Tesis Doctoral Internacional *[International Doctoral Thesis]*

Curso Académico 2021-2022

ACTIVIDAD FÍSICA Y ELECTROFISIOLOGÍA CARDÍACA:
A PROPÓSITO DEL ENVEJECIMIENTO Y LA LONGEVIDAD EXCEPCIONAL

PHYSICAL ACTIVITY AND CARDIAC ELECTROPHYSIOLOGY:
SPECIAL FOCUS ON AGEING AND EXCEPTIONAL LONGEVITY

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Actividad física y electrofisiología cardíaca: A propósito del envejecimiento y la longevidad excepcional

Physical activity and cardiac electrophysiology: Special focus on ageing and exceptional longevity

ADRIÁN HERNÁNDEZ VICENTE

A mi familia y amigos

A mis compañeros de GENUD y BSICoS,

especialmente a Nuria, Germán y Esther

Gracias por ser y estar

La vida y la muerte nacieron juntas.

A.M.B. (centenario)

Spiritus Athletae.

"Sport can fill the human experience with spiritual values

and help in making our lives more meaningful"

Pierre de Coubertin, Ivo Jirásek

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Fdo. Esther Pueyo Paules

Índice

Proyectos y contratos de investigación

La Tesis Doctoral que se presenta a continuación, así como los artículos que la conforman, se enmarcan dentro de diferentes proyectos financiados por las siguientes entidades:

- European Research Council (ERC-StG 638284)
- Ministerio de Ciencia e Innovación (RTI2018-097723-B-I00)
- Ministerio de Ciencia e Innovación (PID2019-105674RB-I00)
- Gobierno de Aragón (T39_20R)
- Gobierno de Aragón (LMP24-18)
- Gobierno de Aragón (LMP44-18)
- Gobierno de Aragón (LMP124-18)

Contratos de investigación

El doctorando Adrián Hernández Vicente fue contratado por la Universidad de Zaragoza gracias a las ayudas para la formación de profesorado universitario del Gobierno de España (FPU16/05879).

Estancias de investigación

A lo largo del periodo de realización de la presente Tesis Doctoral el doctorando Adrián Hernández Vicente, realizó dos estancias de investigación. Las características de las estancias de investigación se detallan a continuación:

I. Estancia de investigación internacional en la Universidad de Stanford, Departamento de medicina (Stanford, California, Estados Unidos). Duración: 3 meses (15/11/2020 - 14/02/2021). Estancia virtual por COVID19. Temática de la estancia: Ejercicio físico y enfermedades cardiovasculares.

II. Estancia de investigación internacional en el Radboud University Medical Center, Departamento de fisiología (Nijmegen, Países Bajos). Duración: 3 meses (15/09/2021 - 14/12/2021). Temática de la estancia: Epidemiología, acelerometría y fisiología cardíaca.

Esta segunda estancia fue financiada por la ayuda EST21/00258 del programa *«Ayudas complementarias para beneficiarios de ayudas (FPU): Estancias Breves y Traslados Temporales. 2021»* del Ministerio de Educación y Formación Profesional del Gobierno de España.

Listado de publicaciones

La presente Tesis Doctoral es un compendio de seis trabajos científicos, de los cuales cuatro han sido publicados, uno se encuentra en proceso de revisión por pares y el artículo restante es presentado como un borrador de resultados preliminares.

A continuación, se detallan las referencias de cada uno de los artículos que componen este documento:

Unidad Temática I. Niveles de actividad física y ejercicio físico: A propósito del envejecimiento y la longevidad excepcional.

I. **Hernández-Vicente A**, Santos-Lozano A, Mayolas-Pi C, Rodríguez-Romo G, Pareja-Galeano H, Bustamante N, Gómez-Trullén EM, Lucia A, Garatachea, N. Physical Activity and Sedentary Behavior at the End of the Human Lifespan. J Aging Phys Act. 2019 May 14;27(6):899–905. doi: 10.1123/japa.2018-0122.

II. **Hernández-Vicente A**, Marín-Puyalto J, Pueyo E, Vicente-Rodríguez G, Garatachea N. Physical activity in centenarians beyond cut-point-based accelerometer metrics. (*Under review at Medicine and Science in Sports and Exercise*).

III. Effects of 12 weeks of resistance exercise training in frail institutionalized centenarians: a randomized controlled trial. (*Draft with preliminary results*)

Unidad Temática II. Electrofisiología cardíaca: A propósito del envejecimiento y la longevidad excepcional.

I. **Hernández-Vicente A**, Hernando D, Marín-Puyalto J, Vicente-Rodríguez G, Garatachea N, Pueyo E, Bailón R. Validity of the polar h7 heart rate sensor for heart rate variability analysis during exercise in different age, body composition and fitness level groups. Sensors (Switzerland). 2021;21(3):902. doi: 10.3390/s21030902.

II. **Hernández-Vicente A**, Hernando D, Santos-Lozano A, Rodríguez-Romo G, Vicente-Rodríguez G, Pueyo E, Bailón R, Garatachea, N. Heart Rate Variability and Exceptional Longevity. Front Physiol. 2020 Sep 17;11:1164. doi: 10.3389/fphys.2020.566399.

