistical analysis

Continuous outcomes were calculated as mean and standard deviation whereas percentages and numbers were used for categorical variables. Normal distribution was checked with the Shapiro-Wilks test. The Student *t* test or ANOVA test was used to compare average grip strength in categorical parameters. Pearson correlation was used to estimate the relation between numeric parameters with grip strength. Partial correlation adjusted by age was also calculated. A multivariable logistic regression model was constructed to analyze possible predictors of dynapenia. After testing several models, we chose the model that had a lower Akaike

TABLE 1. *Biochemical parameters reported as mean ± standard deviation*

Parameters	Estimate ($n = 249$)	95% confidence Interval	Reference ranges
Vitamin D	33.7 ± 11.8	32.2-35.2	30-100 ng/mL
Calcium	9.59 ± 0.35	9.5-9.6	8.7-10.4 mg/dL
Phosphorous	3.6 ± 0.4	3.6-3.7	2.5-4.5 mg/dL
Parathormone	44.0 ± 14.9	42.2-45.9	15-65 pg/mL

Information Criterion, taking into account associated factors: age, adiposity and T-score femoral neck. Results are reported as odds ratios (OR) and 95% confidence interval (95% CI). All analyses were performed using the R software (R Core Team, 2019). All the analyses were exploratory. No formal sample size calculation was performed.

RESULTS

The sample included 249 postmenopausal women, with a mean age of 62.7 ± 6.9 years and a mean age at menopause of 50.1 ± 2.7 years. The estimated average grip strength was 22.4 ± 4.0 kg, the whole BMI sample was 24.7 ± 3.8 kg/m^2, and the total sample adiposity was 40.1 ± 5.3%. Mean of BMD measurements in the lumbar spine, femoral neck, and total hip were in the osteopenia ranges. The values of the biochemical parameters vitamin D, calcium, phosphorus, and parathormone were in the normal range (Table 1).

Table 2 displays the handgrip strength and related outcomes. Women aged ≥65 years had significantly lower HGS than those aged <65 years ($P < 0.001$), and there was a significant association between age at menopause and HGS ($P = 0.005$). Women with age at menopause ≥51 years had higher HGS values than those with age at menopause <50 years. There were no statistically significant differences between HGS and smoking status ($P = 0.846$) and BMI categories ($P = 0.691$). Regarding adiposity, women who had > 40% fat showed a significant association with low HSG ($P < 0.001$). There were no differences between HGS and vitamin D levels ($P = 0.503$). Women who performed low physical activity had lower HGS than those with moderate-high physical activity ($P = 0.027$). We also found that women with normal BMD had higher HGS than women with lumbar spine osteopenia-osteoporosis ($P < 0.001$), femoral neck ($P < 0.001$), and total hip ($P < 0.001$). There were no significant correlations between HGS with BMI and studied hormone and biochemical parameters.

Table 3 shows Pearson correlation coefficients (r) between HGS and covariates. HGS was correlated with age (r = −0.34, $P < 0.001$), age at menopause (r = 0.13, $P = 0.035$), adiposity (r = −0.27, $P < 0.001$), T-score lumbar spine (r = 0.26, $P < 0.001$), T-score femoral neck (r = 0.26, $P < 0.001$), and T-score total hip (r = 0.27, $P < 0.001$) (Fig. 1). Partial correlation adjusted by age has shown the same results.

Finally, a logistic regression analysis was fitted to estimate muscle strength <20 kg (dynapenia). Elderly women were more likely to have dynapenia. For every year in age, risk increased with OR of 1.09 (95% CI: 1.04-1.14). Likewise, women with higher adiposity had higher risk of dynapenia. For each 1% of adiposity, OR was 1.06 (95% CI: 1.00-1.13). On the contrary, women with higher femoral neck T-score were less likely to have dynapenia. OR of 0.53 (95% CI: 0.35-

TABLE 2. *Differences between handgrip strength (mean ± standard deviation) with regards to general characteristics of studied women, and vitamin D status*

Parameters	Categories	$n = 249$	HGS	P value	95% CI (mean differences)
Age (y)	<65	132	23.7 ± 3.8	<0.001	[1.7; 3.7]
	≥65	117	21.0 ± 3.8		
Age at menopause (y)	<51	156	21.9 ± 3.8	0.005	[−2.6; −0.5]
	≥51	93	23.4 ± 4.3		
Smoking	Never	202	22.5 ± 4.1	0.846	[−1.4; 1.2]
	Smoker	47	22.3 ± 4.0		
BMI (kg/m^2)	Low	4	22.1 ± 3.2	0.691	[−4.7; 3.5]
	Normal	143	22.6 ± 4.0		Reference
	High	102	22.2 ± 4.1		[−1.5; 0.6]
Adiposity (%)	<40	109	23.7 ± 4.3	<0.001	[1.1; 3.2]
	≥40	140	21.5 ± 3.5		
Vitamin D (ng/mL)	<30	106	22.2 ± 3.6	0.503	[−1.3; 0.7]
	≥30	143	22.6 ± 4.4		
Physical activity	Low	108	21.8 ± 3.5	0.027	Reference
	Moderate	132	22.8 ± 4.3		[−0.01; 2.1]
	High	9	24.9 ± 5.3		[0.4; 5.9]
BMD Lumbar spine	Normal	68	24.5 ± 3.7	<0.001	Reference
	Osteopenia	116	21.6 ± 4.1		[−4.1; −1.7]
	Osteoporosis	65	21.7 ± 3.6		[−4.2; −1.5]
BMD total hip	Normal	103	23.6 ± 3.9	<0.001	Reference
	Osteopenia	134	21.6 ± 4.1		[−3.0; −0.9]
	Osteoporosis	12	21.2 ± 2.2		[−4.9; −0.1]
BMD Femoral Neck	Normal	83	23.9 ± 3.6	<0.001	reference
	Osteopenia	156	21.7 ± 4.1		[−3.3; −1.1]
	Osteoporosis	10	22.2 ± 3.1		[−4.4; 0.9]

BMD, bone mineral density; BMI, body mass index; CI, confidence interval; HSG, handgrip strength.

TABLE 3. *Pearson correlation analyses between handgrip strength and age, age at menopause, adiposity, and bone mineral density (T-score)*

	Handgrip strength			
Covariates	r (Pearson correlation)	*P* value	Partial correlations	*P* value
Age	−0.340	<0.001	-	-
Age at menopause	0.130	0.035	0.166	0.009
Body mass index	0.002	0.970	0.050	0.432
Adiposity	−0.270	<0.001	−0.225	<0.001
Vitamin D	0.041	0.520	0.120	0.058
Calcium	0.110	0.075	0.124	0.051
Phosphorus	0.042	0.510	−0.005	0.941
Parathormone	−0.010	0.870	0.025	0.699
T-score lumbar spine	0.260	<0.001	0.178	0.005
T-score femoral neck	0.260	<0.001	0.223	<0.001
T-score total hip	0.270	<0.001	0.205	0.001

Partial correlations with Pearson adjusted by age.

0.78) for each standard deviation. The prevalence of dynapenia in the study population was 31.3% (78/249).

DISCUSSION

We demonstrated in postmenopausal women aged 50 to 84 years that low HGS was correlated with age at menopause; adiposity (> 40% fat mass) and low BMD adjusted by age. Factors more likely associated with a higher risk of dynapenia were age and adiposity, whereas women with higher femoral neck T-score were less likely to have dynapenia.

Dynapenia and sarcopenia

Dynapenia is the age-associated loss of muscle strength not caused by muscular or neurologic diseases, whereas the term

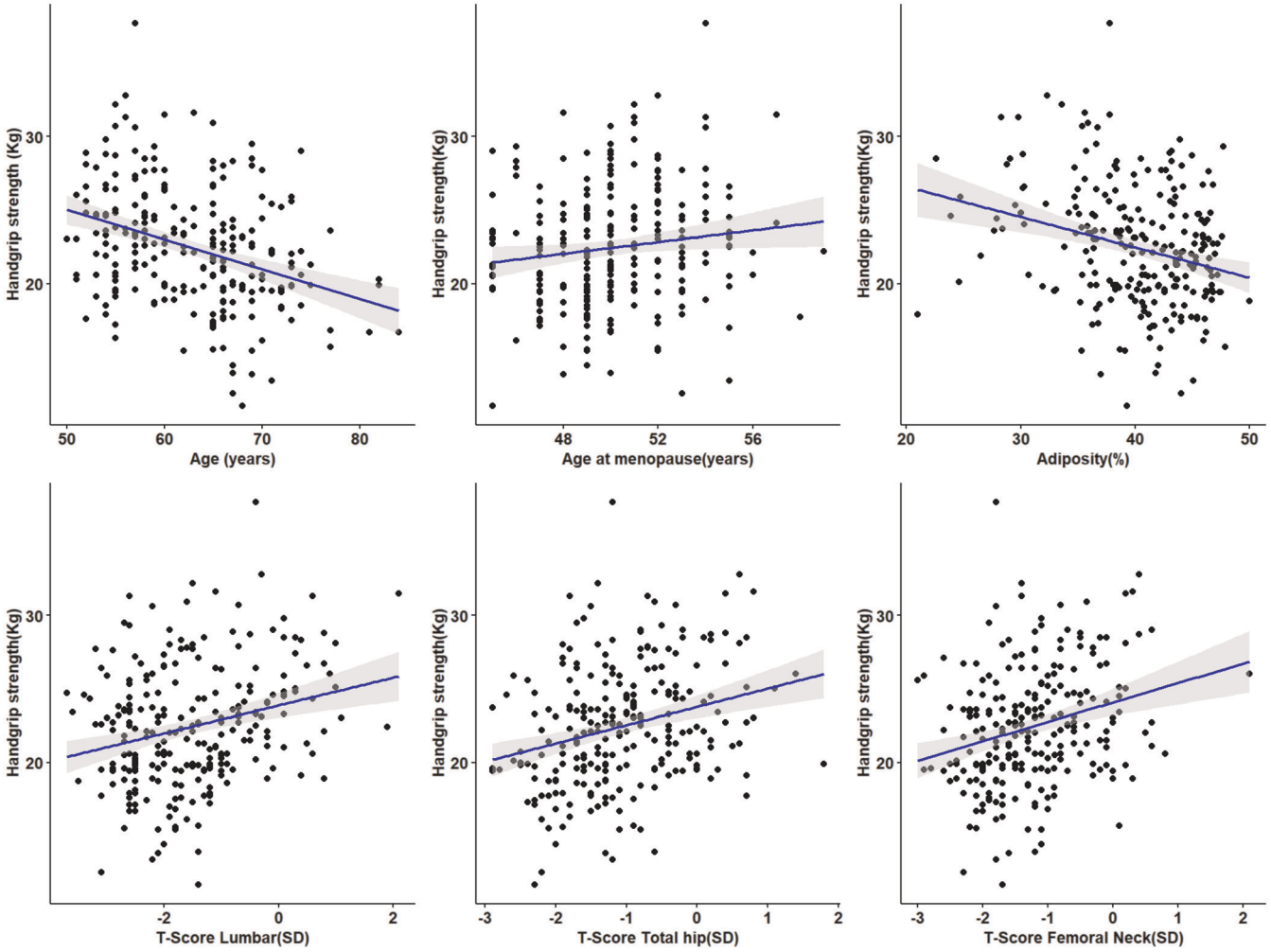

FIG. 1. Correlations between dominant handgrip muscle strength and age, age at menopause, adiposity, and bone mineral density (T-score).

sarcopenia refers to the gradual reduction of muscle mass.[4,8] Dynapenia might be considered an initial step of muscle dysfunction prior to sarcopenia.[10] Barbat-Artigas et al[17] reported that muscle strength is a better outcome of physical capacity than skeletal muscle mass, and dynapenic women have lower cardiorespiratory function than nondynapenic women even if they have the same muscle mass. Women begin the loss of muscle strength around the 5th and 6th decades of age.[18,19] We previously reported in a group of young postmenopausal women (aged <65 y), a weak but significant inverse correlation between HGS and women's age.[20] The present results showed that aging is related with the loss of muscle strength and this loss progressively decreases over the years. The aging process is associated with changes in qualitative and quantitative muscle mass.[21,22] The loss of muscle quality occurs by a progressive decrease in muscle fiber type II, which plays an important role in anaerobic metabolism, likely represents the mechanism that initiates the declining muscle strength.[23] However, age-related loss of muscle strength is only partially explained by the changes in muscle composition and reduction in muscle mass, therefore other physiologic factors would be involved in explaining muscle weakness in older adults,[24] including gonadal ageing and reduction or lack of gonadal steroids[25,26] and loss or dysfunction of motoneurons.[27]

The progressive decline in estrogen levels that occurs during the menopausal transition is also accompanied by significant changes in body composition, loss of muscle strength, and decreased BMD.[10] Age at menopause has been related with HGS and an earlier age at menopause onset has been associated with an increased risk for presenting dynapenia.[20] Thus, menopause could be considered as a factor directly related with muscle strength, regardless of age.[25] The current study revealed that HGS had a weak decline correlated with age at menopause. Although it has been postulated that ovarian hormones protect against age-related loss of muscle strength in postmenopausal women, a meta-analysis suggests that hormone therapy did not protect against age-related lean body mass loss.[28] In addition, the Canadian Longitudinal Study on Aging study reported that postmenopausal women who have never used hormone therapy have higher grip strength compared with those who ever used such treatment.[29]

Previous studies reported the prevalence of dynapenia is within a range of 7.7% to 52.4%. The variations in prevalence depend on the cut-off value, age, geographic location, and ethnicity of the population under study.[30,31] The prevalence of dynapenia in our study population was observed to be 31.3%, so it is within the middle range. In the present study, age and adiposity were significantly associated with a higher risk of dynapenia and interestingly, we found that women with a higher femoral neck T-score were less likely to have dynapenia. There is likely a link between muscle strength and bone strength. In postmenopausal women, regular walking has no significant effect on spine BMD while it has a positive effect on the femoral neck BMD.[32] Furthermore, a recent meta-analysis pointed out that low to moderate exercise is an osteogenic stimulus at the femoral neck.[33] We can speculate that postmenopausal women with higher handgrip strength have, at the same time, some general benefit on femoral neck BMD characteristics due to better muscle function.

In recent years, there has been a growing effort to identifying risk factors contributing to female dynapenia, assessing BMD,[34] vitamin D levels,[35,36] cigarette smoking,[37] bone mineral metabolism,[38] comorbidities, socioeconomic status, and sociodemographic variables,[39-41] with inconsistent results in many of these factors. Smoking which has been associated with increased protein catabolism, induces muscle wasting, reduces oxygen delivery, and impairs mitochondrial function. As a consequence, it increases muscle fatigue, since the ability of the muscular system to obtain energy is compromised.[42] However, the relationship between smoking and muscle strength is unclear. Previous studies have observed the association between smoking and sarcopenia[30] or dynapenia,[43] while other studies found no association.[44] In our study, smoking was not associated with HGS. However, in the Canadian Longitudinal Study on Aging cohort smoking was associated with a higher grip strength.[29]

Links between muscle mass and fat and bone mass

During the menopausal transition there are changes in body composition and in the distribution of fat. The changes in fat mass quantity were attributable predominantly to increasing age and menopause would not have a significant additional influence. After menopause, there is a decrease in the percentage of total fat in the legs and an increase in the measurements of central fat; therefore, it would be indicative of a change in the distribution of fat mass.[45] To asses changes in body composition bioelectrical impedance analysis has been validated and it is considered as a good portable alternative to DXA. Also, it is widely available, rapid, inexpensive, easy to perform, readily reproducible and requires minimal operator training, being appropriate for epidemiological and clinical purposes.[46,47]

Controversies still exist when it comes to assessing the relationship between BMI and hand grip strength. Previous studies have found a weak correlation between BMI and grip strength[48] contrasting with results of other studies which reported that HGS was not related with BMI.[49]

Although assessment of BMI in our study showed that the calculated mean BMI for all women was in the normal range, up to 40.9% of them were overweight-obese, and we did not find any correlation between BMI and handgrip strength. In our study, we observed that 56.2% of women had greater adiposity than the average and found a significant relationship between HGS and adiposity. A plausible reason might be the association between high fat mass content and poor muscle quality. Some previous studies revealed that excessive adipose tissue induces a proinflammatory state by the action of cytokines (e.g., tumor necrosis factor-alpha and interleukin-6) and higher plasma levels of cytokines are associated with lower muscle strength, muscle function, and muscle mass.[50]

Muscle strength and physical activity

Physical activity stimulates muscle protein synthesis and has beneficial effects on muscle mass, muscle strength and physical performance. Exercise has also shown beneficial effects on BMD and to delay the onset of osteoporosis.[51] Exercise during middle age is associated with low prevalence of sarcopenia and is effective in maintaining muscle strength and physical performance in older age.[52] The women in our study who performed moderate-high physical activity had greater HGS.

There are several studies with controversial results regarding the relationship between HGS and BMD. Some studies have reported a positive relationship between HGS and BMD,[53,54] while others have not observed any relationship in postmenopausal women.[55,56] The BMD analysis in our study revealed that mean T-scores were in the range of osteopenia and correlations were observed between HGS and the lumbar spine, femoral neck, and total hip BMD. These results are consistent with previous studies and reinforces the association between handgrip strength and BMD.[34,53,54]

Regarding bone mineral metabolism, some studies found associations between better muscle strength and higher levels of vitamin D, calcium, and phosphorous.[38] In contrast, our study showed no relationship between HGS with bone mineral metabolism status, suggesting that the female phosphocalcic endocrine regulation is adjusted to the aging process. The presence of vitamin D receptors in skeletal muscle implies that vitamin D may directly target this tissue. Vitamin D can act on skeletal muscle through the proliferation and differentiation of muscle cells, and also affecting skeletal muscle contraction.[57,58] Optimal serum 25(OH) vitamin D levels seem to be between 30 and 90 ng/mL (75-225 nmol/L), though there is no international consensus.[16] The effect of vitamin D on muscle strength is not consistent. While some studies reported that vitamin D status has a significant positive correlation with HGS in postmenopausal women,[35,59] other studies showed no significant differences.[36,60] This may be related to vitamin D which affects muscle strength only when it is lower than a certain threshold. Muscle weakness or fatigue is a complaint linked to hypovitaminosis D, especially if serum levels are <15 ng/mL.[61] In our cohort, there weren't any women with extremely low levels of vitamin D.

Limitations and strengths

The present study has several strengths. First, it includes a relatively large sample size of postmenopausal women. Second, all BMD measurements were conducted with the same densitometer with daily quality control of the scanner performed by measurement of a phantom's density. Third, the biochemical determinations were measured in the same laboratory with quality controls in place. However, our study has also certain limitations, including its cross-sectional design and the use of one single technique to assess muscle function. Other measures to assess muscle strength would be necessary to fully assess muscle function, including gait speed and the study of diet protein content.[62,63] Finally, we cannot omit that the studied population is a convenience sample of women with good general health.

CONCLUSIONS

The present study demonstrates that in postmenopausal women, low HGS is associated with age at menopause, low BMD (lumbar spine, femoral neck, and total hip), and adiposity adjusted by age. In the current sample, 31.3% of women had dynapenia. Both age and adiposity were significantly associated with a higher risk of dynapenia, whereas a higher femoral neck T-score was less likely to be associated with dynapenia. Handgrip strength is a standard test to evaluate muscle health status.[64] Therefore, the routine implementation of grip strength measurement can be recommended for the study of changes in body composition and muscle strength under different treatments or physical activity programs in health care services.

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REFERENCES

1. Nelson ME, Rejeski WJ, Blair SN, et al. Physical activity and public health in older adults: recommendation from the American College of Sports Medicine and the American Heart Association. *Med Sci Sports Exerc* 2007;39:1435-1445.
2. Haider S, Grabovac I, Dorner TE. Effects of physical activity interventions in frail and prefrail community-dwelling people on frailty status, muscle strength, physical performance and muscle mass—a narrative review. *Wien Klin Wochenschr* 2019;131:244-254.
3. Warburton DER, Bredin SSD. Health benefits of physical activity: a systematic review of current systematic reviews. *Curr Opin Cardiol* 2017;32:541-556.
4. Manini TM, Clark BC. Dynapenia and aging: an update. *J Gerontol A Biol Sci Med Sci* 2012;67A:28-40.
5. Sayer AA, Kirkwood TBL. Grip strength and mortality: a biomarker of ageing? *Lancet* 2015;386:226-227.
6. Iconaru EI, Ciucurel MM, Georgescu L, Ciucurel C. Hand grip strength as a biomarker of aging from the perspective of a Fibonacci mathematical modeling. *BMC Geriatr* 2018;18:296.
7. Morley JE, Vellas B, Kan GAV, et al. Frailty consensus: a call to action. *J Am Med Dir Assoc* 2013;14:392-397.
8. Clark BC, Manini TM. Sarcopenia = / = dynapenia. *J Gerontol A Biol Sci Med Sci* 2008;63:829-834.
9. Clark BC, Manini TM. What is dynapenia? *Nutrition* 2012;28:495-503.
10. Pérez-López FR. Dynapenia and sarcopenia. In: Cano A, editor. *Menopause: A Comprehensive Approach*. Berlin: Springer; 2017. pp. 317-331; Chapter 20.
11. Cruz-Jentoft AJ, Bahat G, Bauer J, et al; Writing Group for the European Working Group on Sarcopenia in Older People 2 (EWGSOP2), and the Extended Group for EWGSOP2. Sarcopenia: revised European consensus on definition and diagnosis. *Age Ageing* 2019;48:16-31.
12. The IPAQ group. International physical activity questionnaire. 2016. Available at: http://www.ipaq.ki.se (accessed January 10, 2019).
13. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, et al; European Working Group on Sarcopenia in Older People. Sarcopenia: European consensus on definition and diagnosis: report of the European working group on sarcopenia in older people. *Age Ageing* 2010;39:412-423.
14. World Health Organization. Obesity: preventing and managing the global epidemic. Report of a WHO Consultation. WHO Technical Report Series 894, 2002. Geneve, Switzerland.
15. World Health Organization. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. WHO Technical Report Series 843, 1994. Geneve, Switzerland.