III. **Hernández-Vicente A**, Hernando D, Vicente-Rodríguez G, Bailón R, Garatachea N, Pueyo E. ECG Ventricular Repolarization Dynamics during Exercise: Temporal Profile, Relation to Heart Rate Variability and Effects of Age and Physical Health. Int J Environ Res Public Health. 2021;18(18):9497. doi: 10.3390/ijerph18189497.

Listado de abreviaturas

ASR	*Arritmia sinusal respiratoria*
DRP	*Dinámicas de repolarización periódica*
ECG	*Electrocardiograma*
HF	*Alta frecuencia*
LF	*Baja frecuencia*
METs	*Unidad metabólica de reposo*
pNN50	*Porcentaje de intervalos NN adyacentes que distan del anterior >50 ms*
RMSSD	*Raíz cuadrada de la media de las diferencias al cuadrado entre intervalos NN sucesivos*
SDNN	*Desviación estándar de todos los intervalos NN del registro*
SDSD	*Desviación estándar de las diferencias entre intervalos NN adyacentes*
SNA	*Sistema nervioso autónomo*
SNP	*Sistema nervioso parasimpático*
SNS	*Sistema nervioso simpático*
VFC	*Variabilidad de la frecuencia cardíaca*
$\dot{V}O_{2max}$	*Consumo máximo de oxígeno*

Listado de tablas y figuras

Resumen general *[General abstract]*

A nivel mundial, entre 2019-2050, se estima que el número de personas ≥65 años se duplique, el de ≥80 años se triplique y el de ≥100 años se quintuplique. En España, debido a la llegada de las cohortes del *«baby boom»* se prevé que pasemos de 16.303 centenarios en 2019 a 241.059 en 2068. Esto supone un reto para la sociedad, puesto que el envejecimiento se asocia habitualmente con una pérdida de independencia, un gran número de enfermedades crónicas y requiere un incremento del gasto público.

La realización de actividad física y ejercicio a lo largo de la vida puede ayudar a mantener (o al menos atenuar) muchas de las propiedades afectadas por el envejecimiento y en particular en las personas inactivas. Sin embargo, la medición de niveles de actividad física en centenarios podría presentar algunas limitaciones y no existen datos descriptivos en esta población. En lo que respecta al ejercicio físico, únicamente se han reportado intervenciones en dos atletas centenarios de nivel mundial.

Por otra parte, el corazón añoso presenta una serie de alteraciones electrofisiológicas asociadas a procesos de remodelado eléctrico y estructural que ocurren progresivamente con la edad. A pesar de ello, no se conoce si las características de los sujetos podrían afectar las mediciones en reposo, ejercicio y recuperación registradas por los monitores de frecuencia cardíaca ampliamente usados por la población general. Si nos centramos en marcadores tan comunes como la variabilidad de la frecuencia cardíaca, observamos que la evidencia disponible en centenarios es escasa y contradictoria. Y al prestar atención a marcadores más novedosos como las dinámicas de repolarización periódica (DRP), todavía existen muchas incógnitas respecto a su génesis, su relación con la variabilidad de la frecuencia cardíaca, sobre los efectos que tienen las características

del sujeto tales como la edad, o cuál es su perfil temporal en un test de ejercicio incremental.

Por lo tanto, el objetivo de la presente Tesis Doctoral fue analizar las limitaciones de los métodos de medición de los niveles de actividad física y la electrofisiología cardíaca al utilizarse en población mayor, así como aportar resultados descriptivos en personas muy mayores estudiando su relación con diferentes marcadores de salud, para finalmente examinar los efectos de un programa de ejercicio físico en centenarios.

Los principales resultados son los siguientes:

1. El tiempo diario que un centenario pasa en actividad física de intensidad-baja e intensidad moderada-vigorosa varía ampliamente en función del punto de corte utilizado para el cálculo, además se encontró un potencial efecto suelo para la actividad física moderada-vigorosa. Las métricas libres de puntos de corte emergen como una alternativa para proporcionar información más completa y comparable.
2. La banda de pecho *PolarH7* y el electrocardiograma fueron intercambiables en reposo. Sin embargo existió desacuerdo entre los dispositivos al evaluar las oscilaciones de alta frecuencia durante el ejercicio de intensidad moderada-alta y la variabilidad de la frecuencia cardíaca durante la recuperación. Asimismo, la edad, composición corporal y nivel de condición física podrían ser una de las causas de desacuerdo entre dispositivos.
3. Existió un descenso asociado a la edad en el volumen y la intensidad de la actividad física, que parece acelerarse durante las últimas décadas de vida, presentando los centenarios los valores más bajos del espectro de edades en todas las variables. Tanto las medidas basadas en puntos de corte como las libres de puntos de corte estuvieron relacionadas con un estado positivo en diferentes marcadores de salud.

4. Se encontró una clara disminución con la edad en los principales índices de variabilidad de la frecuencia cardíaca que reflejan la actividad parasimpática. Además, una «desviación estándar de todos los intervalos NN del registro» (SDNN) <19ms podría estar relacionado con mortalidad temprana (≤1 año) en personas centenarias.
5. Las oscilaciones de dT ocurren principalmente en la banda de baja frecuencia (es decir DRP) y un 50-70% no están relacionada con la variabilidad de la frecuencia cardíaca. DRP mostró un aumento durante el ejercicio respecto a los valores de reposo y recuperación, observándose variabilidad inter-sujeto durante el test de ejercicio incremental. El análisis de conglomerados identificó un grupo de sujetos con sobrepeso y baja condición física con valores de DRP en reposo significativamente más altos que el resto de la muestra.
6. Es la primera vez que se presenta un ensayo controlado aleatorizado investigando los efectos de 12 semanas de entrenamiento de fuerza en centenarios. Los resultados sugieren que nadie es demasiado mayor para beneficiarse del entrenamiento de fuerza. A pesar de la fragilidad de los centenarios, no se observó ningún efecto adverso importante durante el periodo de intervención.

General abstract

Globally, between 2019-2050, it is estimated that the number of people over ≥65 years will double, the number of people over ≥80 years will triple, and the number of people over ≥100 years will quintuple. In Spain, due to the arrival of the *«baby boom»* cohorts, it is expected that the number of centenarians will increase from 16,303 in 2019 to 241,059 centenarians in 2068. This demographic change represents a challenge for society, since aging is usually associated with a loss of independence, a rise in the number of chronic diseases and requires an increase in public spending.

Physical activity and exercise throughout an individual's life can help to maintain (or at least attenuate) many of the properties affected by aging, particularly in inactive people. However, the measurement of physical activity levels in centenarians could present some limitations and there are no descriptive data in this population. Regarding physical exercise, interventions have only been reported in two world-class centenarian athletes.

On the other hand, the aged heart presents different electrophysiological alterations associated with electrical and structural remodeling processes occurring progressively with age. Despite this, it is not known whether the characteristics of the subjects could affect the measurements at rest, exercise and recovery recorded by the heart rate monitors widely used by the general population. If we focus on commonly used markers such as heart rate variability, we can observe that scientific evidence in centenarians is scarce and contradictory. When paying attention to newer markers such as periodic repolarization dynamics (PRD), there are still many questions regarding its genesis, its relationship with heart rate variability, the effects of the subject's characteristics (including age), or how are its temporal profile during an incremental test.

Therefore, the aim of the present Thesis was to analyze the limitations of the methods for measuring physical activity levels and cardiac electrophysiology when used in the elderly population, as well as to provide descriptive results in the oldest old studying its relationship with different health markers, to finally examine the effects of a physical exercise program in centenarians.

The main results found are the following:

1. The daily time spent by a centenarian in light-intensity physical activity and moderate-to-vigorous physical activity varies greatly depending on the cut-point used for the calculation, moreover a potential floor effect was found for moderate-to-vigorous physical activity. Cut-point-free metrics emerge as an alternative in order to provide more complete and comparable information across groups and populations.
2. The *PolarH7* chest band and the electrocardiogram were interchangeable at rest. However, there was disagreement between devices when evaluating high-frequency heart rate oscillations during moderate-to-high intensity exercise and heart rate variability during post-exercise recovery. In addition, age, body composition and fitness level could represent one of the causes for disagreement between devices.
3. There was an age-related reduction in physical activity volume and intensity, which seems to accelerate during the last decades of life, with the centenarians presenting the lowest values of the age spectrum in all the variables. Both cut-point-based and cut-point-free measures were related with a positive health status in different health markers.
4. A clear decrease with age was found in the main heart rate variability indices reflecting parasympathetic outflow. Additionally, a «standard deviation of all R–R intervals» (SDNN) <19ms could be associated with early mortality (≤1 year) in centenarians.

5. Oscillations in dT mostly occur in the low-frequency band (i.e. PRD) and as much as 50-70% of them are unrelated to heart rate variability. PRD showed an increase during exercise as compared to rest and post-exercise recovery values, with inter-individual variability observed during the incremental exercise test. Clustering analysis identified a group of overweight and unfit individuals with significantly higher PRD values at rest than the rest of the sample.
6. This is the first randomized control trial study investigating the effects of 12-week resistance training in centenarians. The results suggest that no one is too old to benefit from resistance training. Despite the frailty of the centenarians, no major adverse effects were noted over the intervention period.

1. Introducción

La presente Tesis Doctoral tiene como objetivo analizar las limitaciones de los métodos de medición de los niveles de actividad física y la electrofisiología cardíaca al utilizarse en población mayor, así como aportar resultados descriptivos en personas muy mayores estudiando su relación con diferentes marcadores de salud, para finalmente examinar los efectos de un programa de ejercicio físico en centenarios. Por ello, la introducción se estructurará en los siguientes tres apartados: 1) envejecimiento y longevidad excepcional, 2) niveles de actividad física y ejercicio físico y 3) electrofisiología cardíaca.