16. Pérez-López FR, Brincat M, Erel CT, et al. EMAS position statement: vitamin D and postmenopausal health. *Maturitas* 2012;71:83-88.
17. Barbat-Artigas S, Dupontgand S, Fex A, Karelis AD, Aubertin-Leheudre M. Relationship between dynapenia and cardiorespiratory functions in healthy postmenopausal women: novel clinical criteria. *Menopause* 2011;18:400-405.
18. Mitchell KW, Williams J, Atherton P, et al. Sarcopenia, dynapenia and the impact of advancing age on human skeletal muscle size and strength; a quantitative review. *Frontiers Physiol* 2012;3:1-18.
19. Budziarcek. Manini TM, Clark CB. Dynapenia and ageing: an update. *J Gerontol A Biol Sci Med Sci* 2012;67A:28-40.
20. Garcia-Alfaro P, Garcia S, Rodríguez I, Tresserra F, Pérez-López FR. Factors related to muscle strength in postmenopausal women aged younger than 65 years with normal vitamin D status. *Climacteric* 2019;22:390-394.
21. Janssen I, Heymsfield SB, Wang ZM, Ross R. Skeletal muscle mass and distribution in 468 men and women aged 18-88 yr. *J Appl Physiol* 2000;89:81-88.
22. Hughes VA, Frontera WR, Wood M, et al. Longitudinal muscle strength changes in older adults: influence of muscle mass, physical activity, and health. *J Gerontol A Biol Sci Med Sci* 2001;56:B209-B217.
23. Morgan PT, Smeuninx B, Breen L. Exploring the impact of obesity on skeletal muscle function in older age. *Front Nutr* 2020;7:569904.
24. Rizzoli R, Stevenson JC, Bauer JM, et al; ESCEO Task Force. The role of dietary protein and vitamin D in maintaining musculoskeletal health in postmenopausal women: a consensus statement from the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO). *Maturitas* 2014;79:122-132.
25. Cipriani C, Romagnoli E, Carnevale V, et al. Muscle strength and bone in healthy women: effect of age and gonadal status. *Hormones (Athens)* 2012;11:325-332.
26. Borges VS, Lima-Costa MFF, de Andrade FB. A nationwide study on prevalence and factors associated with dynapenia in older adults: ELSI-Brazil. *Cad Saúde Pública* 2020;36:e00107319.
27. Larsson L, Degens H, Li M, et al. Sarcopenia: aging-related loss of muscle mass and function. *Physiol Rev* 2019;99:427-511.
28. Javed AA, Mayhew AJ, Shea AK, Raina P. Association between hormone therapy and muscle mass in postmenopausal women: a systematic review and meta-analysis. *JAMA Netw Open* 2019;2:e1910154.
29. Velez MP, Alvarado BE, Rosendaal N, et al. Age at natural menopause and physical functioning in postmenopausal women: the Canadian Longitudinal Study on Aging. *Menopause* 2019;26:958-965.
30. Neves T, Ferriolli E, Lopes MBM, et al. Prevalence and factors associated with sarcopenia and dynapenia in elderly people. *J Frailty Sarcopenia Falls* 2018;3:194-202.
31. Borim FSA, Alexandre TDS, Neri AL, et al. Combined effect of dynapenia (muscle weakness) and low vitamin d status on incident disability. *J Am Med Dir Assoc* 2019;20:47-52.
32. Martyn-St James M, Carroll S. Meta-analysis of walking for preservation of bone mineral density in postmenopausal women. *Bone* 2008;43:521-531.
33. Kistler-Fischbacher M, Weeks BK, Beck BR. The effect of exercise intensity on bone in postmenopausal women (part 2): a meta-analysis. *Bone* 2021;143:115697.
34. Luo Y, Jiang K, He M. Association between grip strength and bone mineral density in general US population of NHANES 2013-2014. *Arch Osteoporos* 2020;15:47.
35. Aspell N, Laird E, Healy M, Lawlor B, O'Sullivan M. Vitamin D deficiency is associated with impaired muscle strength and physical performance in community-dwelling older adults: findings from the English longitudinal study of ageing. *Clin Interv Aging* 2019;14:1751-1761.
36. Kim BJ, Kwak MK, Lee SH, Koh JM. Lack of association between vitamin D and hand grip strength in Asians: a nationwide population-based study. *Calcif Tissue Int* 2019;104:152-159.
37. Steffl M, Bohannon RW, Petr M, Kohlikova E, Holmerova I. Relation between cigarette smoking and sarcopenia: meta-analysis. *Physiol Res* 2015;64:419-426.
38. Verde Z, Giaquinta A, Sainz CM, Ondina MD, Araque AF. Bone mineral metabolism status, quality of life, and muscle strength in older people. *Nutrients* 2019;11:2748.
39. Sternäng O, Reynolds CA, Finkel D, et al. Factors associated with grip strength decline in older adults. *Age Ageing* 2015;44:269-274.
40. de Lima TR, Silva DAS, de Castro JAC, Christofaro DGD. Handgrip strength and associated sociodemographic and lifestyle factors: a systematic review of the adult population. *J Bodyw Mov Ther* 2017;21:401-413.
41. Kim CR, Jeon Y-J, Jeong T. Risk factors associated with low handgrip strength in the older Korean population. *PLoS One* 2019;14:e0214612.
42. Degens H, Gayan-Ramirez G, van Hees HW. Smoking-induced skeletal muscle dysfunction: from evidence to mechanisms. *Am J Respir Crit Care Med* 2015;191:620-625.
43. Stenholm S, Tiainen K, Rantanen T, et al. Long-term determinants of muscle strength decline: prospective evidence from the 22-year mini-Finland follow-up survey. *J Am Geriatr Soc* 2012;60:77-85.
44. Wiener RC, Findley PA, Shen C, Dwibedi N, Sambamoorthi U. Relationship between smoking status and muscle strength in the United States older adults. *Epidemiol Health* 2020;42:e2020055.
45. Ambikairajah A, Walsh E, Tabatabaei-Jafari H, Cherbuin N. Fat mass changes during menopause: a metaanalysis. *Am J Obstet Gynecol* 2019;221:393-409.
46. Wang JG, Zhang Y, Chen HE, et al. Comparison of two bioelectrical impedance analysis devices with dual energy X-ray absorptiometry and magnetic resonance imaging in the estimation of body composition. *J Strength Cond Res* 2013;27:236-243.
47. Marra M, Sammarco R, De Lorenzo A, et al. Assessment of body composition in health and disease using bioelectrical impedance analysis (BIA) and dual energy x-ray absorptiometry (DXA): a critical overview. *Contrast Media Mol Imaging* 2019;2019:3548284.
48. Lopes J, Grams ST, da Silva EF, et al. Reference equations for handgrip strength: normative values in young adult and middle-aged subjects. *Clin Nutr* 2018;37:914-918.
49. Kamarul T, Ahmad TS, Loh WYC. Handgrip strength in the adult Malaysian population. *J Orthop Surg* 2006;14:122-127.
50. Bian AL, Hu HY, Rong YD, et al. A study on relationship between elderly sarcopenia and inflammatory factors IL-6 and TNF-α. *Eur J Med Res* 2017;22:25.
51. Agostini D, Zeppa Donati S, Lucertini F, et al. Muscle and bone health in postmenopausal women: role of protein and vitamin D supplementation combined with exercise training. *Nutrients* 2018;10:1103.
52. Akune T, Muraki S, Oka H, et al. Exercise habits during middle age are associated with lower prevalence of sarcopenia: the ROAD study. *Osteoporos Int* 2014;25:1081-1088.
53. Kim SW, Lee HA, Cho EH. Low handgrip strength is associated with low bone mineral density and fragility fractures in postmenopausal healthy Korean women. *J Korean Med Sci* 2012;27:744-747.
54. Li YZ, Zhuang HF, Cai SQ, et al. Low grip strength is a strong risk factor of osteoporosis in postmenopausal women. *Orthop Surg* 2018;10:17-22.
55. Bayramoğlu M, Sözay S, Karata M, Kilinç S. Relationships between muscle strength and bone mineral density of three body regions in sedentary postmenopausal women. *Rheumatol Int* 2005;25:513-517.
56. Lindsey C, Brownbill RA, Bohannon RA, Ilich JZ. Association of physical performance measures with bone mineral density in postmenopausal women. *Arch Phys Med Rehabil* 2005;86:1102-1107.
57. Dirks-Naylor AJ, Lennon-Edwards S. The effects of vitamin D on skeletal muscle function and cellular signaling. *J Steroid Biochem Mol Biol* 2011;125:159-168.
58. Gunton JE, Girgis CM. Vitamin D and muscle. *Bone Rep* 2018;8:163-167.
59. Stewart JW, Alekel DL, Ritland LM, et al. Serum 25-hydroxyvitamin D is related to indicators of overall physical fitness in healthy postmenopausal women. *Menopause* 2009;16:1093-1101.
60. Matheï C, Van Pottelbergh G, Vaes B, et al. No relation between vitamin D status and physical performance in the oldest old: results from the Belfrail study. *Age Ageing* 2013;42:186-190.
61. López-Baena MT, Pérez-Roncero GR, Pérez-López FR, Mezones-Holguín E, Chedraui P. Vitamin D, menopause, and aging: quo vadis? *Climacteric* 2020;23:123-129.
62. Filion ME, Barbat-Artigas S, Dupontgand S, et al. Relationship between protein intake and dynapenia in postmenopausal women. *J Nutr Health Aging* 2012;16:616-619.
63. Vafa M, Abiri B, Dehghani M. The association of food intake and physical activity with body composition, muscle strength, and muscle function in postmenopausal women. *Methods Mol Biol* 2020;2138:363-371.
64. Bohannon RW. Grip strength: an indispensable biomarker for older adults. *Clin Interv Aging* 2019;14:1681-1691.

Artículo 2: Objetivo 1

Relationship between handgrip strength and endogenous hormones in postmenopausal women

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ORIGINAL STUDY

Relationship between handgrip strength and endogenous hormones in postmenopausal women

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Abstract

Objectives: This study aimed to evaluate the endogenous hormonal factors related to dominant handgrip strength (HGS) in postmenopausal women.

Methods: A cross-sectional study was performed on 402 postmenopausal women aged 47 to 83 years. The following variables were recorded: age, age at menopause, smoking status, adiposity, HGS, and physical activity. Hormonal parameters (follicle-stimulating hormone, estradiol, testosterone, cortisol, dehydroepiandrosterone sulfate, Δ4 androstenedione, insulin-like growth factor-1 [IGF-1], vitamin D, and parathormone levels) were measured and results reported as odds ratios (ORs), β coefficients and 95% confidence interval (95% CI). A directed acyclic graph was used to identify potential confounding variables and was adjusted in the regression model to assess associations between endogenous hormones and HGS.

Results: The mean dominant HGS was 22.8 ± 3.7 kg, and 25.6% of women had dynapenia. There were significant differences in plasma levels of follicle-stimulating hormone (OR, 0.99; 95% CI, 0.98-1.00), cortisol (OR, 1.07; 95% CI, 1.02-1.12), and dehydroepiandrosterone sulfate (OR, 0.99; 95% CI, 0.98-1.00) between women with normal HGS and those who presented with dynapenia. After adjusting for confounding variables, no significant association was found between endogenous hormones and HGS.

Conclusions: Our results showed that studied ovarian steroids, adrenal hormones, IGF-1, parathormone, and vitamin D were not associated with HGS.

Key Words: Adiposity – Adrenal hormones – Dynapenia – Handgrip strength – Ovarian steroids – Physical activity – Smoking – Vitamin D.

Aging is associated with musculoskeletal changes, initially with decreased functional muscle strength (dynapenia) and, later, the loss of muscle strength is associated with loss of muscle mass (sarcopenia). Thus, dynapenia would be the previous step to sarcopenia.[1] Muscle strength plays a relevant role in overall functional status and particularly in daily activities, maintenance of functional independence, and autonomy. To assess muscle strength, handgrip strength (HGS) is an easily, noninvasive, and accurate technique that only requires no hand disability.[2] HGS is currently considered an independent indicator for the decline in cognition, disability, frailty, and mortality. Grip strength in older adults has been recommended as a biomarker of healthy aging.[3]

In addition to the age-related muscle strength decline, hormonal status is involved in maintaining skeletal muscle. The muscle strength decline occurs at an earlier age in women compared with men and is partly associated with the progressive decline in estrogen levels during the menopausal transition.[4,5] However, there are studies with divergent results regarding the relationship between the effects of estrogens on muscle strength.[6,7] The menopausal transition is also associated with decreased levels of testosterone, dehydroepiandrosterone sulfate (DHEAS), and insulin-like growth factor-1 (IGF-1) that also interact with the intracellular muscle pathway.[8] To assess HGS, other confounding factors should also be taken into account, such as age, age at menopause, adiposity, smoking status, and physical activity. We previously reported that age, age at menopause, adiposity (>40% fat mass), and femoral neck T-score were associated with HGS

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or dynapenia in a cohort of postmenopausal women.[9] The purpose of the current study was to investigate the relationship between HGS with ovarian steroids, adrenal hormones, and other hormonal factors in postmenopausal women.

METHODS

Study design

This cross-sectional study was approved by the institutional review board and the Hospital Ethics Committee. It was performed from January 2021 to December 2021 at the Department of Obstetrics and Gynecology of the Dexeus Women's University Hospital, Barcelona, Spain. Women were excluded if (i) they had an early spontaneous (<45 y) or surgical menopause; (ii) were using menopausal hormonal treatment; or (iii) had cardiovascular, liver, or renal diseases, a history of cancer, or physical disability. Finally, a group of 402 postmenopausal women aged 47 to 83 years participated in the study.

HGS and laboratory parameters

HGS measure was assessed in the dominant hand with a digital dynamometer Camry EH101 (Camry Industries Co. Ltd, Kowloon, Hong Kong) calibrated in kg. After demonstrating how to use the handgrip dynamometer, following the standardized method proposed by the American Society of Hand Therapists.[10] Measurements were carried out sitting with the arm against the side, the elbow bent to a 90° angle, and the forearm in a neutral position. Two measurements were recorded and since the HGS may gradually decreases when repeated, the maximum value was used as the indicator for HGS. For the current study, dynapenia was defined when the hand force was <20 kg, according to the cutoff recommended by the European Working Group on Sarcopenia in Older People (EWGSOP) Consensus.[11]

Blood samples were collected after an overnight fast. Hormonal parameters (follicle-stimulating hormone [FSH], estradiol, testosterone, cortisol, DHEAS, Δ4 androstenedione, IGF-1, vitamin D, and parathormone [PTH] plasma levels) were determined by electrochemiluminescence immunoassay using Roche Elecsys reagents and measured by an automated COBAS 8,000 modular analyzer System (Roche Diagnostics. Pleasanton, CA). The laboratory performs daily quality controls of each parameter according to the International Organization for Standardization, ISO 9001:2015 standard. In the quality control the results must be less than ±2 standard deviations with respect to the daily control value. Coefficients of variation were different depending on the studied hormonal parameter. FSH and testosterone ranged from 1.3% to 1.8%, estradiol ranged from 1.2% to 1.9%, cortisol ranged from 1.6% to 1.7%, DHEAS ranged from 1.5% to 2.3%, Δ4 androstenedione ranged from 1.8% to 2.1%, IGF-1 ranged from 1% to 1.6%, vitamin D ranged from 2.3% to 3% and PTH ranged from 1.4% to 1.7%.

Potential confounders

Age at menopause, adiposity, current smoking status, and physical activity were recorded. Adiposity was assessed by bioelectrical impedance analysis equipment Omron BF 306 monitor (Omrom healthcare Co. Ltd, Kyoto, Japan). Details of the women were entered into the equipment program, including age, height, weight, and sex. Subsequently, the bioelectrical impedance analysis assessment was performed two times in the standing position, with the legs 35° to 45° apart and the arms extended forwards at a 90° angle to the trunk, using a disposable gown. The results were expressed as a percentage of fat. Information on smoking status was recorded and classified as never or smoker. The physical activity was assessed using the Spanish version of the short International Physical Activity Questionnaire. This tool includes seven items that provides information about the average number of days per week, and the average time per day that women spent in moderate and vigorous activities, walking and sitting. With the final score obtained, physical activity was classified as low, moderate, and high level.[12]

Covariate assessment

A directed acyclic graph (DAG)[13] was used to select potential confounding factors adjusted in our analysis. A DAG with endogenous hormones as the main exposure and HGS as the outcome were generated to determine confounding variables. Based on available literature and data collected, age, age at menopause, adiposity, smoking status (never vs smoker), and physical activity (high/moderate vs low) were included as potential confounding in the DAG. According to the minimal sufficiency set of adjustments, all of these variables were identified as confounders Following the DAG, age, age at menopause, adiposity, smoking status and physical activity were considered in the regression model to assess associations between endogenous hormones and HGS (Fig. 1).

Statistical analysis

Continuous variables were shown as mean and standard deviation whereas percentages and numbers were used for categorical variables. The Student's *t* test and χ^2 test were applied to compare continuous and categorical outcomes between women with normal HGS and women with dynapenia. Pearson correlation was used to estimate the relation between numeric parameters with grip strength. A partial correlation adjusted by age was also calculated and results were reported as odds ratios (OR) and 95% confidence interval (95% CI). Finally, a multivariable linear regression analysis, adjusting for potential confounders identified by DAG, was modeled to determine the relationship between endogenous hormones and HGS. Results are reported as β coefficients (β) and 95% CI. All analyses were performed using the R software (R Core Team, 2019). The R package "dagitty" was used.[14] All the analyses were exploratory. No formal a priori sample size calculation was performed.

RESULTS

The sample included 402 postmenopausal women, with a mean age of 62.9 ± 6.6 years and a mean age at menopause of 50.2 ± 2.8 years. The estimated average grip strength was 22.8 ± 3.7 kg, and the total sample adiposity was 40.3% ± 5.2%. The values of the clinical and hormonal parameters are reported in Table 1. Regular physical activity and current smoking habits were reported in 61.9% (249/402) and 19.4% (78/402) of the sample, respectively.

The results of the Pearson correlation coefficients (r) between HGS and covariates are presented in Table 2. We observed a

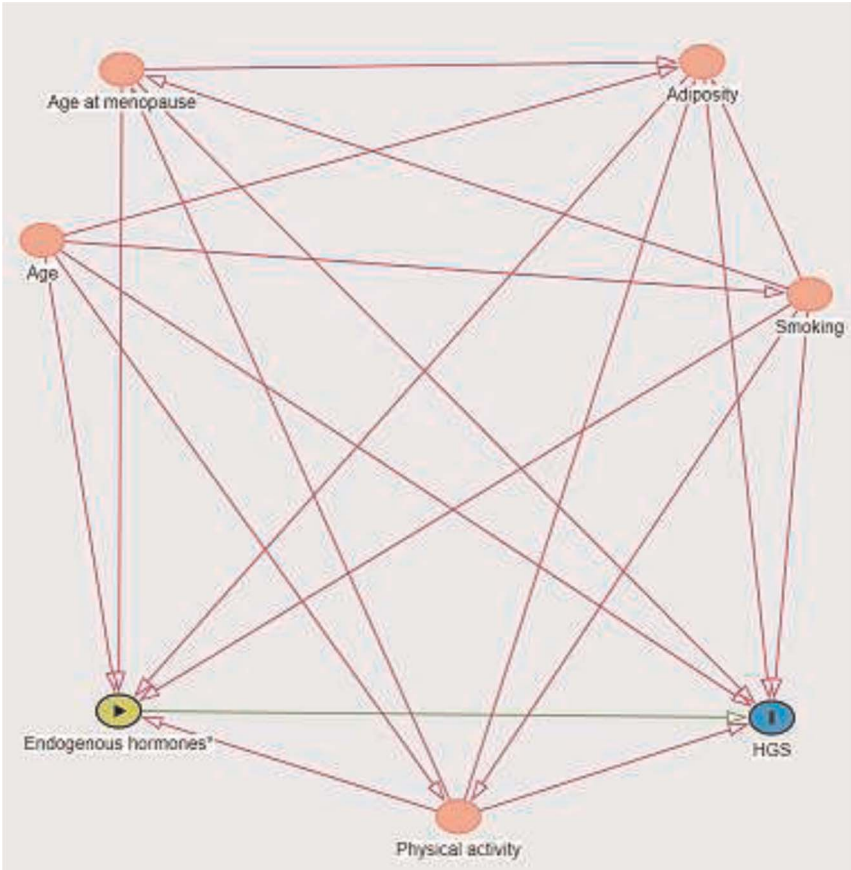

FIG. 1. Directed acyclic graph to distinguish the appropriate set of confounders for estimating our effect of interest. Arrows depict direct effects between variables, whereas the absence of an arrow between two variables represents the assumption of no such direct effect. HGS, handgrip strength.

positive correlation between HGS with plasma DHEAS levels ($r = 0.16$, $P = 0.001$). There were no significant correlations between HGS and other covariates (Fig. 2). After partial correlation adjusted by age the correlation with plasma DHEAS levels was not shown, and the other covariates remained not significantly correlated.

The overall prevalence of dynapenia was 25.6% (103/402). When the differences between women with normal HGS were compared with those who presented dynapenia, there were significant differences in plasma FSH levels with an OR of 0.99 (95% CI, 0.98-1.00), cortisol levels with an OR of 1.07 (95% CI, 1.02-1.12) and DHEAS levels with an OR of 0.99 (95% CI, 0.98-1.00). There were no significant differences among the remaining parameters displayed in Table 3. In women 65 years or younger, 16.8% had dynapenia (44/262) and for women older than 65 years, the prevalence of dynapenia was 42.1% (59/140) with an OR of 3.59 (95% CI, 2.26-5.76; $P < 0.001$). Finally, a multivariable linear regression analysis was used to determine the relationship between endogenous hormones and HGS. After

TABLE 1. *Clinical and hormonal parameters reported as mean ± standard deviation*

Parameters	Estimate (n = 402)	95% CI	Reference ranges
Age	62.9 ± 6.6	62.2-63.5	
Age at menopause	50.2 ± 2.8	49.9-50.5	
Body mass index	24.7 ± 3.8	24.3-25.1	
Adiposity	40.3 ± 5.2	39.8-40.8	
FSH	78.1 ± 25.4	75.6-80.5	40-116 mU/mL
Estradiol	11.6 ± 6.7	10.9-12.2	5-37 pg/mL
Testosterone	0.3 ± 0.2	0.30-0.35	0.02-0.40 ng/mL
Cortisol	14.1 ± 4.9	13.6-14.6	4.3-22.4 µg/dL
DHEAS	83 ± 53.1	77.8-88.2	35-430 µg/dL
Δ4androstenedione	0.9 ± 0.7	0.79-0.92	0.49-1.31 ng/mL
IGF-1	126 ± 41.3	122-130	44-241 ng/mL
Vitamin D	31.8 ± 9.6	30.9-32.7	30-100 ng/mL
Parathormone	42.2 ± 15.4	40.7-43.7	15-65 pg/mL

95% CI, 95% confidence interval; DHEAS, dehydroepiandrosterone sulfate; FSH, follicle-stimulating hormone; IGF-1, insulin-like growth factor 1.