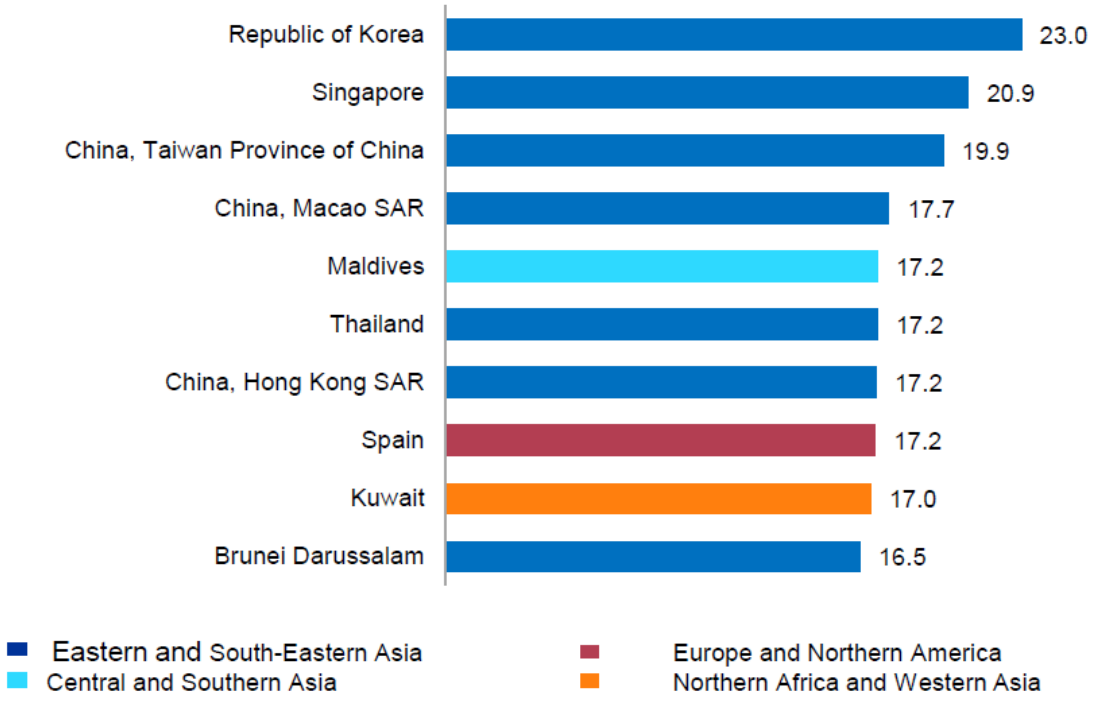
1.1 Envejecimiento y longevidad excepcional

1.1.1 Demografía del envejecimiento

Según las Naciones Unidas las personas de 65 o más años son consideradas como «personas mayores». El envejecimiento de la población es un fenómeno global que sigue avanzando; en 2019 había 703 millones de personas mayores en el mundo y se prevé que esta cifra se doblará, alcanzando los 1.500 millones en 2050 (1). Este hecho es, a priori, un éxito de la humanidad, reflejando los avances de la salud pública, la medicina y el desarrollo económico-social.

España es y será uno de los líderes mundiales en las estadísticas sobre envejecimiento. En 2019 un 19,6% de la población española tenía 65 años o más (9 millones) y según las proyecciones en 2050 supondrán un 36,8% (16 millones). Este incremento de 17,2 puntos porcentuales desde 2019 a 2050 sitúa a España como el único país europeo dentro del «Top 10 mundial» en la proyección del incremento del porcentaje de personas mayores respecto al total de la población (1), ver ***Figura 1***.