TABLE 2. *Pearson correlation analyses between HGS and hormonal parameters*

	HGS			
Covariates (n = 402)	*r* (Pearson correlation)	*P*	Partial correlations	*P*
FSH	0.07	0.168	−0.01	0.911
Estradiol	−0.05	0.301	−0.05	0.325
Testosterone	0.05	0.302	0.04	0.387
Cortisol	−0.09	0.083	−0.05	0.326
DHEAS	0.16	0.001	0.06	0.248
Δ4androstenedione	0.04	0.394	0.01	0.773
IGF-1	0.09	0.088	0.01	0.860
Vitamin D	−0.05	0.299	−0.04	0.470
Parathormona	−0.04	0.404	−0.02	0.690

Partial correlations with Pearson adjusted by age.
DHEAS, dehydroepiandrosterone sulfate; FSH, follicle-stimulating hormone; HGS, handgrip strength; IGF-1, insulin-like growth factor 1.

adjusting for confounder variables, no significant association was found between endogenous hormones and HGS (Table 4).

DISCUSSION

This cross-sectional study of postmenopausal women found no evidence supporting relationships between studied endogenous hormones with HGS.

Human aging is associated with diminished musculoskeletal function related to muscle mass decline in both men and women, reduction of muscle strength, higher fatigability, [15,16] and adiposity.[9] The loss of muscle strength begins around the fifth and sixth decades of age and appears concurrent with the onset of menopause, suggesting that estrogen levels can play a relevant role in muscle strength.[5] However, hormone therapy does not increase or maintain muscle mass or strength during the initial years of menopause.[7] Furthermore, a meta-analysis of randomized clinical trials reported that there is not a significant beneficial or detrimental link between hormone therapy and muscle mass.[17] Thus, the association between estrogen levels and muscle strength in postmenopausal women is ambiguous, probably due to the different ages and study methods, and to the simplification of the involved endocrine factors. Some studies report a relationship between estrogen and muscle strength[4,5] while other studies found no consistent relation.[6,7] Rolland et al[4] evaluated the percentage of loss per year of isometric knee extensor strength in young postmenopausal women, showing that the loss of muscle strength positively correlated with estrone and PTH. Cipriani et al[5] evaluated the isometric grip strength of the upper dominant limb with age and hormonal status, showing that both age and menopause significantly contribute to the loss of grip strength. Schaap et al[6] reported that low levels of estradiol and testosterone were associated with low muscle strength in men, but not in women. In our cohort, the association between estradiol and HGS was not significant, although we interestingly found that higher FSH plasma levels were significantly associated in women with dynapenia, expressing the secondary adjustment of the global hypoestrogenism of postmenopause.

In the same way as estradiol, testosterone decreases with age, starting in perimenopause, and does not differ due to natural menopause.[18] Testosterone levels are related to skeletal muscle mass. However, few studies have evaluated the relationship between testosterone and muscle strength in postmenopausal women. Van Geel et al[19] reported that bioavailable testosterone level was associated with lean body mass and maximum quadriceps extension strength. Recently Kong et al[20] reported that low free testosterone levels and DHEAS were related to weak muscle strength independent of muscle mass. Our cohort was composed only of Spanish White women, and there was no significant

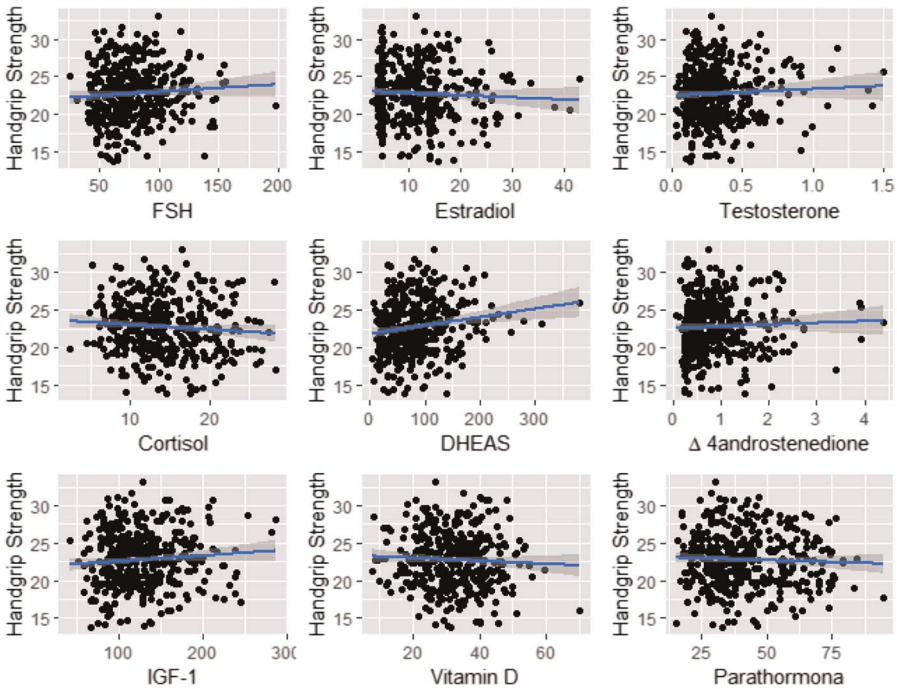

FIG. 2. Pearson correlations between dominant HGS and hormonal parameters. DHEAS, dehydroepiandrosterone sulfate; FSH, follicle-stimulating hormone; HGS, handgrip strength; IGF-1, insulin-like growth factor 1.

TABLE 3. *Mean comparison between hormonal parameters in women with normal HGS (HGS ≥ 20) and dynapenia (HGS <20), OR, 95% CIs, and P values*

Covariates (n = 402)	HGS ≥ 20 kg (n = 299)	HGS < 20 kg (n = 103)	OR [95% CI]	*P*
FSH	79.7 ± 26	73.3 ± 23.1	0.99 [0.98-1.00]	0.019
Estradiol	11.5 ± 6.7	11.9 ± 6.7	1.01 [0.98-1.04]	0.602
Testosterone	0.33 ± 0.23	0.32 ± 0.2	0.79 [0.28-2.21]	0.625
Cortisol	13.7 ± 4.73	15.3 ± 5.19	1.07 [1.02-1.12]	0.005
DHEAS	87 ± 56	71.3 ± 41.8	0.99 [0.99-1.00]	0.003
Δ4androstenedione	0.87 ± 0.67	0.82 ± 0.62	0.87 [0.61-1.25]	0.442
IGF-1	127 ± 41.4	123 ± 41.2	1.00 [0.99-1.00]	0.335
Vitamin D	31.7 ± 9.55	32 ± 9.61	1.00 [0.98-1.03]	0.806
Parathormona	41.7 ± 14.8	43.5 ± 16.9	1.01 [0.99-1.02]	0.338

95% CI, 95% confidence interval; DHEAS, dehydroepiandrosterone sulfate; FSH, follicle-stimulating hormone; HGS, handgrip strength; IGF-1, insulin-like growth factor 1; OR, odds ratio.

association between testosterone and HGS, even after partial correlation adjusted by age.

With aging, there are some changes in the cortisol circadian rhythm with late-day and evening increases in cortisol levels, earlier morning cortisol levels peak, lower circadian amplitude, and more irregular cortisol secretion patterns.[21] Also, aging is associated with higher muscle cortisol generation by local 11β hydroxysteroid dehydrogenase which transforms inactive cortisone into active cortisol.[22] The increased cortisol levels are associated with muscle atrophy and weakness. Different results between muscle strength and cortisol levels may be due to different techniques of cortisol measurement. Plasma cortisol levels are more sensitive to fluctuations than salivary cortisol levels. Peeters et al[23] showed no relationship between plasma cortisol levels and loss of grip strength, although they found that high salivary cortisol levels are associated with an increased risk of reduced grip strength. Bochud et al[24] report a positive association between urinary cortisol metabolites to muscle mass and strength in the upper limbs in younger adults but not in the elderly. Although women with dynapenia had higher plasma cortisol levels, our multivariable analysis did not show a significant association between cortisol levels and HGS.

After menopause, with the decrease in estrogen levels, DHEA becomes the sole source for estrogen and androgen synthesis. DHEA and its sulfate (DHEAS) gradually decrease with age, and at the menopause onset, DHEA has already reduced by an average of 60%. DHEA and DHEAS are themselves inactive, but are present in peripheral tissues and are transformed inside the cells in small amounts of estrogens and androgens.[25] Higher plasma DHEAS levels are associated with psychological wellbeing and physical functioning, including muscle strength. Low plasma DHEAS levels are associated with the development of cardiovascular disease and mortality.[26] In postmenopausal women, low plasma DHEAS and free testosterone are associated with weak muscle strength.[20] Our results showed that DHEAS levels are correlated with HGS but this correlation is lost after adjusting by age. Δ4 androstenedione is a steroid hormone related to hyperandrogenic conditions, such as polycystic ovary syndrome, even though its relationship to HGS in postmenopausal women it has not been studied.[27] The current study did not observe any correlation between Δ4 androstenedione and HGS.

TABLE 4. *Multivariable linear regression model to analyze the relationship between endogenous hormones and HGS (kg) adjusting for age, age at menopause, adiposity, smoking status, and physical activity*

	HGS	
	β	95%CI
FSH	−0.00	−0.01 to 0.01
Estradiol	−0.02	−0.07 to 0.03
Testosterone	0.62	−1.06 to 2.30
Cortisol	−0.05	−0.12 to 0.03
DHEAS	0.00	−0.01 to 0.01
Δ4androstenedione	−0.03	−0.64 to 0.58
IGF-1	−0.00	−0.01 to 0.01
Vitamin D	−0.01	−0.05 to 0.02
Parathormona	−0.00	−0.02 to 0.02
		Adjusted $R^2 = 0.15$

95% CI, 95% confidence interval; DHEAS, dehydroepiandrosterone sulfate; FSH, follicle-stimulating hormone; HGS, handgrip strength; IGF-1, insulin-like growth factor 1.

IGF-1 is a polypeptide hormone with autocrine, paracrine, and endocrine effects that play an important role in skeletal myogenesis, and it is associated with muscle mass and strength development.[28] Plasma IGF-1 levels decreased with aging in both men and women.[29] However, the role of IGF-1 in age-related muscle strength loss is still unclear. Gender is a confounding factor in the association between IGF-1 and muscle strength. Taekema et al[30] found a significant relationship between IGF-1 levels and muscle strength in women but not in men, suggesting a gender-specific influence of IGF-1 on muscle strength. The Cappola et al study[31] showed, after adjustment for age, a significant association between IGF-1 and knee extensor strength, but not anthropometry or other strength measures. They also found a significant correlation between plasma IGF-1 levels and muscle strength, but only below the IGF-1 threshold limit of 50 μg/L. In our studied population, the mean plasma IGF-1 was above this limit, and after correction for confounder variables, we found that IGF-1 plasma levels were not associated with HGS.

Vitamin D plays an important role in preserving muscle function. Nevertheless, the relationship between vitamin D levels and HGS showed conflicting data. Some previous studies reported that low vitamin D levels are related to lower HGS in postmenopausal women,[32] and others showed no significant association.[33] These results may be due to different age ranges in the populations examined and different baseline serum vitamin D levels. In addition, López-Baena et al[34] report that vitamin D

affects muscle strength only when it is lower than a certain threshold. Muscle weakness is linked to vitamin D deficiency, especially if serum levels are less than 15 ng/mL, and the vitamin D supplementation in women who do not have vitamin D deficiency would not obtain significant benefit. Our study showed no association between HGS and vitamin D. However, in our cohort, there were no women with extremely low levels of vitamin D and this fact may be a possible explanation for our finding.

Circulating PTH levels increase with age regardless of renal function and plasma vitamin D, calcium, and phosphorus levels. PTH influences skeletal muscle protein metabolism in animal models and increases intracellular calcium concentration disrupting muscle function.[35] Some studies have examined the relationship between PTH and muscle mass or muscle strength with inconsistent results, some studies found a statistically significant association,[36] whereas others found no association.[37] We have not observed an association between PTH and HGS.

Dynapenia is the age-related reduction of skeletal muscle strength that has been considered an independent factor of mortality.[38] Our current results suggest that studied hormones have no relevant roles in changes in HGS and dynapenia risk in otherwise healthy postmenopausal women. Other factors may be involved in those findings displayed in the studied population. Skeletal muscles are an endocrine organ and source of proteins (myokines) which function as autocrine, paracrine, or endocrine agents to maintain energy homeostasis and are protective of the physical activity of skeletal muscles. Myokines may be also involved in body weight composition, subclinical inflammation, and muscle insulin sensitivity.[39] Physical activity can induce the release of muscle myokines into the circulation to improve glucose metabolism, muscle proteins, and liver fat metabolism.[40,41] Future studies are needed to have evidence about myokines and dynapenia.

On the other hand, adiposity and intramuscular fat accumulation are linked to mitochondrial damage and increased proinflammatory cytokines that induce muscle dysfunction.[42] Therefore, subclinical chronic inflammation may also play a role in HGS reduction and sarcopenia risk.[43] Future research directions should analyze those factors to assess the reduction of HGS and the risk of dynapenia in postmenopausal women.

Limitations and strengths

The present study has certain limitations, including its cross-sectional design, and the longitudinal effect of the studied hormones on muscle strength could not be elucidated. However, it has several strengths. First, it includes a large sample size of postmenopausal women. Second, the biochemical determinations were measured in the same laboratory with quality controls in place. Third, to identify confounding variables we used DAG and a careful adjustment for confounders were carried out in the regression model.

CONCLUSIONS

The present study reports that in postmenopausal women aged 47 to 83 years, our multivariable analysis adjusted for confounder variables did not find a significant association between studied endogenous hormones and HGS. The overall prevalence of dynapenia was 25.6%. Because of the clinical implications of dynapenia, further studies are needed to determine specific factors to assess the HGS reduction, and the risk of dynapenia in postmenopausal women.

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REFERENCES

1. Pérez-López FR. Dynapenia and sarcopenia. In: Cano A (ed). *Menopause: A Comprehensive Approach*. Berlin: Springer; 2017:317-331;Chapter 20.
2. Cruz-Jentoft AJ, Bahat G, Bauer J, et al, Writing Group for the European Working Group on Sarcopenia in Older People 2 (EWGSOP2), and the Extended Group for EWGSOP2. Sarcopenia: revised European consensus on definition and diagnosis. *Age Ageing* 2019;48:16-31. doi: 10.1093/ageing/afy169
3. Bohannon RW. Grip strength: an indispensable biomarker for older adults. *Clin Interv Aging* 2019;14:1681-1691. doi:10.2147/CIA.S194543
4. Rolland YM, Perry HM 3rd, Patrick P, Banks WA, Morley JE. Loss of appendicular muscle mass and loss of muscle strength in young postmenopausal women. *J Gerontol A Biol Sci Med Sci* 2007;62:330-335. doi:10.1093/gerona/62.3.330
5. Cipriani C, Romagnoli E, Carnevale V, et al. Muscle strength and bone in healthy women: effect of age and gonadal status. *Hormones (Athens)* 2012; 11:325-332. doi:10.14310/horm.2002.1361
6. Schaap LA, Pluijm SMF, Smit JH, et al. The association of sex hormone levels with poor mobility, low muscle strength and incidence of falls among older men and women. *Clin Endocrinol* 2005;63:152-160. doi:10.1111/j.1365-2265.2005.02315.x
7. Maddalozzo GF, Cardinal BJ, Li F, Snow CM. The association between hormone therapy use and changes in strength and body composition in early postmenopausal women. *Menopause* 2004;11:438-446. doi:10.1097/01.GME.0000113847.74835.fe
8. Kim YJ, Tamadon A, Park HT, Kim H, Ku SY. The role of sex steroid hormones in the pathophysiology and treatment of sarcopenia. *Osteoporos Sarcopenia* 2016;2:140-155. doi:10.1016/j.afos.2016.06.002
9. García-Alfaro P, García S, Rodríquez I, Pérez-López FR. Handgrip strength, dynapenia, and related factors in postmenopausal women. *Menopause* 2022; 29:16-22. doi:10.1097/GME.0000000000001872
10. Fess EE. Grip strength. In: Casanova JS (ed). *Clinical assessment recommendations*. 2nd ed,. Chicago, IL: American Society of Hand Therapists; 1992:41-45.
11. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, et al, European Working Group on Sarcopenia in Older People. Sarcopenia: European consensus on definition and diagnosis: report of the European Working Group on Sarcopenia in Older People. *Age Ageing* 2010;39:412-423. doi:10.1093/ageing/afq034
12. The IPAQ group. International physical activity questionnaire. 2016. Available at: http://www.ipaq.ki.se. Accessed December 15, 2020.
13. Digitale JC, Martin JN, Glymour MM. Tutorial on directed acyclic graphs. *J Clin Epidemiol* 2022;142:264-267. doi:10.1016/j.jclinepi.2021.08.001
14. Textor J, van der Zander B, Gilthorpe MS, Liskiewicz M, Ellison GT. Robust causal inference using directed acyclic graphs: the R package 'dagitty'. *Int J Epidemiol* 2016;45:1887-1894. doi:10.1093/ije/dyw341
15. Akima H, Kano Y, Enomoto Y, et al. Muscle function in 164 men and women aged 20-84 yr. *Med Sci Sports Exerc* 2001;33:220-226. doi:10.1097/00005768-200102000-00008
16. Faulkner JA, Larkin LM, Claflin DR, Brooks SV. Age-related changes in the structure and function of skeletal muscles. *Clin Exp Pharmacol Physiol* 2007;34:1091-1096. doi:10.1111/j.1440-1681.2007.04752.x
17. Javed AA, Mayhew AJ, Shea AK, Raina P. Association between hormone therapy and muscle mass in postmenopausal women: a systematic review and meta-analysis. *JAMA Netw Open* 2019;2:e1910154. doi:10.1001/jamanetworkopen.2019.10154
18. Davison SL, Bell R, Donath S, et al. Androgen levels in adult females: changes with age, menopause, and oophorectomy. *J Clin Endocrinol Metab* 2005; 90:3847-3853. doi:10.1210/jc.2005-0212
19. van Geel TACM, Geusens PP, Winkens B, Sels J-PJE, Dinant G-J. Measures of bioavailable serum testosterone and estradiol and their relationships with muscle mass, muscle strength and bone mineral density in postmenopausal women: a cross-sectional study. *Eur J Endocrinol* 2009;160:681-687. doi:10.1530/EJE-08-0702

20. Kong SH, Kim JH, Lee JH, et al. Dehydroepiandrosterone sulfate and free testosterone but no estradiol are related to muscle strength and bone microarchitecture in older adults. *Calcif Tissue Int* 2019;105:285-293. doi:10.1007/s00223-019-00566-5
21. Veldhuis JD, Sharma A, Roelfsema F. Age-dependent and gender-dependent regulation of hypothalamic-adrenocorticotropic-adrenal axis. *Endocrinol Metab Clin N Am* 2013;42:201-225. doi:10.1016/j.ecl.2013.02.002
22. Kilgour AH, Gallagher IJ, MacLullich AM, et al. Increased skeletal muscle 11βHSD1 mRNA is associated with lower muscle strength in ageing. *PLoS One* 2013;8:e84057. doi:10.1371/journal.pone.0084057
23. Peeters GMEE, van Schoor NM, van Rossum EFC, Visser M, Lips P. The relationship between cortisol, muscle mass and muscle strength in older persons and the role of genetic variations in the glucocorticoid receptor. *Clin Endocrinol* 2008;69:673-682. doi:10.1111/j.1365-2265.2008.03212.x
24. Bochud M, Ponte B, Pruijim M, et al. Urinary sex steroid and glucocorticoid hormones are associated with muscle mass and strength in healthy adults. *J Clin Endocrinol Metab* 2019;104:2195-2215. doi:10.1210/jc.2018-01942
25. Labrie F. Intracrinology and menopause: the science describing the cell-specific intracellular formation of estrogens and androgens from DHEA and their strictly local action and inactivation in peripheral tissues. *Menopause* 2019;26:220-224. doi:10.1097/GME.0000000000001177
26. Ohlsson C, Vandenput L, Tivesten A. DHEA and mortality: what is the nature of the association? *J Steroid Biochem Mol Biol* 2015;145:248-253. doi:10.1016/j.jsbmb.2014.03.006
27. Badawy MT, Sobeh M, Xiao J, Farag MA. Androstenedione (a natural steroid and a drug supplement): a comprehensive review of its consumption, metabolism, health effects, and toxicity with sex differences. *Molecules* 2021;26:6210. doi:10.3390/molecules26206210
28. Ahmad SS, Ahmad K, Lee EJ, Lee YH, Choi I. Implications of insulin-like growth factor-1 in skeletal muscle and various diseases. *Cell* 2020;9:1773. doi:10.3390/cells9081773
29. Landin-Wilhelmsen K, Lundberg P-A, Lappas G, Wilhelmsen L. Insulin-like growth factor I levels in healthy adults. *Horm Res* 2004;62(Suppl 1):8-16. doi:10.1159/000080753
30. Taekema DG, Ling CH, Blauw GJ, et al. Circulating levels of IGF1 are associated with muscle strength in middle-aged- and oldest-old women. *Eur J Endocrinol* 2011;164:189-196. doi:10.1530/EJE-10-0703
31. Cappola AR, Bandeen-Roche K, Wand GS, et al. Association of IGF-I levels with muscle strength and mobility in older women. *J Clin Endocrinol Metab* 2001;86:4139-4146. doi:10.1210/jcem.86.9.7868
32. Aspell N, Laird E, Healy M, Lawlor B, O'Sullivan M. Vitamin D deficiency is associated with impaired muscle strength and physical performance in community-dwelling older adults: findings from the English Longitudinal Study of Ageing. *Clin Interv Aging* 2019;14:1751-1761. doi:10.2147/CIA.S222143
33. Kim BJ, Kwak MK, Lee SH, Koh JM. Lack of association between vitamin D and hand grip strength in Asians: a nationwide population-based study. *Calcif Tissue Int* 2019;104:152-159. doi:10.1007/s00223-018-0480-7
34. López-Baena MT, Pérez-Roncero GR, Pérez-López FR, Mezones-Holguín E, Chedraui P. Vitamin D, menopause, and aging: quo vadis? *Climacteric* 2020;23:123-129. doi:10.1080/13697137.2019.1682543
35. Visser M, Deeg DJH, Lips P, Longitudinal Aging Study Amsterdam. Low vitamin D and high parathyroid hormone levels as determinants of loss of muscle strength and muscle mass (sarcopenia): the Longitudinal Aging Study Amsterdam. *J Clin Endocrinol Metab* 2003;88:5766-5772. doi:10.1210/jc.2003-030604
36. Dhesi JK, Bearne LM, Moniz C, et al. Neuromuscular and psychomotor function in elderly subjects who fall and the relationship with vitamin D status. *J Bone Miner Res* 2002;17:891-897. doi:10.1359/jbmr.2002.17.5.891
37. Marantes I, Achenbach SJ, Atkinson EJ, et al. Is vitamin D a determinant of muscle mass and strength? *J Bone Miner Res* 2011;26:2860-2871. doi:10.1002/jbmr.510
38. Uchida S, Kamiya K, Hamazaki N, et al. Prognostic utility of dynapenia in patients with cardiovascular disease. *Clin Nutr* 2021;40:2210-2218. doi:10.1016/j.clnu.2020.09.050
39. Hoffmann C, Weigert C. Skeletal muscle as an endocrine organ: the role of myokines in exercise adaptations. *Cold Spring Harb Perspect Med* 2017;7:a029793. doi:10.1101/cshperspect.a029793
40. Aryana GPS, Hapsari AAAR, Kuswardhani RAT. Myokine regulation as marker of sarcopenia in elderly. *Mol Cell Biomed Sci* 2018;2:38-47. doi:10.21705/mcbs.v2i2.32
41. Gonzalez-Gil AM, Elizondo-Montemayor L. The role of exercise in the interplay between myokines, hepatokines, osteokines, adipokines, and modulation of inflammation for energy substrate redistribution and fat mass loss: a review. *Nutrients* 2020;12:1899. doi:10.3390/nu12061899
42. Wiedmer P, Jung T, Castro JP, et al. Sarcopenia—molecular mechanisms and open questions. *Ageing Res Rev* 2021;65:101200. doi:10.1016/j.arr.2020.101200
43. Son D-H, Song S-A, Lee Y-J. Association between c-reactive protein and relative handgrip strength in postmenopausal Korean women aged 45-80 years: a cross-sectional study. *Clin Interv Aging* 2022;17:971-978. doi:10.2147/CIA.S356947

Artículo 3: Objetivo 2

Plasma homocysteine levels and handgrip strength in postmenopausal women

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ORIGINAL ARTICLE

Plasma homocysteine levels and handgrip strength in postmenopausal women

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ABSTRACT

Objective: This study evaluated handgrip strength (HGS), circulating homocysteine levels and related factors in postmenopausal women.

Methods: This study is a sub-analysis of a prospective cohort of 303 postmenopausal women aged 62.7 ± 6.9 years who had HGS measures with a digital dynamometer as the primary outcome, and plasma homocysteine and creatinine levels and glomerular filtration rate (GFR) measures as the secondary outcomes.

Results: The average HGS was 22.5 ± 4.0 kg, 29.4% of women had dynapenia (HGS < 20 kg), adiposity was 40.3 ± 5.4% and 9.57% of women had hyperhomocysteinemia (homocysteine >15 μmol/l). There were no differences between tertiles of homocysteine and HGS ($p = 0.641$). Plasma homocysteine levels were unrelated to HGS ($r = -0.06$) and correlated with age ($r = 0.17$), GFR ($r = -0.28$) and creatinine ($r = 0.23$). Hyperhomocysteinemia was not associated with HGS (odds ratio [OR] = 0.98 [95% confidence interval (CI): 0.89; 1.08]) or dynapenia (OR = 1.10 [95% CI: 0.45; 2.47]). The risk of presenting low HGS were not significantly associated with homocysteine (OR = −0.08 [95% CI: −0.21; 0.06]) and were associated with age (OR = −0.23 [95% CI: −0.29; −0.17]), adiposity (OR = −6.52 [95% CI: −9.53; −3.50]) and creatinine (OR = 6.22 [95% CI: 2.48; 9.97]).

Conclusions: HGS and dynapenia were unrelated to hyperhomocysteinemia. Age, GFR and creatinine were significantly associated with plasma homocysteine levels.

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Introduction

Homocysteine is a non-essential sulfur-containing amino acid produced in the liver by the transmethylation of methionine. It is degraded into cysteine through the irreversible trans-sulfuration pathway, and under normal circumstances, some 50% follow the re-methylation pathway to regenerate methionine. These reaction pathways require the presence of vitamin B6, vitamin B12 and folate as cofactors [1,2]. Plasma homocysteine increases significantly with age and there are gender differences, levels being higher in men than in women [3]. Also, there are lifestyle factors, like smoking and physical inactivity, that may influence their levels [4]; elevated homocysteine levels are associated with oxidative stress, endothelium dysfunction, smooth muscle cell proliferation, reduced Von Willebrand factor and increased fibrinogen levels [5]. Hyperhomocysteinemia has been related to a greater risk for developing acute coronary syndrome, stroke and cardiovascular mortality [6]. Also, it has been associated with cognitive impairment, dementia and Alzheimer's disease [7], increased bone fragility [8] and bone fractures [9]. Other factors associated with hyperhomocysteinemia are chronic kidney disease and the effect of medications such as isoniazid, cyclosporine, methotrexate, phenytoin and theophylline [1]. Recent studies have reported the relationship between plasma homocysteine levels and muscle strength and sarcopenia, although with controversial results [10–13]. Differences in results between studies might be due to the use of different cut-off values of muscle strength and homocysteine, and the ages of studied populations.

We previously reported the relationship between homocysteine and bone mineral density [14] and general results related to handgrip strength (HGS) or dynapenia [15] in a cohort of postmenopausal women. The purpose of this sub-analysis is to assess the relationship between HGS with plasma homocysteine, glomerular filtration, creatinine and related factors in postmenopausal women.

Materials and methods

Study design

This cross-sectional study was approved by the Institutional Review Board and the Hospital Ethics Committee. It was performed from May 2020 to May 2021 at the Department of Obstetrics and Gynecology of the Dexeus Women's University Hospital, Barcelona, Spain. This sub-analysis examine the impact of hyperhomocysteinemia on the risk of dynapenia as measured by HGS. Inclusion criteria were spontaneous postmenopausal women with amenorrhea of 1 year or more before the initiation of the study, and no physical disability. Women were excluded if they had an early

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(age <45 years) or surgical menopause; were using menopausal hormonal treatment or other known treatments influencing the levels of homocysteine (folates and vitamin B12 supplements, isoniazid, cyclosporine, methotrexate, phenytoin, theophylline, carbamazepine, nicotinic acid and fibric acid derivates); or had cardiovascular, liver or renal diseases, cancer or physical disability. Finally, 303 postmenopausal women aged 50–84 years participated in the study.

Anthropometry, physical activity and muscle strength measurements

Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters after weighing and measuring participants without shoes and wearing a disposable gown. Adiposity was assessed by bioelectrical impedance analysis equipment (Omron BF 306 monitor; Omrom Healthcare Co. Ltd, Japan). The assessment was performed two times in the standing position, with the legs 35°–45° apart and the arms extended forwards at a 90° angle concerning the trunk. The results were expressed as a percentage of fat.

The physical activity was assessed using the Spanish version of the short International Physical Activity Questionnaire. This tool includes seven items that provide information about the average number of days per week and the average time per day that subjects spent in moderate and vigorous activities, walking and sitting. With the final score, the physical activity was classified as low, moderate or high [16]. The muscle strength was assessed by HGS measured with a digital dynamometer (Camry EH101; Camry Industries Co. Ltd, Hong Kong) calibrated in kilograms. The measurement was carried out with the woman seated, with the arm against the side and the elbow bent to a 90° angle. The women performed two trials with the dominant hand and the maximum value was recorded [17]. According to the cut-off recommended by the European Working Group on Sarcopenia in Older People (EWGSOP) Consensus, dynapenia was diagnosed when the dominant hand force was <20 kg [18].

Laboratory parameters

Blood samples were collected after an overnight fast. The mean corpuscular volume (MCV), glomerular filtration rate (GFR), plasma homocysteine, creatinine and vitamin D were measured by an automated COBAS® 8000 modular analyzer System (Roche Diagnostics. Pleasanton, CA, USA). The laboratory performs daily quality controls following the International Organization for Standardization ISO 9001:2015 standard for each parameter. The results must be less than ±2 standard deviations concerning the daily control value. The GFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation [19]. Hyperhomocysteinemia was considered for homocysteine levels >15 μmol/l, and hypovitaminosis D for levels <30 ng/ml [20,21].

Statistical analysis

Women were categorized into tertiles according to their plasma homocysteine levels. The lower tertile of homocysteine was considered the reference group. Analysis of variance was used to compare continuous variables between tertiles and the chi-square test was applied for categorical variables. The association between continuous variables was performed using the Pearson correlation. Logistic regression analysis was fitted to estimate the effects of various covariates over the risk of presenting hyperhomocysteinemia and low HGS. The statistical analyses were performed using R software version 2.10.1 (The R Foundation for Statistical Computing: http://www.r-project.org/). All of the analyses were exploratory. No formal sample size calculation was performed.

Results

The studied sample included 303 postmenopausal women aged 62.7 ± 6.9 years with a mean age at menopause of 50.1 ± 2.7 years. The whole sample BMI was 24.6 ± 3.7 kg/m^2, the adiposity was 40.3 ± 5.4% and the average grip strength was 22.5 ± 4.0 kg. The mean values of the laboratory parameters homocysteine, MCV, GFR, creatinine and vitamin D were in the normal range (Table 1).

The characteristics of the participating women according to tertiles of homocysteine are presented in Table 2. We observed that women in the upper homocysteine tertile have lower GFR levels ($p = 0.003$) and higher plasma creatinine levels ($p = 0.009$). There were no significant associations between plasma homocysteine with either age, age at menopause, smoking status, BMI, adiposity, MCV, vitamin D levels, HGS and degree of physical activity.

When we compared women with normal plasma homocysteine levels with those who presented hyperhomocysteinemia, there were significant differences in MCV (odds ratio [OR] = 1.23 [95% confidence interval (CI): 1.07; 1.42]), GFR (OR = 0.94 [95% CI: 0.91; 0.98]) and plasma creatinine (OR = 24.5 [95% CI: 0.5; 1125]) levels. There were no significant differences among HGS (OR = 0.98 [95% CI: 0.89; 1.08]) or dynapenia (OR = 1.10 [95% CI: 0.45; 2.47]) and the remaining parameters presented in Table 3. Concerning the Pearson correlation coefficients (r) between homocysteine and covariates, Figure 1 shows that homocysteine levels were correlated with age ($r = 0.17$), GFR ($r = -0.28$) and creatinine ($r = 0.23$). There were no significant correlations between homocysteine and HGS ($r = -0.06$) and other covariates.

A logistic regression analysis was fitted to estimate the effect of various covariates over the risk of presenting

Table 1. Laboratory parameters.

Parameter	*Estimate (n = 303)*	*95% CI*	*Reference range*
Homocysteine (μmol/l)	11.4 ± 3.18	11.0; 11.7	5–15
Mean corpuscular volume (fl)	92.4 ± 2.91	92.1; 92.8	80–100
Glomerular filtration rate (ml/min)	84.4 ± 12.0	83.1; 85.8	>60
Creatinine (mg/dl)	0.75 ± 0.11	0.74; 0.76	0.5–1.01
Vitamin D (ng/ml)	33.7 ± 11.3	32.4; 35.0	30–100

Data presented as mean ± standard deviation. CI, confidence interval.

Table 2. Characteristics of the studied population according to tertiles of homocysteine.

Parameter	*Homocysteine tertile 1 [≥5.1 to ≤10 μmol/l] (n = 105)*	*Homocysteine tertile 2 [>10 to ≤12 μmol/l] (n = 99)*	*Homocysteine tertile 3 [>12 to ≤28.7 μmol/l] (n = 99)*	p-*Value*
Age (years)	61.7 ± 6.5	63.4 ± 6.6	63.1 ± 7.4	0.169
Age at menopause (years)	50.0 ± 2.9	49.9 ± 2.8	50.4 ± 2.4	0.465
Smoking status				
Smoker	15 (26.3%)	19 (33.3%)	23 (40.4%)	0.261
Never	90 (36.6%)	80 (32.5%)	76 (30.9%)	
BMI (kg/m^2)	24.3 ± 3.7	24.7 ± 3.6	24.7 ± 3.9	0.733
Adiposity (%)	40.9 ± 4.7	39.4 ± 5.6	40.5 ± 5.8	0.141
MCV (fl)	92.3 ± 2.8	92.2 ± 2.9	92.7 ± 2.9	0.429
GFR (ml/min)	87.4 ± 10.8	84.1 ± 11.3	81.7 ± 13.2	0.003
Creatinine (mg/dl)	0.72 ± 0.1	0.75 ± 0.1	0.77 ± 0.1	0.009
Vitamin D (ng/ml)	34.6 ± 10.7	32.0 ± 11.7	34.3 ± 11.5	0.190
Handgrip strength (kg)	22.8 ± 3.4	22.4 ± 4.2	22.3 ± 4.3	0.641
Physical activity				
Low	44 (32.4%)	42 (30.9%)	50 (36.8%)	0.390
Moderate/high	61 (36.5%)	57 (34.1%)	49 (29.3%)	

Data presented as mean ± standard deviation or *n* (%). BMI, body mass index; GFR, glomerular filtration rate; MCV, mean corpuscular volume.

Table 3. General characteristics of the studied parameters in women with normal homocysteine levels (homocysteine ≤15 μmol/l) and hyperhomocysteinemia (homocysteine >15 μmol/l).

Parameter	*Homocysteine ≤15 μmol/l (n = 274)*	*Homocysteine >15 μmol/l (n = 29)*	*Odds ratio [95% CI]*	p-*Value*
Age (years)	62.4 ± 6.7	65.6 ± 8.1	1.07 [1.01; 1.13]	0.051
Age at menopause (years)	50.0 ± 2.8	50.3 ± 2.36	1.04 [0.91; 1.19]	0.526
Smoking status				
Smoker	52 (91.2%)	5 (8.7%)	Reference	0.999
Never	222 (90.2%)	24 (9.7%)	1.10 [0.43; 3.45]	
BMI (kg/m^2)	24.4 ± 3.7	26.2 ± 3.9	1.13 [1.02; 1.24]	0.024
Adiposity (%)	40.2 ± 5.3	40.6 ± 5.4	1.01 [0.94; 1.09]	0.712
MCV (fl)	92.3 ± 2.8	93.9 ± 2.8	1.23 [1.07; 1.42]	0.004
GFR (ml/min)	85.2 ± 11.7	77.2 ± 12.3	0.94 [0.91; 0.98]	0.002
Creatinine (mg/dl)	0.74 ± 0.1	0.80 ± 0.1	24.5 [0.5; 1125]	0.004
Vitamin D (ng/ml)	33.5 ± 11.3	35.4 ± 11.8	1.01 [0.98; 1.05]	0.407
Mean handgrip strength (kg)	22.5 ± 4.0	22.2 ± 3.6	0.98 [0.89; 1.08]	0.713
Handgrip strength (kg)				
≥20 (normal)	194 (90.7%)	20 (9.3%)	Reference	0.999
<20 (dynapenia)	80 (89.9%)	9 (10.1%)	1.10 [0.45; 2.47]	
Physical activity				
Low	122 (89.7%)	14 (10.3%)	Reference	0.849
Moderate/high	152 (91.1%)	15 (8.9%)	0.86 [0.40; 1.88]	

Data presented as mean ± standard deviation or *n* (%). BMI, body mass index; CI, confidence interval; GFR, glomerular filtration rate; MCV, mean corpuscular volume.

hyperhomocysteinemia. A significant association was found between BMI (OR = 1.14 [95% CI: 1.01; 1.29]), MCV (OR = 1.23 [95% CI: 1.07; 1.43]) and creatinine (OR = 1.88 [95% CI: 1.18; 3.10]) and the risk of presenting hyperhomocysteinemia. No other significant association was found with the remaining variables (Table 4). Finally, a logistic regression model examined the effect of plasma homocysteine levels and other variables over the risk of presenting low HGS showing that there were significant association with age (OR = −0.23 [95% CI: −0.29; −0.17]), adiposity (OR = −6.52 [95% CI: −9.53; −3.50]) and creatinine (OR = 6.22 [95% CI: 2.48; 9.97]). There was no significant association with homocysteine (OR = −0.08 [95% CI: −0.21; 0.06]). The overall prevalence of dynapenia was 29.4% (89/303).

Discussion

This cross-sectional study showed that hyperhomocysteinemia is not associated with HGS or dynapenia in postmenopausal women. In this cohort, the high levels of homocysteine were associated with low GFR and MCV, and increased creatinine values.

Homocysteine is a significant marker for overall health status [22]. In older women, the hyperhomocysteinemia prevalence reported in different studies is within a range of 4.13–48.1% [23,24]. Aging is associated with increased homocysteine concentrations due to renal function decline [25] and the high prevalence of vitamin B12 and folate deficiency [26]. In our cohort of postmenopausal women, the hyperhomocysteinemia prevalence was 9.57%, within the published low range. We also found that plasma homocysteine levels were significantly correlated with advanced age, which is consistent with previous studies [3]. During the menopausal transition, there is a progressive decrease in estrogen levels, a redistribution of body fat from the periphery to the abdominal region, changes in plasma lipoprotein levels and insulin resistance, and increasing prevalence of metabolic syndrome, obesity, dyslipidemia, hyperinsulinemia, hypertension and hyperhomocysteinemia [27–29]. It seems that adequate estradiol levels may minimize homocysteine-mediated oxidative DNA damage [30]. In addition,

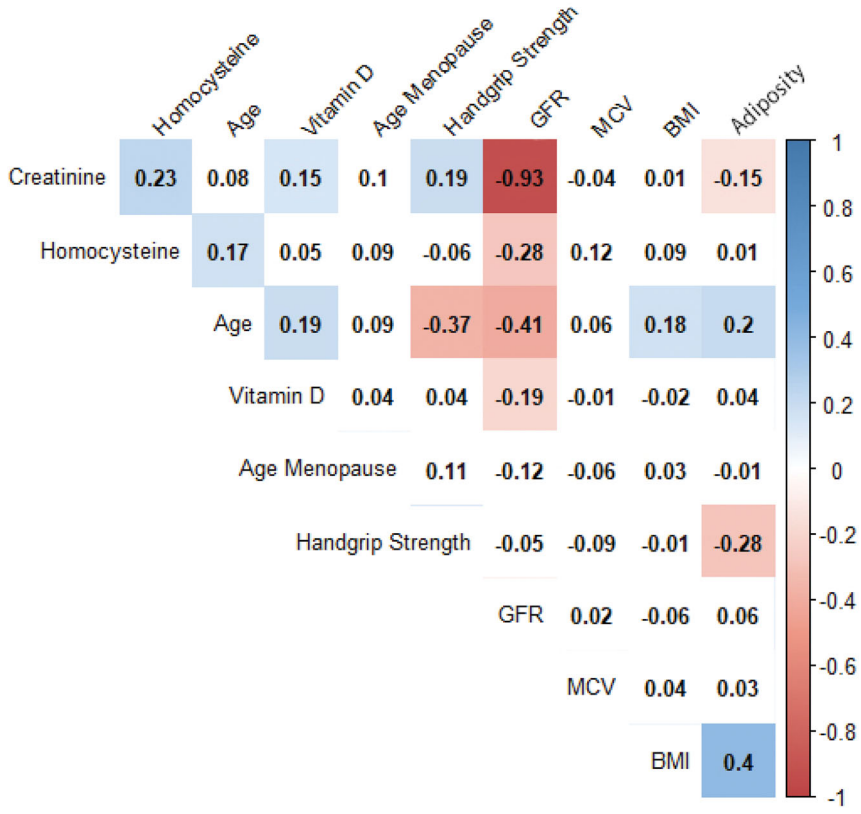

Figure 1. Pearson correlation between age, age at menopause, adiposity, body mass index (BMI), creatinine, glomerular filtration rate (GFR), handgrip strength (HGS), mean corpuscular volume, vitamin D and homocysteine.

Table 4. Logistic regression model analyzing the effect of covariates over the risk of presenting hyperhomocysteinemia (homocysteine >15 μmol/l).

	Hyperhomocysteinemia	
Covariate	*Odds ratio*	*95% confidence interval*
Age	1.05	[0.98; 1.13]
Age at menopause	1.03	[0.89; 1.19]
Smoking	0.95	[0.33; 3.17]
Body mass index	1.14	[1.01; 1.29]
Adiposity	0.98	[0.89; 1.08]
Mean corpuscular volume	1.23	[1.07; 1.43]
Creatinine	1.88	[1.18; 3.10]
Vitamin D	1.01	[0.97; 1.04]
Handgrip strength	0.99	[0.88; 1.11]
Physical activity, low vs. moderate/high	1.21	[0.52; 2.90]

postmenopausal women with hormone replacement therapy had decreased plasma homocysteine [31].

Muscle strength decreases with age and has a predictive value of the individual's brain function, physical vulnerability and even mortality in the long term [32]. Aging is associated with an increased prevalence of muscle alterations: first appears a loss of muscle strength without significant changes in muscle mass (dynapenia), and later the loss of muscle strength is accompanied by decreased muscle mass (sarcopenia) [33]. Some studies have reported the relationship between homocysteine and muscle strength and physical function with controversial or inconsistent results [10,34]. Swart et al. revealed that elevated plasma homocysteine levels are associated with lower grip strength only in older men and the three-year follow-up revealed no significant association between quartiles of homocysteine and HGS in both men and women [11]. The Baltimore Longitudinal Study of Aging reported a significant inverse relationship between homocysteine and grip strength in women, while results were not significant in men [12]. We observed no significant association between plasma homocysteine levels and HGS when compared with the tertiles of homocysteine. In our studied population, the dynapenia prevalence was 29.4%, and hyperhomocysteinemia was not associated with dynapenia.

Kidney function declines with aging, causing a reduction in GFR and consequently a decrease in creatinine and homocysteine clearance [2]. The relationship between homocysteine levels with renal function and creatinine level has been reported in the literature, showing that the plasma homocysteine level was negatively associated with renal function [23]. Our results were in line with previous studies which reported that the GFR and creatinine are associated with hyperhomocysteinemia.

Vitamin B12 and folate deficiency causes macrocytic anemia and hyperhomocysteinemia [35]. In our study, we have used the MCV as a surrogate marker of plasma vitamin B12 and folate levels, and no case of macrocytosis was identified. Interestingly, our study found that the MCV was associated with a higher risk of hyperhomocysteinemia. Vitamin D has been also associated with homocysteine levels [36,37]; however, others have not found such relationships [38]. Amer and Qayyum reported a non-linear relationship between vitamin D and homocysteine, as it was only observed among those with plasma vitamin D levels that were ≤21 ng/ml [36]. In our cohort, there was no association between homocysteine and plasma vitamin D levels. This

may be attributed to the fact that vitamin D levels were in the normal range and there were no women with very low plasma vitamin D levels.

The relationship between BMI and homocysteine levels showed conflicting data. Some previous studies reported a negative correlation between BMI and homocysteine levels [39], and others have found that hyperhomocysteinemia was associated with an increase in the percentage of adipose tissue [40]. Conversely, some studies reported no relationship between BMI and hyperhomocysteinemia [3]. Our results show an association between plasma homocysteine levels and BMI, and no relationship with adiposity. Of interest is that BMI was significantly associated with a higher risk of hyperhomocysteinemia.