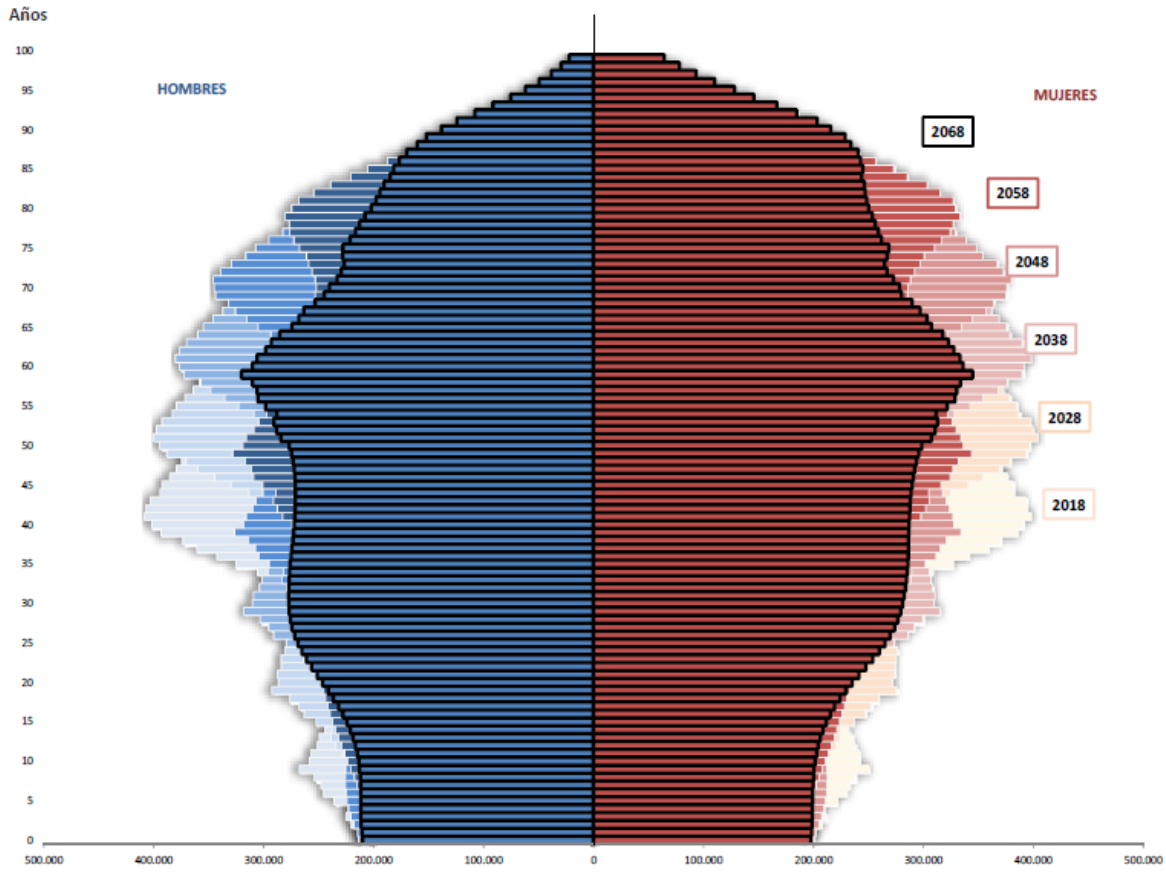
Figura 1. Países y áreas con el mayor crecimiento del porcentaje de personas de 65 o más años respecto al total de la población, 2019-2050 (puntos porcentuales).



Fuente: Figura obtenida del informe *«World Population Ageing 2019»* que utiliza datos del Departamento de Asuntos Económicos y Sociales de las Naciones Unidas, División de Población (2019). Perspectivas de la población mundial para 2019 (1). Licencia: Creative Commons (CC BY 3.0 IGO).

Para ver la causa de este cambio demográfico tenemos que retroceder hasta el denominado *«baby boom»* español que se dio entre los años 1958-1977. Durante ese periodo de tiempo nacieron más de 650.000 niños por año, lo que supone 4,5 millones más que en los 20 años siguientes y 2,5 millones más que en los 20 años anteriores (2). Por otra parte, según las proyecciones del Instituto Nacional de Estadística, entre 2019 y 2068 la población total aumentará únicamente en 1,5 millones de habitantes, con la consiguiente pirámide de población en forma de «pilar de población», ver ***Figura 2***. Consecuentemente, la llegada a la jubilación de las cohortes del «*baby boom*» a partir del año 2024 supondrá una notable presión sobre los sistemas de protección social entre 2040 y 2050 (2).

Figura 2. Proyecciones de población según edad y sexo, 2018-2068.



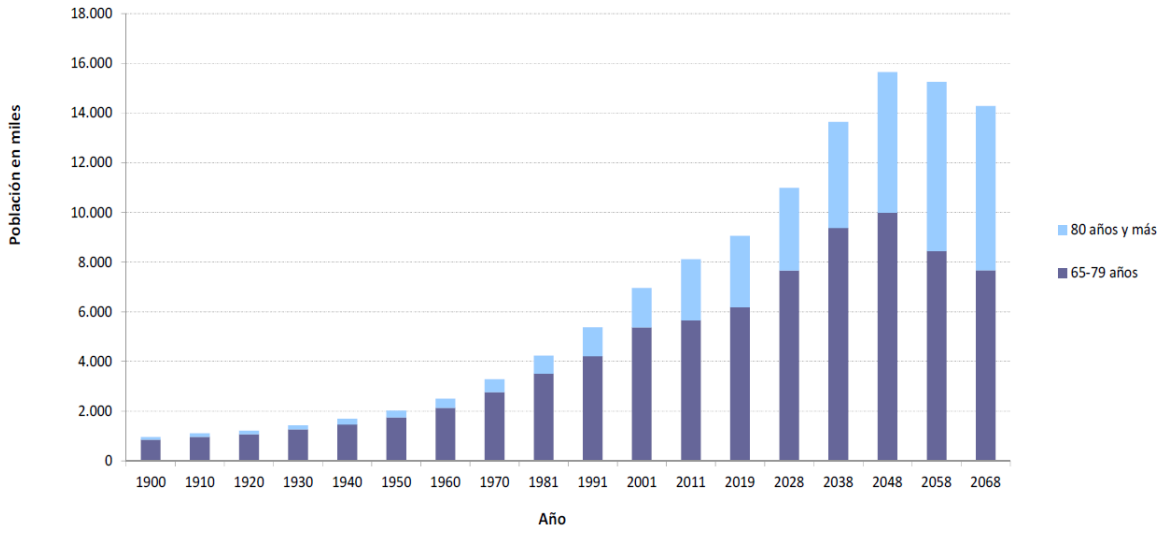
Nota: de 1900 a 2019 los datos son reales; de 2019 a 2068 se trata de proyecciones del INE. Fuente: El gráfico pertenece al informe «Envejecimiento en red nº 25, un perfil de las personas mayores de España (2020)» que utiliza datos del padron contínuo del INE y sus proyecciones de población (2). Licencia: Creative Commons (CC BY-SA).