Physical activity has been associated with plasma homocysteine levels but with controversial results. Acute exercise, independent of the duration or intensity of the exercise performed, usually induces increases in plasma homocysteine levels. The HERITAGE Family Study observed that regular aerobic exercise reduced plasma homocysteine levels in subjects with hyperhomocysteinemia, with no difference between men and women, but in subjects with a normal range of homocysteine there is a slight increase [41]. Regarding the effects of different exercise programs on plasma homocysteine levels, there is no consensus – this is due to the large variety of exercise interventions, with different durations and intensities, and different populations making it difficult to have consistent conclusions [42]. In our study, we observed that physical activity had no relationship with plasma homocysteine levels. Cigarette smoking has been associated with hyperhomocysteinemia [43]. The related mechanism is not fully known, but one possible explanation would be that smokers have lower vitamin B6, vitamin B12 and folate levels due to intake differences in fruit, vegetables and vitamin supplements between smokers and non-smokers [44]. In our study, we have not observed an association between smoking status and plasma homocysteine levels.

Limitations and strengths

Our study has certain limitations, including its cross-sectional design and the lack of data regarding the folate and vitamin B12 status. The strengths of the present study include a relatively large sample size of postmenopausal women and that the biochemical parameters were analyzed in a single laboratory with quality controls in place. We also included multiple potential risk factors that have been separately analyzed in previous publications. Furthermore, homogeneity in ethnicity and non-institutionalized women reduces potential confounding by different ethnicities or submission to the life regime of an institution.

Conclusions

In summary, the present study reports that in postmenopausal women, plasma homocysteine levels are not related to HGS. Hyperhomocysteinemia was not associated with HGS and dynapenia. Results showed that age, GFR and creatinine were correlated with plasma homocysteine levels. A total of 9.57% of women had hyperhomocysteinemia and 29.4% had dynapenia.

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References

[1] Kaye AD, Jeha GM, Pham AD, et al. Folic acid supplementation in patients with elevated homocysteine levels. Adv Ther. 2020;37(10):4149–4164.
[2] Friedman AN, Bostom AG, Selhub J, et al. The kidney and homocysteine metabolism. J Am Soc Nephrol. 2001;12(10):2181–2189.
[3] Chen S, Guo X, Dong S, et al. Relationship between lifestyle factors and hyperhomocysteinemia in general chinese population: a cross-sectional study. Postgrad Med. 2017;129(2):216–223.
[4] Panagiotakos DB, Pitsavos C, Zeimbekis A, et al. The association between lifestyle-related factors and plasma homocysteine levels in healthy individuals from the "ATTICA" study. Int J Cardiol. 2005;98(3):471–477.
[5] Škovierová H, Vidomanová E, Mahmood S, et al. The molecular and cellular effect of homocysteine metabolism imbalance on human health. Int J Mol Sci. 2016;17(10):1733.
[6] Chrysant SG, Chrysant GS. The current status of homocysteine as a risk factor for cardiovascular disease: a mini review. Expert Rev Cardiovasc Ther. 2018;16(8):559–565.
[7] Ma F, Wu T, Zhao J, et al. Plasma homocysteine and serum folate and vitamin B12 levels in mild cognitive impairment and Alzheimer's disease: a case-control study. Nutrients. 2017;9(7):725.
[8] Behera J, Bala J, Nuru M, et al. Homocysteine as a pathological biomarker for bone disease. J Cell Physiol. 2017;232(10):2704–2709.
[9] Wang P, Liu L, Lei SF. Causal effects of homocysteine levels on the changes of bone mineral density and risk for bone fracture: a two-sample Mendelian randomization study. Clin Nutr. 2021;40(4):1588–1595.
[10] van Schoor NM, Swart KM, Pluijm SM, et al. Cross-sectional and longitudinal association between homocysteine, vitamin B12 and physical performance in older persons. Eur J Clin Nutr. 2012;66(2):174–181.
[11] Swart KM, van Schoor NM, Heymans MW, et al. Elevated homocysteine levels are associated with low muscle strength and functional limitations in older persons. J Nutr Health Aging. 2013;17(6):578–584.
[12] Vidoni ML, Pettee Gabriel K, Luo ST, et al. Relationship between homocysteine and muscle strength decline: the Baltimore longitudinal study of aging. J Gerontol A Biol Sci Med Sci. 2018;73(4):546–551.

[13] De Giuseppe R, Tomasinelli CE, Vincenti A, et al. Sarcopenia and homocysteine: is there a possible association in the elderly? A narrative review. Nutr Res Rev. 2021;2021:1–14.
[14] García-Alfaro P, Rodriguez I, Pascual MA. Evaluation of the relationship between homocysteine levels and bone mineral density in postmenopausal women. Climacteric. 2022;25(2):179–185.
[15] García-Alfaro P, García S, Rodríquez I, et al. Handgrip strength, dynapenia, and related factors in postmenopausal women. Menopause. 2022;29(1):16–22.
[16] The IPAQ group. International physical activity questionnaire. 2016; [cited 2020 Mar 20]. Available from: http://www.ipaq.ki.se.
[17] Garcia-Alfaro P, Garcia S, Rodríguez I, et al. Factors related to muscle strength in postmenopausal women aged younger than 65 years with normal vitamin D status. Climacteric. 2019;22(4):390–394.
[18] Cruz-Jentoft AJ, Baeyens JP, Bauer JM, et al. European working group on sarcopenia in older people. Sarcopenia: European consensus on definition and diagnosis: report of the European working group on sarcopenia in older people. Age Ageing. 2010;39(4):412–423.
[19] Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate [published correction appears. Ann Intern Med. 2009;150(9):604–612.
[20] Lussana F, Betti S, D'Angelo A, et al. Evaluation of the prevalence of severe hyperhomocysteinemia in adult patients with thrombosis who underwent screening for thrombophilia. Thromb Res. 2013;132(6):681–684.
[21] Pérez-López FR, Brincat M, Erel CT, et al. EMAS position statement: Vitamin D and postmenopausal health. Maturitas. 2012;71(1):83–88.
[22] Azzini E, Ruggeri S, Polito A. Homocysteine: its possible emerging role in at-risk population groups. Int J Mol Sci. 2020;21(4):1421.
[23] Marti F, Vollenweider P, Marques-Vidal PM, et al. Hyperhomocysteinemia is independently associated with albuminuria in the population-based CoLaus study. BMC Public Health. 2011;11(1):733.
[24] Karger AB, Steffen BT, Nomura SO, et al. Association between homocysteine and vascular calcification incidence, prevalence, and progression in the MESA cohort. J Am Heart Assoc. 2020;9(3):e013934.
[25] Arnadottir M, Hultberg B, Nilsson-Ehle P, et al. The effect of reduced glomerular filtration rate on plasma total homocysteine concentration. Scand J Clin Lab Invest. 1996;56(1):41–46.
[26] Fakhrzadeh H, Ghotbi S, Pourebrahim R, et al. Total plasma homocysteine, folate, and vitamin B12 status in healthy Iranian adults: the Tehran homocysteine survey (2003–2004)/a cross-sectional population based study. BMC Public Health. 2006;6(1):29.
[27] Pérez-López FR, Chedraui P, Gilbert JJ, et al. Cardiovascular risk in menopausal women and prevalent related co-morbid conditions: facing the post-Women's health initiative era. Fertil Steril. 2009;92(4):1171–1186.
[28] Pérez-López FR, Larrad-Mur L, Kallen A, et al. Gender differences in cardiovascular disease: hormonal and biochemical influences. Reprod Sci. 2010;17(6):511–531.
[29] Zhu Z, Jiang S, Li C, et al. Relationship between serum homocysteine and different menopausal stage. Climacteric. 2020;23(1):59–64.
[30] Mohan IK, Khan SA, Jacob R, et al. Application of adaptive neuro-fuzzy inference systems (ANFIS) to delineate estradiol, glutathione and homocysteine interactions. Clin. Nutr. ESPEN. 2017;20:41–46.
[31] Chiantera V, Sarti CD, Fornaro F, et al. Long-term effects of oral and transdermal hormone replacement therapy on plasma homocysteine levels. Menopause. 2003;10(4):286–291.
[32] Cruz-Jentoft AJ, Bahat G, Bauer J, et al. Sarcopenia: revised european consensus on definition and diagnosis. Age Ageing. 2019;48(1):16–31.
[33] Pérez-López FR. Dynapenia and sarcopenia., In: Cano A, editor. Menopause: a comprehensive approach. Berlin: Springer; 2017. Chapter 20 pp. 317–331. DOI10.1007/978-3-319-59318-0_20.
[34] Kado DM, Bucur A, Selhub J, et al. Homocysteine levels and decline in physical function: MacArthur studies of successful aging. Am J Med. 2002;113(7):537–542.
[35] Nagao T, Hirokawa M. Diagnosis and treatment of macrocytic anemias in adults. J Gen Fam Med. 2017;18(5):200–204.
[36] Amer M, Qayyum R. The relationship between 25-hydroxyvitamin D and homocysteine in asymptomatic adults. J Clin Endocrinol Metab. 2014;99(2):633–638.
[37] Verdoia M, Nardin M, Gioscia R, et al. Association between vitamin D deficiency and serum homocysteine levels and its relationship with coronary artery disease. J Thromb Thrombolysis. 2021;52(2):523–529.
[38] García-Bailo B, Da Costa LA, Arora P, et al. Plasma vitamin D and biomarkers of cardiometabolic disease risk in adult Canadians, 2007–2009. Prev Chronic Dis. 2013;10:E91.
[39] Elshorbagy AK, Nurk E, Gjesdal CG, et al. Homocysteine, cysteine, and body composition in the Hordaland homocysteine study: does cysteine link amino acid and lipid metabolism. Am J Clin Nutr. 2008;88(3):738–746.
[40] Park SB, Georgiades A. Changes in body composition predict homocysteine changes and hyperhomocysteinemia in Korea. J Korean Med Sci. 2013;28(7):1015–1020.
[41] Okura T, Rankinen T, Gagnon J, et al. Effect of regular exercise on homocysteine concentrations: the HERITAGE Family Study. Eur J Appl Physiol. 2006;98(4):394–401.
[42] Maroto-Sanchez B, Lopez-Torres O, Palacios G, et al. What do we know about homocysteine and exercise? A review from the literature. Clin Chem Lab Med. 2016;54(10):1561–1577.
[43] Omoike OE, Paul TK, Ridner SL, et al. Association between smoking status and homocysteine levels and possible effect modification by cholesterol and oestradiol. Biomarkers. 2020;25(2):126–130.
[44] Subar AF, Harlan LC, Mattson ME. Food and nutrient intake differences between smokers and non-smokers in the US. Am J Public Health. 1990;80(11):1323–1329.

Artículo 4: Objetivo 2

Evaluation of the relationship between homocysteine levels and bone mineral density in postmenopausal women

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ORIGINAL ARTICLE

Evaluation of the relationship between homocysteine levels and bone mineral density in postmenopausal women

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ABSTRACT

Objective: The current study aimed to evaluate the relationship between homocysteine (Hcy) levels and bone mineral density (BMD) in postmenopausal women.

Methods: The present, cross-sectional study included 760 postmenopausal women. The following variables were recorded: age, age at menopause, body mass index (BMI), BMD (measured by dual-energy X-ray absorptiometry [DXA] scanning and expressed as lumbar, femoral neck and total hip *T*-scores), smoking status, biochemical parameters (Hcy, creatinine, calcium, phosphorus, vitamin D and parathormone levels) and vitamin D supplementation.

Results: The mean age of the sample population was 56.4 ± 5.77 years and the mean age at menopause was 49.9 ± 3.62 years. The mean BMI was 25.2 ± 4.49 kg/m^2. In the current study, a comparison of the subjects with osteoporosis, osteopenia and normal BMD revealed that the subjects in the low BMD group were significantly older ($p < 0.001$), had a lower age at menopause ($p < 0.001$) and had lower BMI ($p < 0.001$). There was no statistically significant difference among the groups with regard to the plasma levels of Hcy ($p = 0.946$). The levels of Hcy were positively correlated to the creatinine levels ($r = 0.21$). The present study did not observe any significant correlations between the Hcy levels and other parameters.

Conclusions: In the present study, 15.3% of the subjects had hyperhomocysteinemia and 62.11% had low BMD. The current results obtained from a group of postmenopausal women suggest that the plasma levels of Hcy are not related to BMD in the lumbar spine (L1–L4), femoral neck and total hip. In the current study, age, age at menopause and low BMI were observed to be associated with low BMD.

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Introduction

Homocysteine (Hcy) is a sulfur-containing amino acid produced by our body from methionine retrieved from the diet. Hcy can be converted into cysteine or remethylated to regenerate methionine through enzymatic reactions that depend on the presence of cofactors, such as folic acid and vitamins B6 and B12. The deficiency of enzymatic activity or its cofactors could result in an elevation of the plasma levels of Hcy [1].

Hyperhomocysteinemia (>15 μmol/l) has been recognized as a risk factor for atherosclerosis, hypercoagulability and atherothrombosis, and has been associated with a greater risk of adverse cardiovascular events, such as acute coronary syndrome, stroke and cardiovascular mortality [2,3]. Moreover, the condition has been related to cognitive impairment, dementia and Alzheimer's disease [4].

Several recent studies have shown that the serum levels of Hcy affect the formation of bone tissue by means of several mechanisms. Hcy reduces blood flow to the bone [5], increases osteoclast activity [6], activates matrix metalloproteinases [7], causes oxidative damage and interferes with the enzymatic cross-link formation in normal collagen. Consequently, Hcy decreases bone mineralization and reduces bone strength [8,9].

Osteoporosis is a skeletal disorder that causes considerable morbidity and mortality, owing to fractures, and is currently considered a major public health problem [10]. Previous studies have reported aging, female gender, low body weight, cigarette smoking, alcohol consumption, early menopause, prior low-trauma fracture as an adult and a history of hip fracture in a first-degree relative as the important risk factors for osteoporosis [11].

In the literature, certain previous studies have observed a relationship between the increased plasma levels of Hcy and bone mineral density (BMD) and an increased risk of osteoporotic fractures in postmenopausal women, independent of the BMD [12–16]. In contrast, some other studies have not observed any relationship between the levels of Hcy and BMD [17–19].

The purpose of the current study was to investigate whether the plasma levels of Hcy are related to BMD in a group of postmenopausal women who visited the Obstetrics and Gynecology Department at the Hospital Universitario Dexeus (Barcelona, Spain).

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Materials and methods

Study design

The current, cross-sectional study was performed during the time period from April 2019 to March 2020 in our Department and included 760 postmenopausal women aged 50–80 years. The following variables were recorded: the subject's age at menopause, biochemical laboratory parameters (Hcy, creatinine, calcium, phosphorus, vitamin D and parathormone [PTH] levels), body mass index (BMI) and BMD measured using dual-energy X-ray absorptiometry (DXA), smoking status and vitamin D supplementation. Postmenopausal status was defined as amenorrhea for a minimum duration of 1 year prior to initiation of the study. The exclusion criteria are stated as follows: women with primary ovarian failure, early menopause, cardiovascular, liver or renal diseases, surgical menopause or history of cancer, and patients under treatments that are known to influence the levels of Hcy or bone mineralization (folates or vitamin B12 supplements, menopausal hormone therapy, corticosteroids, anticonvulsants, heparin, thiazide diuretics and antiresorptive agents).

The present study was approved by the Institutional Review Board of our Clinical Center and registered in ClinicalTrials.gov (NCT04821492).

BMI measurements

BMI was calculated as the weight in kilograms divided by the square of the height in meters after assessing the weight and height of the participants wearing a disposable gown without footwear.

BMD measurements

BMD was determined by DXA using the Lunar iDXA system (GE Healthcare, Chicago, IL, USA) in the lumbar spine (L1–L4), femoral neck and total hip. The quality control of the scanner was performed by the measurement of a phantom's density on a daily basis. The phantom measurements showed stable results during the course of the study. Coefficients of variation of DXA measurements ranged from 0.23% to 0.38%. The evaluation of BMD was performed using only the raw data generated by the DXA. The values were expressed in absolute values as grams of mineral content per square centimeters of bone area (g/cm^2) and *T*-scores. In accordance with the World Health Organization (WHO) classification system, the subjects were classified on the basis of the lowest *T*-score as follows: normal BMD with a *T*-score > -1 standard deviation (SD); osteopenia with *T*-scores ranging from -1 SD to -2.5 SD; and osteoporosis with a *T*-score ≤ -2.5 SD [20].

Biochemical laboratory parameters

The blood samples were obtained during the time period between 8:00 a.m. and 9:00 a.m. after an overnight fast without any prior exercise or smoking. The plasma levels of Hcy, creatinine, calcium, phosphorus, vitamin D and PTH were evaluated using an automated analyzer system (COBAS® 8000; Roche Diagnostics, Pleasanton, CA, USA). Hyperhomocysteinemia was defined as a total serum level of Hcy $\geq$15 μmol/l [21].

Statistical analysis

In the present study, continuous variables are presented as the mean and SD, and categorical variables are presented as the percentage and number. The subjects were categorized into the following three groups: osteoporotic, osteopenic and normal groups. The continuous variables pertaining to the groups were compared using the analysis of variance test, and the Shapiro–Wilks test was used to check the normality of the variables. Pearson correlation was used to evaluate the association between continuous variables. The chi-square test or Fisher's exact test was used to compare categorical variables. The multivariate linear model was used to evaluate the association between BMD and age, age at menopause, BMI and the plasma levels of Hcy and vitamin D. $p < 0.05$ was considered statistically significant for all of the comparisons. The statistical analyses were performed using R software version 2.10.1 (The R Foundation for Statistical Computing: http://www.r-project.org/). All analyses were exploratory. No formal sample size calculation was performed.

Results

The mean age of participants was 56.4 ± 5.77 years and the mean age at menopause was 49.9 ± 3.62 years. The mean BMI of the whole sample was 25.2 ± 4.49 kg/m^2. The values of the clinical and biochemical parameters pertaining to the subjects, expressed as the mean ± SD and *n* (%), are presented in Table 1.

Table 1. Clinical and biochemical parameters pertaining to the 760 postmenopausal women.

Parameter	*Mean ± SD or* N (%)	*Reference range*
Homocysteine (μmol/l)	11.9 ± 3.62	5–15
Creatinine (mg/dl)	0.74 ± 0.12	0.50–1.02
Ca (mg/dl)	9.57 ± 0.45	8.7–10.4
P (mg/dl)	3.46 ± 0.47	2.5–4.5
Vitamin D (ng/ml)	27.0 ± 12.0	20–50
PTH (pg/ml)	41.2 ± 15.7	15–65
BMD (g/cm^2)		
Lumbar spine	1.11 ± 0.17	
Femoral neck	0.88 ± 0.13	
Total hip	0.92 ± 0.13	
T-score (SD)		
Lumbar spine	−0.75 ± 1.41	
Femoral neck	−0.80 ± 1.02	
Total hip	−0.66 ± 1.04	
Smoking		
Never	622 (81.9)	
Smoker	138 (18.1)	
Vitamin D supplementation		
No	447 (58.8)	
Yes	313 (41.2)	

BMD, bone mineral density; Ca, calcium; P, phosphorus; PTH: parathormone; SD, standard deviation.

Table 2. Clinical and biochemical parameters, according to the lowest *T*-score.

Parameter	*Osteoporosis* (n = *86*)	*Osteopenia* (n = *386*)	*Normal* (n = *288*)	p-*Value*
Age	59.5 ± 5.73	57.3 ± 6.12	54.3 ± 4.49	<0.001
Age at menopause	48.8 ± 4.32	49.8 ± 3.65	50.7 ± 2.92	<0.001
BMI (kg/m^2)	23.3 ± 4.21	24.6 ± 3.93	26.5 ± 4.92	<0.001
Homocysteine (μmol/l)	12.0 ± 3.62	11.9 ± 3.59	11.8 ± 3.68	0.946
Creatinine (mg/dl)	0.72 ± 0.12	0.73 ± 0.11	0.76 ± 0.13	<0.05
Ca (mg/dl)	9.52 ± 0.44	9.59 ± 0.43	9.56 ± 0.49	0.681
P (mg/dl)	3.56 ± 0.42	3.48 ± 0.49	3.40 ± 0.46	0.149
Vitamin D (ng/ml)	30.8 ± 15.1	27.2 ± 11.6	25.5 ± 11.2	<0.05
PTH (pg/ml)	41.3 ± 17.0	41.3 ± 15.9	41.3 ± 14.7	1.000
BMD (g/cm^2)				
Lumbar spine	0.87 ± 0.07	1.05 ± 0.10	1.27 ± 0.13	<0.001
Femoral neck	0.74 ± 0.07	0.83 ± 0.08	0.99 ± 0.11	<0.001
Total hip	0.77 ± 0.08	0.86 ± 0.08	1.03 ± 0.12	<0.001
T-score (SD)				
Lumbar spine	−2.70 ± 0.61	−1.21 ± 0.85	0.58 ± 1.02	<0.001
Femoral neck	−1.91 ± 0.61	−1.23 ± 0.62	0.12 ± 0.77	<0.001
Total hip	−1.88 ± 0.66	−1.09 ± 0.63	0.25 ± 0.79	<0.001
Smoking	19 (22.1%)	70 (18.1%)	49 (17.0%)	0.563
Vitamin D supplementation	36 (41.9%)	166 (43.0%)	111 (38.5%)	0.503

BMD, bone mineral density; BMI, body mass index; Ca, calcium; P, phosphorus; PTH, parathormone; SD, standard deviation.

Table 3. Mean comparison between the plasma levels of Hcy levels and BMD.

BMD (g/cm^2)	*Hcy ≤15 μmol/l (n = 643)*	*Hcy >15 μmol/l (n = 117)*	*95% CI for mean difference*	p-*Value*
Lumbar spine	1.11 ± 0.17	1.09 ± 0.19	0.02 [−0.02 to 0.05]	0.359
Femoral neck	0.88 ± 0.13	0.86 ± 0.12	0.02 [−0.00 to 0.04]	0.149
Total hip	0.92 ± 0.13	0.91 ± 0.13	0.01 [−0.01 to 0.03]	0.416

BMD, bone mineral density; CI, confidence interval; Hcy, homocysteine.

In accordance with the lowest *T*-score pertaining to any of the three sites used for the assessment of BMD, the current study observed that, among the study subjects, 86 had osteoporosis (11.32%), 386 had osteopenia (50.79%) and 288 displayed normal BMD (37.89%).

A comparison of the subjects with osteoporosis, osteopenia and normal BMD revealed that the osteoporotic and osteopenic groups were significantly older ($p < 0.001$). The present study compared the mean age at menopause with regard to BMD and observed that the age at menopause was higher in the normal BMD group. The difference was statistically significant ($p < 0.001$). The mean BMI of the subjects with normal BMD was significantly higher, compared to the subjects with osteopenia and osteoporosis ($p < 0.001$).