Otro hecho destacado por los demógrafos de todo el mundo es el gran aumento de los «muy mayores» u *«oldest old»*, es decir, aquellas personas que han cumplido 80 años o más. Al principio del apartado atendíamos a que el número de personas mayores con ≥65 años va a doblarse desde 2019-2050, en el caso de los ≥80 años se estima que la población se triplicará entre 2019-2050, siendo por tanto el subgrupo de población que más rápido crece. En otras palabras, en el año 2050 habrá 1.500 millones de personas ≥65 años, de los cuales 426 millones serán ≥80 años (1). Y es que según datos de 2015-2020,

a nivel global, una persona que cumple 65 años puede esperar vivir 17 años adicionales (1).

España no es una excepción, en 2019 un 6,1% de la población española tenía 80 años o más y según las proyecciones este grupo seguirá ganando peso entre la población mayor. Si atendemos a la ***Figura 3***, podemos observar cómo en 2068 se espera que haya más de 14 millones de personas mayores, de los cuales más de 6 millones tendrán ≥80 años (2).

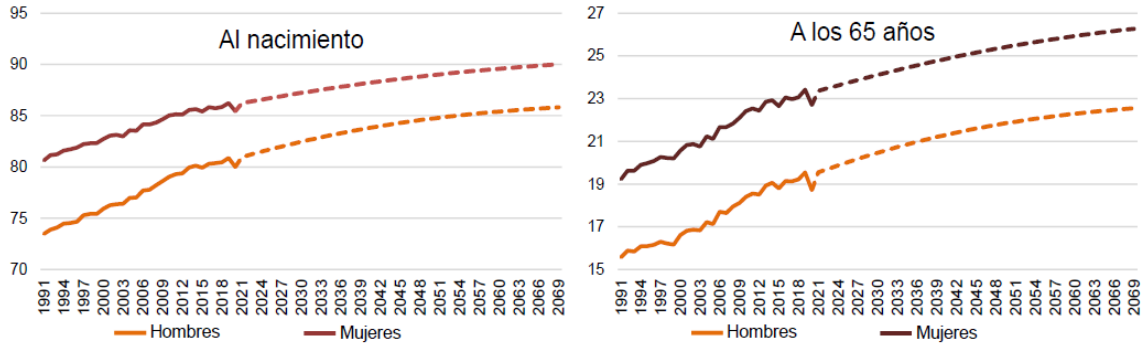
Figura 3. Evolución de la población de 65 y más años. España, 1900-2068.



Nota: de 1900 a 2019 los datos son reales; de 2019 a 2068 se trata de proyecciones del INE. Fuente: El gráfico pertenece al informe «Envejecimiento en red nº 25, un perfil de las personas mayores de España (2020)» que utiliza datos del padron contínuo del INE y sus proyecciones de población (2). Licencia: Creative Commons (CC BY-SA).

En lo que respecta a la esperanza de vida a los 65 años, según datos de 2018, España se encuentra en cabeza a nivel europeo y mundial, con medias de 19,2 años adicionales para los españoles y de 23,1 años adicionales para las españolas, ver ***Figura 4*** (2,3).

Figura 4. Esperanza de vida observada (1991-2019) y proyectada (2020-2069) en España por sexo.



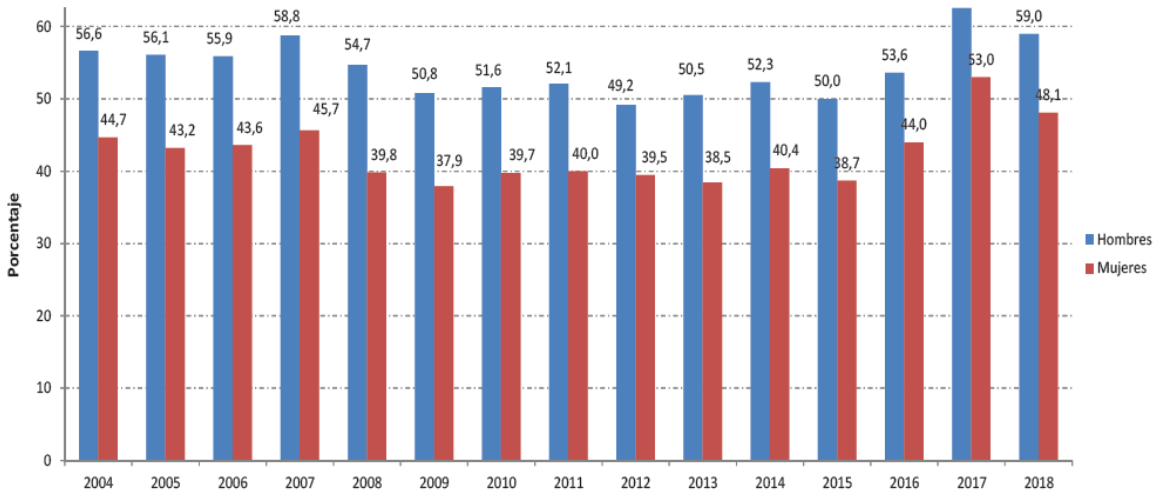
Fuente: El gráfico pertenece al informe «Proyecciones de Población 2020-2070» que utiliza las tablas de mortalidad del INE para los años 1991-2019 (3). Licencia: Creative Commons (CC BY-SA).