Regarding the difference among the groups in terms of BMD, the current study observed that the differences in the plasma levels of Hcy were not statistically significant ($p = 0.946$). Among the subjects involved in the current study, 18.1% were smokers and there was no significant difference among the groups with regard to smoking status ($p = 0.563$). Regarding the biochemical parameters, the plasma levels of creatinine were lower in the osteopenic and osteoporotic groups, compared to the normal group, and the differences were statistically significant ($p < 0.05$). The plasma level of vitamin D was significantly higher in the osteoporotic group ($p < 0.05$). However, there was no statistically significant difference among the groups with regard to vitamin D supplementation ($p = 0.503$) and the plasma levels of calcium, phosphorus and PTH. The results are summarized in Table 2.

The prevalence of hyperhomocysteinemia (Hcy >15 μmol/l) in the study population was observed to be 15.3%. The present study performed a mean comparison of the plasma levels of Hcy and BMD in the lumbar spine, femoral neck and total hip. The plasma level of Hcy was dichotomized into ≤15 μmol/l and >15 μmol/l. The differences among the groups in terms of the plasma levels of Hcy with respect to BMD were not statistically significant (Table 3).

The Pearson correlation coefficients (r) of the parameters pertaining to all of the subjects are shown in Figure 1. The present study observed that the levels of Hcy were positively correlated to the levels of creatinine ($r = 0.21$). No significant correlations were observed between the levels of Hcy and other parameters. Age was positively correlated with BMI ($r = 0.35$), and negatively correlated with the levels of phosphorus ($r = -0.29$) and the *T*-score of the lumbar spine ($r = -0.26$). BMI was negatively correlated with the levels of phosphate ($r = -0.27$) and positively correlated with the *T*-score of the total hip ($r = 0.26$).

Finally, multiple regression analysis was used to determine the possible influence of age, age at menopause, BMI and the plasma levels of Hcy and vitamin D in predicting BMD. According to the β-coefficients, age and BMI were the main predictors of BMD in the lumbar spine, femoral neck and total hip, whereas age at menopause was a predictor of BMD in the lumbar spine and total hip. The plasma levels of Hcy and vitamin D were not significant predictors of BMD (Table 4).

Discussion

The current study analyzed the association between plasma levels of Hcy and BMD in postmenopausal women. The present results did not show any significant correlation between the plasma levels of Hcy and BMD in the lumbar spine (L1–L4), femoral neck or total hip.

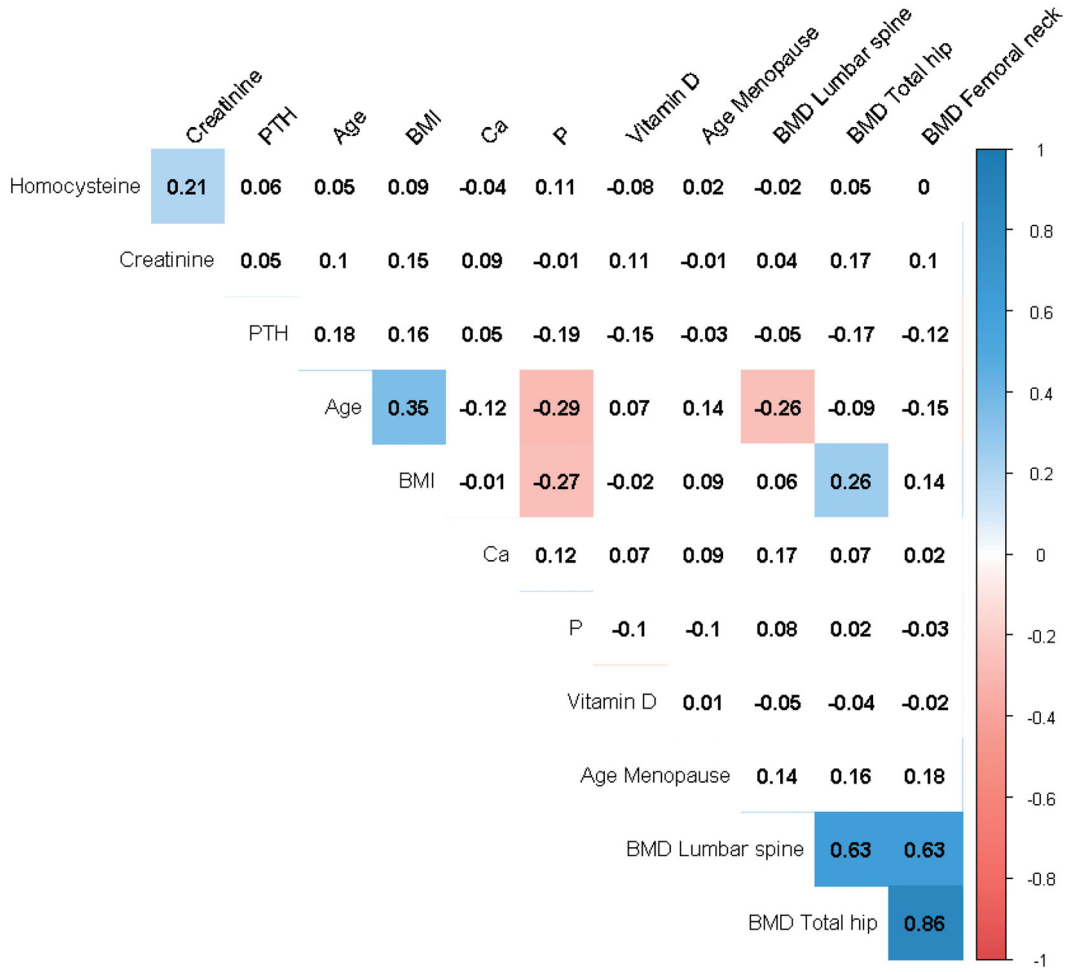

Figure 1. Pearson correlation between the parameters and bone mineral density (BMD). BMI, body mass index; Ca, calcium; P, phosphorus; PTH, parathormone.

Table 4. Multiple regression analysis to determine the predictors of BMD (g/cm^2).

	Lumbar spine BMD		*Femoral neck BMD*		*Total hip BMD*	
Predictor	*β*	*95% CI*	*β*	*95% CI*	*β*	*95% CI*
Age	−0.01[a]	0.99–0.99	−0.01[a]	0.99–1.00	−0.00[a]	0.99–1.00
Age at menopause	0.01[a]	1.00–1.01	0.00	1.00–1.01	0.00[b]	1.00–1.01
BMI	0.01[a]	1.00–1.01	0.01[a]	1.00–1.01	0.01[a]	1.01–1.01
Hcy	0.00	1.00–1.01	0.00	1.00–1.00	0.00	1.00–1.00
Vitamin D	−0.00	1.00–1.00	−0.00	1.00–1.00	−0.00	1.00–1.00
		Adjusted $R^2 = 0.11$		Adjusted $R^2 = 0.10$		Adjusted $R^2 = 0.14$

[a] $p < 0.001$.
[b] $p < 0.05$.
BMD, bone mineral density; BMI, body mass index; CI, confidence interval; Hcy, homocysteine.

Previous studies have reported a significant positive association between the plasma levels of Hcy and the risk of fractures [15,16,22,23]. Moreover, the severity of vertebral fractures [24] is currently considered a new, independent risk factor for decreased bone strength and fractures [25]. However, the relationship between the plasma levels of Hcy and BMD remains controversial. Some previous studies have reported that the elevated levels of Hcy are not related to low BMD [17–19,26], whereas other studies have observed a significant association between the levels of Hcy and BMD [12–14,27,28]. The present study was conducted to investigate the relationship between the levels of Hcy and BMD, and no correlation was observed.

A comparison of the previous reports with the current study revealed certain differences in the menopausal status of the subjects and the bones involved in the evaluations. In the present study, all of the subjects were postmenopausal women with mean age at menopause within the normal range. Some of the previous studies included subjects in the stages of early menopause or perimenopausal women. Regarding the evaluation of BMD in different bones, the current study analyzed the lumbar spine, femoral neck and total hip, whereas other studies evaluated only the lumbar spine or hip.

A systematic review and meta-analyses by van Wijngaarden et al. showed that vitamin B12, folate and

plasma levels of Hcy are not associated with BMD. Nevertheless, there was evidence of the association between elevated plasma levels of Hcy and fracture risk [23]. This highlights the importance of the documentation of major osteoporotic fractures.

A recent study by Su et al. regarding the sex-based differences between the levels of Hcy and BMD reported that a higher fracture risk and a greater decline in BMD with higher levels of Hcy were observed in males, but not in females [29]. Accordingly, the sex of the subjects should be taken into consideration during assessment of the association between levels of Hcy and BMD.

A cross-sectional study by Kim et al. stratified the subjects in accordance with their age into three groups, and reported that Hcy was negatively correlated with BMD only in female subjects above the age of 50 years [30]. The aforementioned results suggest that the age of the subjects may influence the results.

Considering the relationship between the levels of Hcy and increased osteoclast activity, the results regarding the bone turnover markers of bone resorption and levels of Hcy are controversial. Mittal et al. reported that there was no direct correlation between Hcy and cathepsin K [19]. In addition, the study by Ohishi et al. did not observe any relationship between Hcy and N-terminal telopeptide of type I collagen [31]. However, a recent study by De Martinis et al. reported a significant relation between higher levels of Hcy and higher C-terminal telopeptide of type I collagen levels [32], thereby indicating the importance of including bone turnover markers in the research.

Elevated levels of Hcy contribute to the development of osteoporosis through the following mechanisms: increasing osteoclast activity, inhibiting osteoblast activity, reducing blood flow to the bone, activating matrix metalloproteinases, increasing oxidative damage by the production of reactive oxygen species and interfering with collagen cross-linking. Consequently, the aforementioned mechanisms reduce the strength of the bone microarchitecture, damage the biomechanical properties of the bone, reduce bone quality and increase the risk of fractures [33]. BMD assessments detect the changes in bone metabolism with a significant delay and provide poor information regarding the microarchitecture of the bone matrix (e.g. the cross-linking of collagen molecules or the integration of collagen molecules in the bone matrix) that might be crucial for mechanical stability [34]. One of the pathophysiological mechanisms of osteoporosis in women with higher levels of Hcy may be damage to the quality of bone, which cannot be detected by BMD evaluation using DXA [35]. In relation to this fact, the current study did not observe any correlation between the group of subjects with hyperhomocysteinemia and BMD.

Hcy levels increase with age and are higher in postmenopausal women, compared to perimenopausal women [36]. Hcy levels are also associated with renal function and the creatinine level is correlated with the concentration of Hcy [31]. In the present study, all of the subjects were postmenopausal women and age was not observed to be related to the levels of Hcy. The levels of Hcy were related to the creatinine levels. Age, menopausal status and renal function should be considered during the assessment of levels of Hcy.

Furthermore, there are important differences in the levels of Hcy, in accordance with variation in geographical locations. Lower values of Hcy levels were reported in the Mediterranean countries, compared to central or northern European countries [37]. The data reported by various previous studies suggest that the prevalence of hyperhomocysteinemia is within a range of 4.13–39.1% in the adult female population and the variations depend on the cut-off value of Hcy levels, geographic location, age and ethnicity of the population under study [38–40]. The prevalence of hyperhomocysteinemia among the subjects in the current study is within the low range, compared to the previous results reported in the literature.

Several factors play key roles in the development of osteoporosis, such as low BMI, aging, age at menopause and vitamin D levels. These factors are associated with low BMD [41,42]. In accordance with these data, the current study observed that the osteopenic and osteoporotic subjects displayed lower BMI, were significantly older and had a lower age at menopause, compared to the subjects with normal BMD. However, the vitamin D levels were higher in the subjects with low BMD, compared to the subjects with normal BMD, which can be attributed to the fact that in our department, vitamin D supplementation is recommended for all postmenopausal women diagnosed with low levels of vitamin D. However, there were no significant differences among the groups based on BMD with regard to vitamin D supplementation.

The strengths of the current study include the large sample size of postmenopausal women and the fact that all of the BMD evaluations were performed with DXA using the same densitometer and the biochemical parameters were analyzed in the same biochemical laboratory with quality controls in place. However, the present study has certain limitations. The retrospective nature of the study and the lack of data regarding the folate and vitamin B12 status of the subjects are limitations. Furthermore, the lack of data regarding the osteoporotic fractures and biochemical bone turnover markers is another limitation, which made the assessment of the possible influence of the levels of Hcy on these variables impossible.

Conclusions

The results of the present study suggest that plasma levels of Hcy are not related to BMD in the lumbar spine (L1–L4), femoral neck and total hip in a group of postmenopausal women. Hence, it can be concluded that the plasma level of Hcy is not a good predictor of BMD status. In the current study, 15.3% of the subjects had hyperhomocysteinemia and 62.11% had low BMD. Age, low BMI and age at menopause were observed to be associated with low BMD.

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No linked research data sets are associated with this article. Data will be made available on request.

References

[1] Swart KM, van Schoor NM, Lips P. Vitamin B12, folic acid, and bone. Curr Osteoporos Rep. 2013;11(3):213–218.
[2] Djuric D, Jakovljevic V, Zivkovic V, et al. Homocysteine and homocysteine-related compounds: an overview of the roles in the pathology of the cardiovascular and nervous systems. Can J Physiol Pharmacol. 2018;96(10):991–1003.
[3] Chrysant SG, Chrysant GS. The current status of homocysteine as a risk factor for cardiovascular disease: a mini review. Expert Rev Cardiovasc Ther. 2018;16(8):559–565.
[4] Ma F, Wu T, Zhao J, et al. Plasma homocysteine and serum folate and vitamin B12 levels in mild cognitive impairment and Alzheimer's disease: a case-control study. Nutrients. 2017;9(7):725.
[5] Tyagi N, Kandel M, Munjal C, et al. Homocysteine mediated decrease in bone blood flow and remodeling: role of folic acid. J Orthop Res. 2011;29:1511–1516.
[6] Koh J-M, Lee Y-S, Kim YS, et al. Homocysteine enhances bone resorption by stimulation of osteoclast formation and activity through increased intracellular ROS generation. J Bone Miner Res. 2006;21(7):1003–1011.
[7] Vacek TP, Kalani A, Voor MJ, et al. The role of homocysteine in bone remodeling. Clin Chem Lab Med. 2013;51(3):579–590.
[8] Saito M, Marumo K. Collagen cross-links as a determinant of bone quality: a possible explanation for bone fragility in aging, osteoporosis, and diabetes mellitus. Osteoporos Int. 2010;21:195–214.
[9] Fratoni V, Brandi ML. B Vitamins, Homocysteine and Bone Health. Nutrients. 2015;7:2176–2192.
[10] De Martinis M, Sirufo MM, Ginaldi L. Osteoporosis: current and emerging therapies targeted to immunological checkpoints. Curr Med Chem. 2020;27(37):6356–6372.
[11] Akkawi I, Zmerly H. Osteoporosis: current concepts. Joints. 2018;6:122–127.
[12] Bozkurt N, Erdem M, Yilmaz E, et al. The relationship of homocysteine, B12 and folic acid with the bone mineral density of the femur and lumbar spine in Turkish postmenopausal women. Arch Gynecol Obstet. 2009;280(3):381–387.
[13] Bucciarelli P, Martini G, Martinelli I, et al. The relationship between plasma homocysteine levels and bone mineral density in post-menopausal women. Eur J Intern Med. 2010;21(4):301–305.
[14] Ouzzif Z, Oumghar K, Sbai K, et al. Relation of plasma total homocysteine, folate and vitamin B12 levels to bone mineral density in Moroccan healthy postmenopausal women. Rheumatol Int. 2012;32(1):123–128.
[15] LeBoff MS, Narweker R, LaCroix A, et al. Homocysteine levels and risk of hip fracture in postmenopausal women. J Clin Endocrinol Metab. 2009;94:1207–1213.
[16] Bailey RL, van Wijngaarden JP. The role of B-vitamins in bone health and disease in older adults. Curr Osteoporos Rep. 2015;13(4):256–261.
[17] Cagnacci A, Bagni B, Zini A, et al. Relation of folates, vitamin B12 and homocysteine to vertebral bone density change in postmenopausal women. A five-year longitudinal evaluation. Bone. 2008;42:314–320.
[18] Haliloglu B, Aksungar FB, Ilter E, et al. Relationship between bone mineral density, bone turnover markers and homocysteine, folate and vitamin B12 levels in postmenopausal women. Arch Gynecol Obstet. 2010;281(4):663–668.
[19] Mittal M, Verma R, Mishra A, et al. Relation of bone mineral density with homocysteine and cathepsin K levels in postmenopausal women. Indian J Endocr Metab. 2018;22(2):261–266.
[20] World Health Organization. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. WHO Technical Report Series 843. Geneva (Switzerland): World Health Organization; 1994.
[21] Zaric BL, Obradovic M, Bajic V, et al. Homocysteine and hyperhomocysteinaemia. CMC. 2019;26(16):2948–2961.
[22] Yang J, Hu X, Zhang Q, et al. Homocysteine level and risk of fracture: a meta-analysis and systematic review. Bone. 2012;51(3):376–382.
[23] van Wijngaarden JP, Doets EL, Szczecińska A, et al. Vitamin B12, folate, homocysteine, and bone health in adults and elderly people: a systematic review with meta-analyses. J Nutr Metab. 2013;2013:486186.
[24] Kuroda T, Tanaka S, Saito M, et al. Plasma level of homocysteine associated with severe vertebral fracture in postmenopausal women. Calcif Tissue Int. 2013;93(3):269–275.
[25] Behera J, Bala J, Nuru M, et al. Homocysteine as a pathological biomarker for bone disease. J Cell Physiol. 2017;232(10):2704–2709.
[26] Rumbak I, Zižić V, Sokolić L, et al. Bone mineral density is not associated with homocysteine level, folate and vitamin B12 status. Arch Gynecol Obstet. 2012;285:991–1000.
[27] Enneman AW, Swart KM, Zillikens MC, et al. The association between plasma homocysteine levels and bone quality and bone mineral density parameters in older persons. Bone. 2014;63:141–146.
[28] Bahtiri E, Islami H, Rexhepi S, et al. Relationship of homocysteine levels with lumbar spine and femur neck BMD in postmenopausal women. Acta Reumatol Port. 2015;40(4):355–362.
[29] Su Y, Elshorbagy A, Turner C, et al. Circulating amino acids are associated with bone mineral density decline and ten-year major osteoporotic fracture risk in older community-dwelling adults. Bone. 2019;129:115082.
[30] Kim JI, Moon JH, Chung HW, et al. Association between homocysteine and bone mineral density according to age and sex in healthy adults. J Bone Metab. 2016;23(3):129–134.
[31] Ohishi T, Fujita T, Nishida T, et al. Factors influencing serum homocysteine levels in postmenopausal osteoporotic females – comparison to urinary collagen crosslinks. Endocr Res. 2019;44(3):117–125.
[32] De Martinis M, Sirufo MM, Nocelli C, et al. Hyperhomocysteinemia is associated with inflammation, bone resorption, vitamin B12 and folate deficiency and MTHFR C677T polymorphism in postmenopausal women with decreased bone mineral density. IJERPH. 2020;17(12):4260.
[33] Saito M, Marumo K. The effects of homocysteine on the skeleton. Curr Osteoporos Rep. 2018;16(5):554–560.
[34] Harvey NC, Glüer CC, Binkley N, et al. Trabecular bone score (TBS) as a new complementary approach for osteoporosis evaluation in clinical practice. Bone. 2015;78:216–224.
[35] Shevroja E, Lamy O, Kohlmeier L, et al. Use of Trabecular Bone Score (TBS) as a complementary approach to dual-energy X-ray absorptiometry (DXA) for fracture risk assessment in clinical practice. J Clin Densitom. 2017;20(3):334–345.
[36] Zhu Z, Jiang S, Li C, et al. Relationship between serum homocysteine and different menopausal stage. Climacteric. 2020;23(1):59–64.

[37] Varela-Moreiras G, Escudero JM, Alonso-Aperte E. Homocysteine related vitamins and lifestyles in the elderly people: SENECA study. Nutr Hosp. 2007;22(3):363–370.
[38] Marti F, Vollenweider P, Marques-Vidal PM, Mooser V, et al. Hyperhomocysteinemia is independently associated with albuminuria in the population-based CoLaus study. BMC Public Health. 2011;11(1):733.
[39] Sellos-Moura M, Glavin F, Lapidus D, Evans KA, et al. Estimated prevalence of moderate to severely elevated total homocysteine levels in the United States: a missed opportunity for diagnosis of homocystinuria? Mol Genet Metab. 2020;130(1): 36–40.
[40] Karger AB, Steffen BT, Nomura SO, et al. Association between homocysteine and vascular calcification incidence, prevalence, and progression in the MESA cohort. J Am Heart Assoc. 2020;9(3):e013934.
[41] Aspray TJ, Hill TR. Osteoporosis and the ageing skeleton. Subcell Biochem. 2019;91:453–476.
[42] Kanis JA, Cooper C, Rizzoli R, et al. Scientific Advisory Board of the European Society for Clinical and Economic Aspects of Osteoporosis (ESCEO) and the Committees of Scientific Advisors and National Societies of the International Osteoporosis Foundation (IOF). European guidance for the diagnosis and management of osteoporosis in postmenopausal women. Osteoporos Int. 2019;30(1):3–44.

5. DISCUSIÓN

Con nuestro trabajo hemos querido determinar los factores generales y hormonales asociados a la fuerza muscular de agarre y la DMO, así como valorar si la homocisteína y su relación con la fuerza muscular de agarre y la DMO, podría tener utilidad en la práctica clínica diaria a la hora de evaluar a la mujer postmenopáusica.

La discusión se ha estructurado siguiendo la línea argumental de los objetivos establecidos.

5.1 Factores generales relacionados con la fuerza muscular de agarre y la DMO

El proceso de envejecimiento está asociado a cambios cualitativos y cuantitativos en la masa muscular, así como en la masa ósea (Hughes et al., 2001; Seeman., 2002). Con la edad, se produce una pérdida de calidad muscular debido a una disminución progresiva de la fibra muscular tipo II, que juega un papel importante en el metabolismo anaeróbico, representando probablemente el mecanismo que inicia la disminución de la fuerza muscular (Morgan et al., 2020).

Para el mantenimiento de las propiedades óseas es necesario el recambio óseo y cuando hay desequilibrio entre los procesos de resorción ósea y formación ósea, se desarrolla la osteoporosis. Este proceso está influenciado por la edad, los esteroides sexuales, la vitamina D, la hormona paratiroidea (PTH) y factores plasmáticos involucrados en el crecimiento celular (Cheung et al., 2004; Hadjidakis et al., 2006).

En nuestro trabajo (García-Alfaro et al., 2022[a]) en consonancia con estudios previos, observamos que la edad está relacionada con la pérdida de fuerza muscular y esta pérdida va disminuyendo progresivamente con el paso de los años. Además, observamos que la edad está asociada a mayor riesgo de presentar dinapenia. Respecto a la DMO, los grupos de mujeres con osteopenia y osteoporosis eran significativamente mayores respecto al grupo de mujeres con DMO normal (García-Alfaro et al., 2022[b]).

La edad de la menopausia se ha relacionado con la fuerza muscular de agarre y la DMO. Una edad más temprana de inicio de la menopausia se asocia con un mayor riesgo de presentar dinapenia (García-Alfaro et al., 2019), menor DMO y una mayor tasa de fracturas (Gallagher., 2007). En este sentido nuestro estudio revela que la fuerza muscular de agarre tiene una correlación con la edad de la menopausia. Además se observa que existe una diferencia en la media de edad de la menopausia entre los grupos de mujeres con DMO normal, osteopenia y osteoporosis, teniendo una menor edad de la menopausia las mujeres con osteoporosis (García-Alfaro et al., 2022[b]).

La relación entre fumar y la fuerza muscular no está clara. Estudios previos han observado la asociación entre el tabaquismo y la sarcopenia (Neves et al., 2018) o la dinapenia (Stenholm et al., 2012), mientras que otros estudios no encuentran asociación (Wiener et al., 2020). Incluso, se ha observado en el Estudio Longitudinal Canadiense sobre el Envejecimiento que fumar se asocia con una mayor fuerza de prensión (Velez et al., 2019). En relación con la DMO, el tabaquismo comporta una disminución de la

DMO y un aumento del riesgo de fracturas osteoporóticas (Trevisan et al., 2020). En nuestro estudio, el tabaquismo no se asoció con la fuerza muscular de agarre ni con la DMO. Estos resultados podrían explicarse por el relativo bajo porcentaje de fumadoras de nuestro trabajo, situándose entre el 18.8% y el 19.4%.(García-Alfaro et al., 2022[a]; García-Alfaro et al., 2023).

Todavía existen controversias a la hora de evaluar la relación entre el IMC y la fuerza muscular de agarre. Estudios previos han encontrado una débil correlación entre el IMC y la fuerza muscular de agarre (Lopes et al., 2018) y contrastan con el resultado de otros estudios donde informan que la fuerza muscular de agarre no está relacionada con el IMC (Kamarul et al., 2006). En relación con la DMO, presentar un IMC bajo (< 19 kg/m^2), comporta un mayor riesgo de osteoporosis y riesgo de fractura, sin embargo se ha observado que la obesidad, puede aumentar el riesgo de algún tipo de fractura periférica (Riancho et al., 2022). Aunque la evaluación del IMC en nuestro estudio mostró que el IMC medio calculado para todo el grupo de mujeres estaba en el rango normal, hasta el 40,9% de ellas tenían sobrepeso-obesidad. En nuestros resultados, no encontramos ninguna correlación entre el IMC y la fuerza muscular de agarre (García-Alfaro et al., 2022[a]), pero si observamos que el IMC medio de las mujeres con osteoporosis fue significativamente menor, en comparación con las mujeres con osteopenia y DMO normal (García-Alfaro et al., 2022[b])

La adiposidad induce un estado proinflamatorio que se asocia con una menor fuerza muscular y masa muscular. También estaría asociada con un impacto negativo en la calidad ósea (Bian et al.,

2017; Gkastaris et al., 2020). En nuestro estudio observamos que el 56,2% de las mujeres tenían mayor adiposidad que la media. Encontramos una relación significativa entre la fuerza muscular de agarre y la adiposidad. Además, la adiposidad está asociada a mayor riesgo de presentar dinapenia (García-Alfaro et al., 2022[a]). Una razón plausible podría estar en la asociación entre un alto contenido de masa grasa y una mala calidad muscular.

La actividad física tiene efectos beneficiosos sobre la masa muscular, la fuerza muscular y el rendimiento físico al estimular la síntesis de proteínas musculares. El ejercicio físico también tiene efectos beneficiosos para alcanzar y mantener una buena masa ósea, así como para retrasar la aparición de la osteoporosis (Agostini et al., 2018). Realizar ejercicio regularmente durante la edad media de la vida es eficaz para mantener la fuerza muscular y el rendimiento físico en etapas de edad avanzada (Akune et al., 2014). Las mujeres de nuestro estudio que realizaban actividad física moderada-alta tenían mayor fuerza muscular de agarre en comparación con las mujeres que realizaban una baja actividad física (García-Alfaro et al., 2022[a]).

Existen varios estudios con resultados controvertidos en cuanto a la relación entre la fuerza muscular de agarre y la DMO. Algunos estudios informaron una relación positiva entre la fuerza muscular de agarre y la DMO (Kim et al., 2012; Li et al., 2018), mientras que otros no observaron ninguna relación en mujeres posmenopáusicas (Bayramoğlu et al., 2005; Lindsey et al., 2005). El análisis de la DMO en nuestro estudio reveló que las puntuaciones medias del T-score estaban en el rango de osteopenia. La fuerza de prensión se

correlacionó con la DMO en columna lumbar, cuello femoral y cadera total. Estos resultados son consistentes con estudios previos. Además, en nuestro trabajo las mujeres con T-score más alto en cuello femoral presentan menor riesgo de tener dinapenia, reforzando la asociación entre la fuerza muscular de agarre y la DMO (García-Alfaro et al., 2022[a]).

Con respecto al metabolismo mineral óseo, algún estudio ha encontrado asociación entre una mejor fuerza muscular y niveles más altos de vitamina D, calcio y fósforo (Verde et al., 2019). Por el contrario, nuestro estudio no ha mostrado relación entre la fuerza muscular de agarre con el estado del metabolismo mineral óseo, lo que sugiere que la regulación endocrina fosfocálcica femenina se ajusta al proceso de envejecimiento.

5.2 Vínculos entre las hormonas endógenas con la fuerza muscular de agarre y la DMO

La asociación entre los niveles de estrógenos y la fuerza muscular en mujeres postmenopáusicas es ambigua, probablemente debido a las diferentes edades y métodos de estudio y a la simplificación de los factores endocrinos involucrados. Algunos estudios reportan una relación entre los estrógenos y la fuerza muscular (Rolland et al., 2007; Ciprinani et al., 2012), mientras que otros estudios no encuentran una relación consistente (Schaap et al., 2005; Maddalozzo et al., 2004). En nuestra cohorte, la asociación entre estradiol y la fuerza muscular de agarre no fue significativa, aunque curiosamente encontramos que los niveles plasmáticos más altos

de hormona folículo estimulante (FSH) se asociaron significativamente en mujeres con dinapenia, expresando el ajuste secundario del hipoestrogenismo global de la postmenopausia (García-Alfaro et al., 2023).

La disminución de los niveles de estrógeno que se produce durante la transición de la menopausia se asocia con la disminución de la DMO (Riggs et al., 2003; Rogers et al., 2002) y un mayor riesgo de fracturas osteoporóticas (Finigan et al., 2012). Sin embargo, durante el período postmenopáusico, el efecto de los niveles séricos de estradiol solo explica un pequeño porcentaje de la pérdida ósea, y otros factores como la edad, la edad de la menopausia, los años transcurridos desde la menopausia, el IMC, la adiposidad, el tabaquismo y la actividad física pueden afectar la DMO (Akdeniz et al., 2009; Sullivan et al., 2017).

El efecto de los andrógenos sobre la fuerza muscular de agarre en mujeres postmenopáusicas no está bien aclarado. Los niveles de testosterona están asociados con la masa muscular y algunos estudios informan sobre la relación entre los niveles de testosterona y la fuerza muscular (van Geel et al., 2009; Kong et al., 2019). Unos bajos niveles plasmáticos de DHEAS se han asociado con el desarrollo de enfermedad cardiovascular y mortalidad, así como a una débil fuerza muscular (Ohlsson et al., 2015; Kong et al., 2019)
La Δ4 androstendione, es una hormona esteroidea relacionada con los estados de hiperandrogenismo, pero su relación con la fuerza muscular de agarre en mujeres postmenopáusicas no ha sido estudiada (Badawy et al., 2021). Nuestro análisis muestra que los únicos niveles de andrógenos asociados con la fuerza muscular de

agarre, fueron los niveles de DHEAS, pero esta relación se pierde tras el ajuste por la edad. Por lo tanto no hemos encontrado asociación entre la fuerza muscular de agarre y los andrógenos estudiados (García-Alfaro et al., 2023).

La relación entre los niveles de cortisol y la fuerza muscular de agarre no está aclarada. Peeters et al. (2008) no mostraron relación entre los niveles de cortisol plasmático y la pérdida de la fuerza muscular de agarre, aunque encontraron que los niveles altos de cortisol salival están asociados con un mayor riesgo de fuerza de prensión disminuida. También se ha encontrado una asociación positiva entre los metabolitos del cortisol urinario con la masa muscular y la fuerza en las extremidades superiores en adultos jóvenes pero no en ancianos (Bochud et al., 2019). Respecto a los diferentes resultados que se han encontrado entre los niveles de cortisol y la fuerza muscular, podrían explicarse por el uso de diferentes técnicas de determinación de los niveles de cortisol, al ser valorado en plasma, orina o saliva, según cada estudio. En nuestros resultados se observó que las mujeres con dinapenia tenían niveles de cortisol en plasma más altos, pero tras realizar el análisis multivariable no mostró una asociación significativa entre los niveles de cortisol y la fuerza manual de agarre (García-Alfaro et al., 2023).

Los niveles plasmáticos de IGF-1 disminuyen con la edad, tanto en hombres como en mujeres (Landin-Wilhelmsen et al., 2004). Sin embargo, el papel del IGF-1 en la pérdida de fuerza muscular relacionada con la edad aún no está claro. El género es un factor de confusión en la asociación entre IGF-1 y la fuerza muscular, al

encontrarse una relación significativa entre los niveles de IGF-1 y la fuerza muscular en mujeres pero no en hombres (Taekema et al., 2011). Después del ajuste por edad, Cappola et al. (2001) observaron una asociación significativa entre IGF-1 y la fuerza extensora de la rodilla, pero no con la antropometría y otras medidas de fuerza. En su estudio, también encontraron una correlación significativa entre los niveles plasmáticos de IGF-1 y la fuerza muscular, pero solo por debajo del umbral de IGF-1 de 50 μg/L. En nuestra población estudiada, la media del nivel plasmático de IGF-1 está por encima del umbral descrito previamente y después de la corrección por las variables de confusión, encontramos que los niveles plasmáticos de IGF-1 no estaban asociados con la fuerza muscular de agarre (García-Alfaro et al., 2023).

A diferencia con otros estudios, para analizar la asociación entre las hormonas endógenas y la fuerza muscular de agarre, en nuestro estudio hemos utilizado un gráfico acíclico directo para identificar las variables de confusión y se realizó un ajuste cuidadoso en el modelo de regresión.

La relación entre los niveles de vitamina D y la fuerza muscular de agarrre muestran datos contradictorios. Algunos estudios previos informan que los niveles bajos de vitamina D están relacionados con una fuerza de prensión más baja en mujeres posmenopáusicas (Aspell et al., 2019) y otros no muestran una asociación significativa (Kim et al., 2019). Estos resultados pueden deberse a diferentes rangos de edad en las poblaciones examinadas y diferentes niveles

basales de vitamina D en suero. Además, la vitamina D afecta a la fuerza muscular sólo cuando está por debajo de un determinado umbral, especialmente si los niveles séricos son <15 ng/ml y la suplementación con vitamina D en mujeres que no tienen deficiencia de vitamina D no obtendría un beneficio significativo (López-Baena et al., 2020). La deficiencia de vitamina D es un factor de riesgo asociado con el desarrollo de osteoporosis y tener un umbral de vitamina D < 12 ng/ml (30 nmol/l) sería compatible con la presencia de efectos adversos sobre la salud ósea (Institute of Medicine., 2011). Nuestro trabajo no mostró asociación entre los niveles de vitamina D con la fuerza muscular de agarre y paradójicamente, las mujeres con osteoporosis tenían niveles más altos de vitamina D que las mujeres con osteopenia y con un DMO normal. En nuestra cohorte, no hubo mujeres con niveles extremadamente bajos de vitamina D y este hecho puede ser una posible explicación de nuestros hallazgos (García-Alfaro et al., 2023).

Diversos estudios han examinado la relación entre la PTH y la fuerza muscular con resultados inconsistentes, debido a que algunos estudios encuentran una asociación estadísticamente significativa (Dhesi et at., 2002), mientras que otros no la encuentran (Marantes et al., 2011). En nuestro trabajo, no hemos observado una asociación entre PTH y la fuerza muscular de agarre. Algunos estudios asocian los niveles de PTH con la reducción de la DMO (Bruce et al., 1999), pero nuestros resultados identificaron que no había diferencias en los niveles de PTH entre las mujeres con osteoporosis, osteopenia y DMO normal, por lo que

este hecho podría justificar nuestros hallazgos (García-Alfaro et al., 2022[b])

5.3 Relación entre la homocisteína con la fuerza de prensión y la DMO

La relación entre la homocisteína y la fuerza muscular presenta resultados controvertidos o inconsistentes. Swart et al. (2013) observaron que los niveles elevados de homocisteína en plasma se asocian con una menor fuerza de agarre solo en hombres mayores y el seguimiento de tres años no reveló una asociación significativa entre los cuartiles de homocisteína y la fuerza de agarre tanto en hombres como en mujeres. En cambio, el Estudio Longitudinal de Envejecimiento de Baltimore informó una relación inversa significativa entre la homocisteína y la fuerza de prensión en mujeres, mientras que los resultados no fueron significativos en hombres (Vidoni et al., 2018). En nuestros resultados, no observamos una asociación significativa entre los niveles de homocisteína en plasma y la fuerza de agarre cuando se comparan los terciles de homocisteína y tampoco observamos asociación entre la hiperhomocisteinemia y la dinapenia (García-Alfaro et al., 2022[c]). Una posible explicación de nuestros resultados podría ser el hecho de que nuestra cohorte presentaba una hiperhomocisteinemia leve (< 30 µmol/l).

Del mismo modo, la relación entre los niveles plasmáticos de homocisteína y la DMO sigue siendo controvertida. Algunos estudios previos han reportado que los niveles elevados de homocisteina no están relacionados con una DMO baja (Cagnacci

et al., 2008; Rumbak et al., 2012; Mittal et al., 2018), mientras que otros estudios han observado una asociación significativa entre los niveles de homocisteina y la DMO (Bucciarelli et al., 2010; Ouzzif et al., 2012; Bahtiri et al., 2015). Los resultados de nuestro estudio no observó relación entre los niveles de homocisteina y la DMO (García-Alfaro et al., 2022[b]).

Cuando comparamos los informes de los estudios anteriores con nuestro estudio, se observa que existen ciertas diferencias. Primero, en el estado menopáusico de las mujeres, al incluir mujeres con menopausia temprana o mujeres perimenopáusicas. En nuestro estudio, eran mujeres potsmenopáusicas con una edad media de menopausia dentro del rango normal. Segundo, respecto a las zonas de interés óseas involucradas en las evaluaciones. Nuestro estudio analizó la columna lumbar, el cuello femoral y la cadera total, mientras que otros estudios evaluaron solo la columna lumbar o la cadera.

A partir de los resultados, incorporar la determinación de la homocisteína en la práctica clínica diaria como marcador de la fuerza muscular y la DMO, no tendría utilidad.

Limitaciones

El presente estudio tiene varias limitaciones:

- Su diseño transversal.
- El uso de solo una técnica para evaluar la función muscular.
- La falta de datos sobre el estado de folato y vitamina B12.
- El efecto longitudinal de las hormonas estudiadas sobre la fuerza muscular que no ha sido posible analizar.

Puntos fuertes

Sin embargo, nuestro trabajo también tiene ciertos puntos fuertes:

- Incluye una muestra relativamente grande de mujeres postmenopáusicas.
- Las mediciones de la fuerza muscular de agarre se realizaron con el mismo dinamómetro, siendo una sencilla forma de medida para llevar a cabo esta determinación.
- Todas las mediciones de la DMO se realizaron con el mismo densitómetro con un control de calidad mediante un estándar de referencia o fantoma de densidad conocida.
- Las determinaciones bioquímicas se midieron en el mismo laboratorio con controles de calidad establecidos y uniformes.
- En alguno de nuestros estudios, para identificar las variables de confusión utilizamos un gráfico acíclico directo y se realizó un ajuste cuidadoso para los factores de confusión en el modelo de regresión.

6. CONCLUSIONES

1. La fuerza muscular de agarre está asociada con la edad de la menopausia, la adiposidad y la baja DMO ajustada por la edad.

2. La edad y la adiposidad están asociadas de forma directa a un mayor riesgo de dinapenia.

3. La DMO en cuello femoral está asociada de forma inversa con el riesgo de dinapenia.

4. La fuerza muscular de agarre no está asociada con las hormonas endógenas estudiadas: FSH, estradiol, testosterona, Cortisol DHEAS, ∆4 androstendiona, IGF-1, Vitamina D y PTH.

5. Las mujeres con osteoporosis tienen mayor edad que las mujeres con osteopenia y DMO normal.

6. Las mujeres con osteoporosis tienen menor edad de la menopausia y menor IMC que las mujeres con osteopenia y DMO normal.

7. Los niveles de homocisteina no están relacionados con la fuerza muscular de agarre.

8. Los niveles de homocisteina no están relacionados con la DMO a nivel lumbar, cuello femoral o cadera total.

7. REFERENCIAS

A

Agostini D, Zeppa Donati S, Lucertini F, et al. Muscle and Bone Health in Postmenopausal Women: Role of Protein and Vitamin D Supplementation Combined with Exercise Training. Nutrients. 2018;10(8):1103.

Ahmad SS, Ahmad K, Lee EJ, Lee YH, Choi I. Implications of Insulin-Like Growth Factor-1 in Skeletal Muscle and Various Diseases. Cells. 2020;9(8):1773.

Akune T, Muraki S, Oka H, et al. Exercise habits during middle age are associated with lower prevalence of sarcopenia: the ROAD study. Osteoporos Int. 2014;25(3):1081-8.

Ambikairajah A, Walsh E, Tabatabaei-Jafari H, Cherbuin N. Fat mass changes during menopause: a metaanalysis. Am J Obstet Gynecol. 2019;221(5):393-409.e50.

Aspell N, Laird E, Healy M, Lawlor B, O'Sullivan M. Vitamin D Deficiency Is Associated With Impaired Muscle Strength And Physical Performance In Community-Dwelling Older Adults: Findings From The English Longitudinal Study Of Ageing. Clin Interv Aging. 2019;14:1751-1761.

Avin KG, Bloomfield SA, Gross TS, Warten SJ. Biomechanical aspects of the Muscle-Bone interaction. Curr Osteoporos Rep. 2015; 13(1):1-8.

Akdeniz N, Akpolat V, Kale A, Erdemoglu M, Kuyumcuoglu U, Celik Y. Risk factors for postmenopausal osteoporosis: anthropometric measurements, age, age at menopause and the time elapsed after menopause onset. Gynecol Endocrinol. 2009;25:125-9.

B

Badawy MT, Sobeh M, Xiao J, Farag MA. Androstenedione (a Natural Steroid and a Drug Supplement): A comprehensive review of its consumption, metabolism, health effects, and toxicity with sex differences. Molecules. 2021;26(20):6210.

Bahtiri E, Islami H, Rexhepi S, Qorraj-Bytyqi H, Thaçi K, Thaçi S, Karakulak Ç, Hoxha R. Relationship of homocysteine levels with lumbar spine and femur neck BMD in postmenopausal women. Acta Reumatol Port. 2015;40(4):355-362.

Barbat-Artigas S, Dupontgand S, Fex A, Karelis AD, Aubertin-Leheudre M. Relationship between dynapenia and cardiorespiratory functions in healthy postmenopausal women: novel clinical criteria. Menopause. 2011;18(4):400-5.

Barbat-Artigas S,Dupontgand S, Pion CH, Feiter-Murphy Y, Aubertin-Leheudre M. Identifying recreational physical activities associated with muscle quality in men and women aged 50 years and over. J Cachexia Sarcopenia Muscle. 2014;5(3):221-8.

Bayramoğlu M, Sözay S, Karata M, Kilinç S. Relationships between muscle strength and bone mineral density of three body regions in sedentary postmenopausal women. Rheumatol Int. 2005;25:513-517.

Bian AL, Hu HY, Rong YD, et al. A study on relationship between elderly sarcopenia and inflammatory factors IL-6 and TNF-α. Eur J Med Res. 2017;22(1):25.

Bijelic R, Milicevic S, Balaban J. Risk factors for osteoporosis in postmenopausal women. Med Arch. 2017;71(1):25-28.

Bochud M, Ponte B, Pruijim M, et al. Urinary sex steroid and glucocorticoid hormones are associated with muscle mass and strength in healthy adults. J Clin Endocrinol Metab. 2019;104(6):2195-2215.

Bohannon RW. Hand-grip dynamometry predicts future outcomes in aging adults. J Geriatr Phys Ther. 2008;31(1):3-10.

Bohannon RW. Grip Strength: An Indispensable Biomarker For Older Adults. Clin Interv Aging. 2019;14:1681-1691.

Borges VS, Lima-Costa MFF, de Andrade FB. A nationwide study on prevalence and factors associated with dynapenia in older adults: ELSI-Brazil. Cad. Saúde Pública. 2020;36(4):e00107319.

Brandi ML. Microarchitecture, the key to bone quality. Rheumatology (Oxford). 2009;48(Suppl 4):iv3-8.

Bruce DG, St John A, Nicklason F, Goldswain PR. Secondary hyperparathyroidism in patients from Western Australia with hip fracture: relationship to type of hip fracture, renal function, and vitamin D deficiency. J Am Geriatr Soc. 1999;47:354-59.

Bucciarelli P, Martini G, Martinelli I, Ceccarelli E, Gennari L, et al. The relationship between plasma homocysteine levels and bone mineral density in post-menopausal women. Eur J Intern Med. 2010; 21(4):301–305.

Buchman AS, Wilson RS, Boyle PA et al., Grip strength and the risk of incident Alzheimer's disease. Neuroepidemiology. 2007;29(1-2):66-73.

C

Cagnacci A, Bagni B, Zini A, Cannoletta M, Generali M, Volpe A. Relation of folates, vitamin B12 and homocysteine to vertebral bone density change in postmenopausal women. A five-year longitudinal evaluation. Bone. 2008;42:314-320.

Cappola AR, Bandeen-Roche K, Wand GS, et al. Association of IGF-I levels with muscle strength and mobility in older women. J Clin Endocrinol Metab. 2001;86:4139-4146.