Este importante envejecimiento de la población, que se asocia habitualmente con un gran número de enfermedades crónicas, requiere un incremento de gasto para los recursos públicos y constituye una gran carga para los cuidados informales (4). Ante este reto de la sociedad, Naciones Unidas ha declarado la «Década del Envejecimiento Saludable (2020-2030)», con el objetivo de impulsar la acción internacional para mejorar, tanto la vida de las personas mayores, como la de sus familias y comunidades.

1.1.2 Envejecimiento y función

Aunque la esperanza de vida total sea cada vez más alta, no se corresponde con la «esperanza de vida saludable», ya que la morbilidad está presente durante el último periodo de nuestra vida. La esperanza de vida saludable suele construirse a partir de datos sobre morbilidad crónica y salud auto-percibida y se define como el número de años que se puede esperar que una persona viva con completa salud. Por ejemplo, cuando observamos el caso de España, a pesar del aumento en la esperanza de vida (***Figura 4***), la esperanza de vida saludable se ha mantenido más o menos constante durante los últimos años (***Figura 5***). También cabe destacar, que aunque los hombres viven menos años, el porcentaje de vida que viven en buenas condiciones de salud es mayor en relación al de las mujeres, ver ***Figura 5*** (2).

Figura 5. Esperanza de vida saludable a los 65 años respecto del total de esperanza de vida (%), por sexo. España 2004-2018.



Fuente: El gráfico pertenece al informe «Envejecimiento en red nº 25» que utiliza datos de Eurostat (2).

Dado que añadir años a la vida, no implica añadir vida a los años, en 2015 la Organización Mundial de la Salud redefinió el «envejecimiento saludable» entorno a la capacidad funcional, entendiendo esta como la combinación de la capacidad intrínseca

(compuesta por las capacidades físicas, mentales y psicosociales), el entorno del individuo y las interacciones entre individuo-entorno (5).

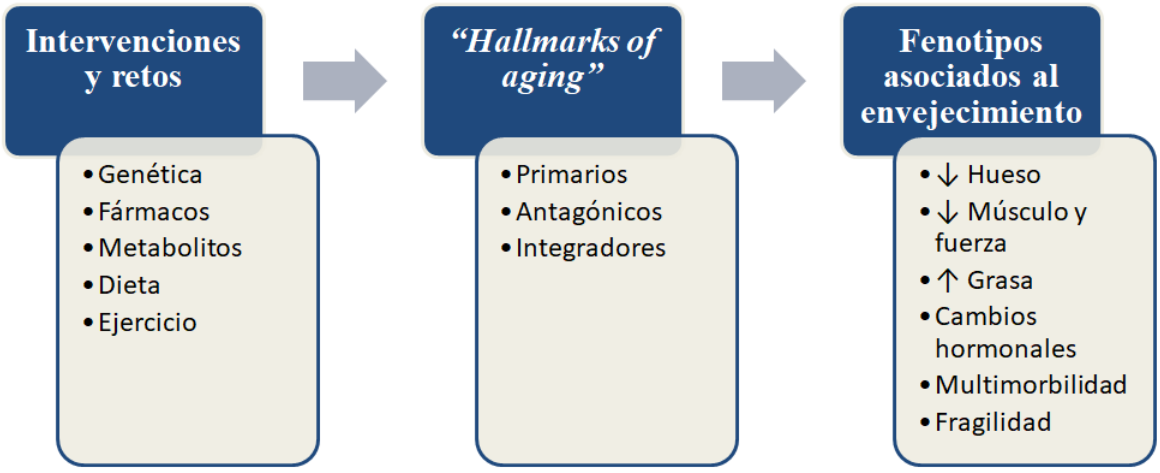
El envejecimiento es un proceso inexorable, caracterizado por una pérdida progresiva de la integridad fisiológica que conduce a un deterioro en la función de todos los sistemas y finalmente a la muerte. Este deterioro asociado al envejecimiento es el principal factor etiológico de la patología en el ser humano, y está asociado con una serie de mecanismos celulares y moleculares (6), ver ***Tabla 1***.

Tabla 1. Los marcadores del envejecimiento *«Hallmarks of aging»* (6).