Cawthon PM, Fox KM, Gandra SR, Delmonico MJ, Chiou CF, et al. Do muscle mass, muscle density, strength, and physical function similarly influence risk of hospitalization in older adults? J Am Geriatr Soc. 2009;57(8):1411-9.

Chen S, Guo X, Dong S, Li Z, Sun Y. Relationship between lifestyle factors and hyperhomocysteinemia in general Chinese population: a cross-sectional study. Postgrad Med. 2017;129(2):216-223.

Cheung AM, Chaudhry R, Kapral M, Jackevicius C, Robinson G. Perimenopausal and postmenopausal health. BMC Womens Health. 2004;4:S1- S23.

Chrysant SG, Chrysant GS. The current status of homocysteine as a risk factor for cardiovascular disease: a mini review. Expert Rev Cardiovasc Ther. 2018;16(8):559-565.

Cianferotti L, Brandi ML. Muscle-bone interactions: basic and clinical aspects. Endocrine. 2014;45(2):165-77.

Cipriani C, Romagnoli E, Carnevale V, et al. Muscle strength and bone in healthy women: effect of age and gonadal status. Hormones (Athens). 2012;11(3):325-32.

Clark BC, Manini TM. Sarcopenia =/= dynapenia. J Gerontol A Biol Sci Med Sci. 2008;63(8):829-34.

Clark BC, Manini TM. What is dynapenia?. Nutrition. 2012;28:495-503.

Clynes MA, Harvey NC, Curtis EM, Fuggle NR, Dennison EM, Cooper C. The epidemiology of Osteoporosis. Br Med Bull. 2020;133(1):105-117.

Compston J. Clinical and therapeutic aspects of osteoporosis.
Eur J Radiol. 2009;71:388-91.

Cosman F, de Beur SJ, LeBoff MS, et al. Clinician's Guide to Prevention and Treatment of Osteoporosis [published correction appears in Osteoporos Int. 2015;26(7):2045-7. Osteoporos Int. 2014;25(10):2359-2381

Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003;35:1381-95.

Cruz-Jentoft AJ, Baeyens JP, Bauer JM, et al.; European Working Group on Sarcopenia in Older People. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. Age Ageing. 2010;39(4):412-23.

Cruz-Jentoft AJ, Bahat G, Bauer J, et al; Writing Group for the European Working Group on Sarcopenia in Older People 2 (EWGSOP2), and the Extended Group for EWGSOP2. Sarcopenia: revised European consensus on definition and diagnosis. Age Ageing. 2019;48(1):16-31.

D

De Giuseppe R, Tomaselli C, Vincenti A, Di Napoli I, Negro M, Cena H. Sarcopenia and homocysteine: Is there a possible association in the elderly? A narrative review. Nutrition Research Reviews. 2021;1-14.

de Souza Macêdo PR, Rocha TN, Gomes SG, Apolinário MC, Jerez-Roig J, et al. Possible association of early menopause with worse physical function: a systematic review. Menopause. 2021;28:467-475.

Degens H, Gayan-Ramirez G, van Hees HW. Smoking-induced skeletal muscle dysfunction: from evidence to mechanisms. Am J Respir Crit Care Med. 2015;191(6):620-5.

Del Pino J. Osteoporosis: Concepto e importancia. Cuadro clínico. Rev Osteoporos Metab Miner. 2010;2(4): S15-S20.

Dhesi JK, Bearne LM, Moniz C, et al. Neuromuscular and psychomotor function in elderly subjects who fall and the relationship with vitamin D status. J Bone Miner Res. 2002;17(5):891-7.

F

Fess, EE. Grip strength. In: Casanova, JS., editor. Clinical assessment recommendations. 2nd ed. Chicago: American Society of Hand Therapists; 1992. p. 41-45.

Finigan J, Gossiel F, Glüer CC, Felsenberg D, Reid DM, Roux C, Eastell R. Endogenous estradiol and the risk of incident fracture in postmenopausal women: the OPUS study. Calcif Tissue Int. 2012;91(1):59-68.

Fratoni V, Brandi ML, B Vitamins, Homocysteine and Bone Health. Nutrients. 2015;7:2176-2192.

G

Gallagher JC, Effect of early menopause on bone mineral density and fractures. Menopause. 2007;14:567-571.

Garcia-Alfaro P, Garcia S, Rodríguez I, Tresserra F, Pérez-López FR. Factors related to muscle strength in postmenopausal women aged younger than 65 years with normal vitamin D status. Climacteric. 2019;22:390-394.

García-Alfaro P, García S, Rodríguez I, Pérez-López FR. Handgrip strength, dynapenia, and related factors in postmenopausal women. Menopause. 2022[a];29(1):16-22.

Garcia-Alfaro P, Rodríguez I, Pascual MA. Evaluation of the relationship between homocysteine levels and bone mineral density in postmenopausal women. Climacteric. 2022[b];25(2):179-185.

García-Alfaro P, Rodríguez I, Pérez-López FR. Plasma homocysteine levels and handgrip strength in postmenopausal women. Climacteric. 2022[c];25(5):504-509.

Garcia-Alfaro P, Garcia S, Rodríguez I, Bergamaschi L, Pérez-López FR. Relationship between handgrip strength and endogenous hormones in postmenopausal women. Menopause. 2023;22:390-394.

Gibson AL, Heyward VH, Mermier CM. Predictive accuracy of Omron body logic analyzer in estimating relative body fat of adults. Int J Sport Nutr Exerc Metab. 2000;10:216-227.

Gkastaris K, Goulis DG, Potoupnis M, Anastasilakis AD, Kapetanos G. Obesity, osteoporosis and bone metabolism. J Musculoskelet Neuronal Interact. 2020;20(3):372-381.

Godos J, Giampieri F, Chisari E, Micek A, Paladino N, et al. Alcohol Consumption, Bone Mineral Density, and Risk of Osteoporotic Fractures: A Dose-Response Meta-Analysis. Int J Envirom Res Public Health. 2022;19(3):1515.

González- Macías J, Martín F, Vila J, Díez Pérez A, Abizanda M, et al.; proyecto ECOSAP. Factores de riesgo de osteoporosis y de fractura osteoporótica en 5.195 mujeres de más de 65 años. Med Clin (Barc). 2004;123:85-9.

Goodpaster BH, Park SW, Harris TB, Kritchevsky SB, Nevitt M, et al. The loss of skeletal muscle strength, mass, and quality in older adults: the health, aging and body composition study. J Gerontol A Biol Sci Med Sci, 2006;61(10):1059-64.

Gopinath B, Kifley A, Liew G, et al. Handgrip strength and its association with functional independence, depressive symptoms and quality of life in older adults. Maturitas. 2017;106:92-94.

Guinot M, Manubens M. Osteoporosis postmenopáusica. Factores de riesgo y prevención de los mismos. En: Sánchez Borrego R,

Palacios S, editores. Prevención de fracturas osteoporóticas: la osteopenia como factor de riesgo. Madrid: Habe Editores. 2005;p.35-6.

H

Hadjidakis DJ, Androulakis II. Bone remodeling. Ann N Y Acad Sci. 2006;1092:385-96.

Haider S, Grabovac I, Dorner TE. Effects of physical activity interventions in frail and prefrail community-dwelling people on frailty status, muscle strength, physical performance and muscle mass-a narrative review. Wien Klin Wochenschr. 2019;131(11-12):244-254.

Hughes VA, Frontera WR, Wood M, et al. Longitudinal muscle strength changes in older adults: influence of muscle mass, physical activity, and health. J Gerontol A Biol Sci Med Sci. 2001;56(5):B209-B217.

I

Institute of Medicine. Dietary Reference Intakes for Calcium and Vitamin D. Washington, DC; The National Academie Press. 2011.

J

Johnell O, Kanis J. Epidemiology of osteoporotic fractures. Osteoporos Int. 2005;16(Suppl 2):S3-S7.

Johnell O, Kanis J. An estimate of the worldwide prevalence and disability associated with osteoporotic fractures. Osteoporos Int. 2006;17:1726-33.

K

Kamarul T, Ahmad TS, Loh WYC. Handgrip strength in the adult Malaysian population. J Orthop Surg. 2006;14(2):122-7.

Kaye AD, Jeha GM, Pham AD, et al. Folic Acid Supplementation in Patients with Elevated Homocysteine Levels. Adv Ther. 2020;37(10):4149-4164.

Kim SW, Lee HA, Cho EH. Low handgrip strength is associated with low bone mineral density and fragility fractures in postmenopausal healthy Korean women. J Korean Med Sci. 2012;27(7):744-7.

Kim BJ, Kwak MK, Lee SH, Koh JM. Lack of Association between Vitamin D and Hand Grip Strength in Asians: A Nationwide Population-Based Study. Calcif Tissue Int. 2019;104(2):152-159.

Koh, J.; Lee, Y.; Kim, Y. Homocysteine enhances bone resorption by stimulation of osteoclast formation and activity through increased intracellular ROS generation. J. Bone Miner. Res. 2006;2:1003-1011.

Kong SH, Kim JH, Lee JH, et al. Dehydroepiandrosterone sulfate and free testosterone but no estradiol are related to muscle strength and bone microarchitecture in older adults. Calcif Tissue Int. 2019;105(3):285-293.

L

Labrie F. Intracinology and menopause: the science describing the cell-specific intracelular formation of estrogens and androgens from DHEA and their strictly local action and inactivation in peripheral tissues. Menopause. 2019;26(2):220-224.

Landin-Wilhelmsen K, Lundberg PA, Lappas G, Wilhelmsen L. Insulin-like growth factor I levels in healthy adults. Horm Res. 2004;62(1):8-16.

Larsson L, Degens H, Li M, et al. Sarcopenia: Aging-Related Loss of Muscle Mass and Function. Physiol Rev. 2019;99(1):427-511.

Leblanc A, Pescatello LS, Taylor BA, Capizzi JA, Clarkson PM et al., Relationships between physical activity and muscular strength among healthy adults across the lifespan. Springerplus. 2015;4:557.

Leong DP, Teo KK, Rangarajan S, et al; Prospective Urban Rural Epidemiology (PURE) Study investigators. Prognostic value of grip strength: findings from the prospective urban rural epidemiology (PURE) study. Lancet. 2015;386:266-273.

Li YZ, Zhuang HF, Cai SQ, et al. Low grip strength is a strong risk factor of osteoporosis in postmenopausal women. Orthop Surg. 2018;10:17-22.

Lindsey C, Brownbill RA, Bohannon RA, Ilich JZ. Association of physical performance measures with bone mineral density in postmenopausal women. Arch Phys Med Rehabil. 2005;86:1102 1107.

Lopes J, Grams ST, da Silva EF, et al. Reference equations for handgrip strength: Normative values in young adult and middle-aged subjects. Clin Nutr. 2018;37:914-918.

López-Baena MT, Pérez-Roncero GR, Pérez-López FR, Mezones-Holguín E, Chedraui P. Vitamin D, menopause, and aging: quo vadis? Climacteric. 2020;23(2):123-129.

M

Ma F, Wu T, Zhao J, Ji L, Song A, Zhang M, Huang G. Plasma Homocysteine and Serum Folate and Vitamin B12 Levels in Mild Cognitive Impairment and Alzheimer's Disease: A Case-Control Study. Nutrients. 2017;9(7):725.

Maddalozzo GF, Cardinal BJ, Li F, Snow CM. The association between hormone therapy use and changes in strength and body composition in early postmenopausal women. Menopause. 2004;11:438-46.

Manini TM, Clark BC. Dynapenia and aging: an update. J Gerontol A Biol Sci Med Sci. 2012;67A(1):28-40.

Mankowski R, Anton SD, Aubertin-Leheudre M.The Role of Muscle Mass, Muscle Quality, and Body Composition in Risk for the Metabolic Syndrome and Functional Decline in Older Adults. Curr Geri Rep. 2015;4:221-228.

Marantes I, Achenbach SJ, Atkinson EJ, et al. Is vitamin D a determinant of muscle mass and strength?. J Bone Miner Res. 2011;26(12):2860-71.

Marra M, Sammarco R, De Lorenzo A, et al. Assessment of Body Composition in Health and Disease Using Bioelectrical Impedance Analysis (BIA) and Dual Energy X-Ray Absorptiometry (DXA): A Critical Overview. Contrast Media Mol Imaging. 2019:3548284.

Martin-Ruiz C, von Zglinicki T. Biomarkers of healthy ageing: expectations and validation. Proc Nutr Soc. 2014;73:422-429.

McGrath R, Johnson N, Klawitter L, et al. What are the association patterns between handgrip strength and adverse health conditions? A topical review. SAGE Open Med. 2020;8:1-12.

Mittal M, Verma R, Mishra A, Singh A, Kumar V, Sawlani KK, et al. Relation of bone mineral density with homocysteine and cathepsin K levels in postmenopausal women. Indian J Endocr Metab. 2018;22:261-6.

Mora S, Gilsanz V. Establishment of peak bone mass. Endocrinol Metab Clin North Am. 2003;32:39-63.

Morgan PT, Smeuninx B, Breen L. Exploring the Impact of Obesity on Skeletal Muscle Function in Older Age. Front Nutr. 2020;7:569904.

N

NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy. Osteoporosis prevention, diagnosis, and therapy. JAMA. 2001;285(6):785-95.

Neves T, Ferriolli E, Lopes MBM, et al. Prevalence and factors associated with sarcopenia and dynapenia in elderly people. J Frailty Sarcopenia Falls. 2018;3(4):194-202.

Newman AB, Kupelian V, Visser M, Simonsick EM, Goodpaster BH, et al. Strength, but not muscle mass, is associated with mortality in the health, aging and body composition study cohort. J Gerontol A Biol Sci Med Sci. 2006;61(1):72-7.

O

Ohlsson C , Vandenput L , Tivesten A . DHEA and mortality: what is the nature of the association?. J Steroid Biochem Mol Biol. 2015;145:248–53.

Ouzzif Z, Oumghar K, Sbai K, Mounach A, Derouiche EM, El Maghraoui A. Relation of plasma total homocysteine, folate and vitamin B12 levels to bone mineral density in Moroccan healthy postmenopausal women. Rheumatol Int. 2012;32(1):123–128.

P

Papaioannou A, Kennedy CC, Ioannidis G, et al. The impact of incident fractures on health-related quality of life: 5 years of data from the Canadian Multicentre Osteoporosis Study. Osteoporos Int. 2009;20:703-14.

Peeters GM, van Schoor NM, van Rossum EF, Visser M, Lips P. The relationship between cortisol, muscle mass and muscle strength in older persons and the role of genetic variations in the glucocorticoid receptor. Clinical Endocrinology. 2008;69:673-682.

Pérez-López FR. Dynapenia and sarcopenia, In: Cano A (Ed.), Menopause: A comprehensive approach, Springer, Berlin, 2017 Chapter 20;pp: 317-331.

Presa JC, García-Alfaro P, Martínez M, Baró F, Fasero M, et al. 2022. Osteoporosis, Menoguía AEEM. Segunda edición. ISBN: 978-84-338-6989-0.

R

Ralston SH, de Crombrugghe B. Genetic regulation of bone mass and susceptibility to osteoporosis. Genes Dev. 2006;20:2492-506.

Riancho JA, Peris P, González-Macías J, Pérez-Castrillón JL, et al. Guías de práctica clínica en las osteoporosis postmenopáusica, glucocorticoidea y del varón actualización 2022). Sociedad Española de Investigación Ósea y del Metabolismo Mineral. Rev Osteoporos Metab Miner. 2022;14 (1):13-33.

Riggs BL, Khosla S, Atkinson EJ, Dunstan CR, Melton LJ 3rd. Evidence that type I osteoporosis results from enhanced responsiveness of bone to estrogen deficiency. Osteoporos Int. 2003;14:728–733.

Rizzoli R, Stevenson JC, Bauer JM, et al; ESCEO Task Force. The role of dietary protein and vitamin D in maintaining musculoskeletal health in postmenopausal women: a consensus statement from the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO). Maturitas. 2014;79(1):122-32.

Rogers A, Saleh G, Hannon RA, Greenfield D, Eastell R. Circulating estradiol and osteoprotegerin as determinants of bone turnover and bone density in postmenopausal women. J Clin Endocrinol Metab. 2002;87:4470-4475.

Rolland YM, Perry HM 3rd, Patrick P, Banks WA, Morley JE. Loss of appendicular muscle mass and loss of muscle strength in young postmenopausal women. J Gerontol A Biol Sci Med Sci. 2007;62:330-5.

Rumbak I, Zižić V, Sokolić L, Cvijetić S, Kajfež R, Colić Barić I. Bone mineral density is not associated with homocysteine level, folate and vitamin B12 status. Arch Gynecol Obstet. 2012;285:991-1000.

S

Saito M, Marumo K. Collagen cross-links as a determinant of bone quality: a possible explanation for bone fragility in aging, osteoporosis, and diabetes mellitus. Osteoporos Int. 2010;21:195-214.

Sayer AA, Kirkwood TBL. Grip strength and mortality: a biomarker of ageing?. Lancet. 2015;386(9990):226–227.

Schaap LA, Pluijm SM, Smit JH, et al. The association of sex hormone levels with poor mobility, low muscle strength and incidence of falls among older men and women. Clin Endocrinol (Oxf).2005;63:152-60.

Seeman E. Pathogenesis of bone fragility in women and men. Lancet. 2002;359:1841-50.

Shuhart CR, Yeap SS, Anderson PA, Jankowski LG, Lewieck EM, Morse LR, et al. Executive Summary of the 2019 ISCD Position Development Conference on Monitoring Treatment, DXA Cross-calibration and Least Significant Change, Spinal Cord Injury, Peri-prosthetic and Orthopedic Bone Heath, Transgender Medicine, and Pediatrics. J Clin Densitom. 2019;22(4):453-471.

Smith AD, Refsum H. Homocysteine – from disease biomarker to disease prevention. J Intern Med. 2021;290(4):826-854.

Stenholm S, Tiainen K, Rantanen T, et al. Long-term determinants of muscle strength decline: prospective evidence from the 22-year mini-Finland follow-up survey. J Am Geriatr Soc.2012; 60:77–85.

Sullivan SD, Lehman A, Nathan NK, Thomson CA, Howard BV. Age of menopause and fracture risk in postmenopausal women randomized to calcium + vitamin D, hormone therapy, or the combination: results from the Women's Health Initiative Clinical Trials. Menopause. 2017;24(4):371-378.

Swart KM, van Schoor NM, Heymans MW,et al. Elevated homocysteine levels are associated with low muscle strength and

functional limitations in older persons. J Nutr Health Aging. 2013;17(6):578-584.

T

Taekema DG, Ling CH, Blauw GJ, et al., Circulating levels of IGF1 are associated with muscle strength in middle-aged- and oldest-old women. Eur J Endocrinol. 2011;164(2):189-96.

The IPAQ group. International physical activity questionnaire. 2016. http://www.ipaq.ki.se (accessed 10 January 2019).

Trevisan C, Alessi A, Girotti G, Zanforlini BM, Bertocc A, et al. The impact of Smoking on Bone Metabolism, Bone Mineral Density and Vertebral Fractures in Postmenopausal Women. J Clin Densitom. 2020;23(3):381-389.

Tyagi N, Kandel M, Munjal C, Vacek JC, Qipshidze N, Pushpakumar SB, Metreveli N, Tyagi SC. Homocysteine mediated decrease in bone blood flow and remodeling: role of folic acid. J Orthop Res. 2011;29:1511-1516.

V

Vacek TP, Kalani A, Voor MJ, Tyagi SC, Tyagi N. The role of homocysteine in bone remodeling. Clin Chem Lab Med. 2013;51(3):579-90.

van Geel TA, Geusens PP, Winkens B, Sels JP, Dinant GJ. Measures of bioavailable serum testosterone and estradiol and their relationships with muscle mass, muscle strength and bone mineral

density in postmenopausal women: a cross-sectional study. Eur J Endocrinol. 2009;160(4):681–687.

van Schoor NM, Swart KM, Pluijm SM, et al. Cross-sectional and longitudinal association between homocysteine, vitamin B12 and physical performance in older persons. Eur J Clin Nutr. 2012;66(2):174-181.

Veldhuis JD, Sharma A, Roelfsema F. Age-dependent and gender-dependent regulation of hypothalamic-adrenocorticotropic-adrenal axis. Endocrinol Metab Clin North Am. 2013;42: 201-25.

Velez MP, Alvarado BE, Rosendaal N, et al. Age at natural menopause and physical functioning in postmenopausal women: the Canadian Longitudinal Study on Aging. Menopause. 2019;26(9):958-965. *Erratum in*: Menopause. 2020;27(1):121.

Verde Z, Giaquinta A, Sainz CM, Ondina MD, Araque AF. Bone Mineral Metabolism Status, Quality of Life, and Muscle Strength in Older People. Nutrients. 2019;11(11):2748.

Vidoni ML, Pettee Gabriel K, Luo ST, Simonsick EM, Day RS. Relationship between Homocysteine and Muscle Strength Decline: The Baltimore Longitudinal Study of Aging. J Gerontol A Biol Sci Med Sci. 2018;73(4):546-551.

Visser M, Deeg DJH, Lips P. Low vitamin D and high parathyroid hormone levels as determinants of loss of muscle strength and

muscle mass (sarcopenia): The Longitudinal Aging Study Amsterdam. J Clin Endocrinol Metab. 2003;88(12):5766-72.

W

Warburton DER, Bredin SSD. Health benefits of physical activity: a systematic review of current systematic reviews. Curr Opin Cardiol.2017;32(5):541-556.

Wiener RC, Findley PA, Shen C, Dwibedi N, Sambamoorthi U. Relationship between smoking status and muscle strength in the United States older adults. Epidemiol Health. 2020;42:e2020055.

Wilson PW, D'Agostino RB, Parise H, et al. Metabolic syndrome as a precursor of cardiovascular disease and tipe 2 diabetes mellitus. Circulation. 2005;112(20):3066-3072.

World Health Organization. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Report of a WHO Study Group. World Health Organ Tech Rep Ser. 1994;843:1-129.

World Health Organization. Obesity: preventing and managing the global epidemic. Report of a WHO Consultation. WHO Technical Report Series 894, 2002. Geneve, Switzerland.

World Health Organization. Assessment of osteoporosis at the primare health care level: summary report of a WHO Scientific Group. World Health Organization: 2007.

Z

Zysset P, Qin L, Lang T, Khosla S, Leslie WD, Shepherd JA, Schousboe JT, Engelke K. Clinical Use of Quantitative Computed Tomography-Based Finite Element Analysis of the Hip and Spine in the Management of Osteoporosis in Adults: the 2015 ISCD Official Positions-Part II. J Clin Densitom. 2015;18(3):359-92.

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