Causas primarias del daño celular	**Respuestas compensatorias antagónicas al daño**	**Biomarcadores integradores**
Inestabilidad genómica	Desregulación de los sistemas de detección de nutrientes	Agotamiento de células madre
Desgaste telomérico		Alteración de la comunicación intercelular
Alteraciones epigenéticas	Disfunción mitocondrial	
Pérdida de proteostasis	Senescencia celular	

Sin embargo, la longevidad es plástica, puesto que se puede modular mediante diferentes intervenciones, tales como la manipulación genética, la farmacología, la nutrición o el ejercicio físico. Las intervenciones que tienen por objetivo prevenir o mejorar los signos del envejecimiento lo harían a través de los diferentes «*Hallmarks of aging*», ver ***Figura 6***; de este modo se puede explicar que la velocidad de envejecimiento difiera entre organismos y en diferentes células, tejidos, órganos y sistemas de un mismo organismo (7).

Figura 6. Mecanismos del envejecimiento.



Fuente: Figura del autor a partir del artículo *«Facing up to the global challenges of ageing»* publicado en 2018 en la revista *«Nature»* (7).

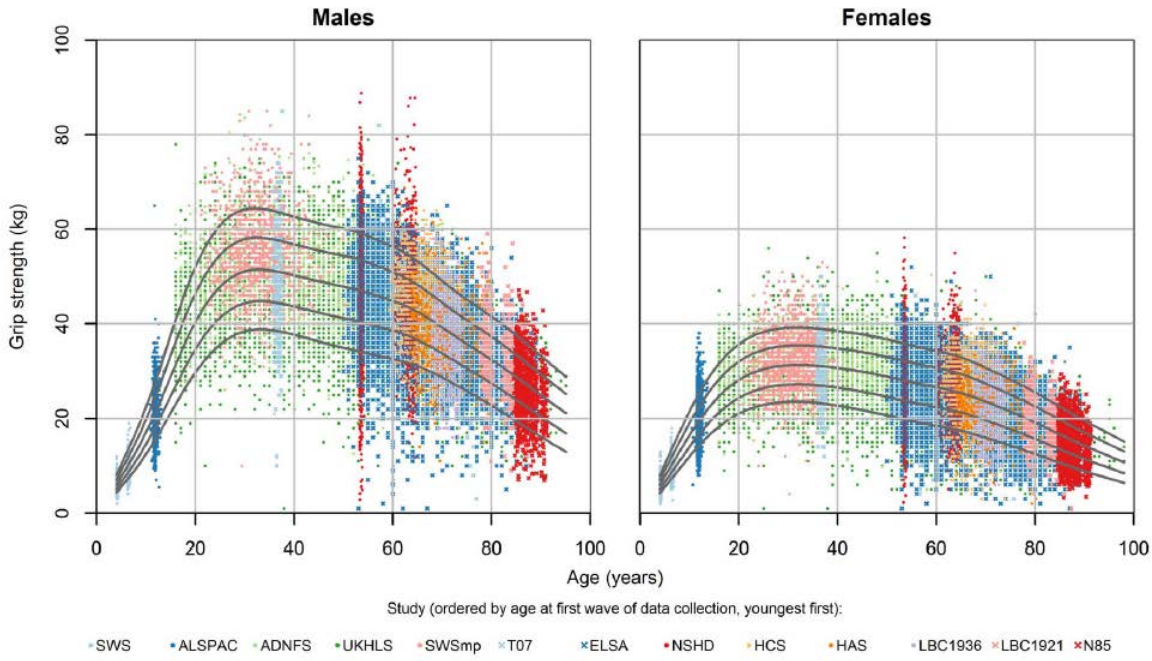
En lo que respecta a las intervenciones con ejercicio físico, el artículo publicado por Garatachea et al. en 2015 supone un punto de referencia al enlazar los *«hallmarks of aging»* publicados previamente por López-Otín con los mecanismos moleculares que median los beneficios del ejercicio físico (8). Tal es el papel del ejercicio físico en el envejecimiento que algunos autores afirman que ser físicamente activo es la condición biológica humana por defecto; y por tanto el proceso de envejecimiento humano solamente puede ser estudiado en las personas físicamente activas (9).

En la actualidad, el ejercicio físico regular es una de las principales herramientas anti-envejecimiento a nivel multisistémico, este hecho queda patente tanto en el plano cuantitativo como en el cualitativo. Si atendemos a la cantidad de vida, los atletas de élite viven más que la población general, es decir aquellos humanos que han mantenido altas dosis de ejercicio suelen ser más longevos (10). Además, incluso ligeros incrementos en los niveles de actividad física a los 80 años de edad (pasar de realizar 0-0.5h/sem a 0.5-1.9h/sem) han sido asociados con incrementos de >2 años en la esperanza de vida total, lo que apunta a que nunca es tarde para beneficiarse del ejercicio físico (11).

En el plano cualitativo, vimos anteriormente que el «envejecimiento saludable» se define entorno a la funcionalidad, siendo un reto de la sociedad mantener la independencia de los mayores (5). La condición física es imprescindible para una óptima capacidad intrínseca, por lo tanto la independencia funcional es directamente dependiente de la condición física. La condición física se define como la capacidad de llevar a cabo las actividades de la vida diaria con vigor y diligencia, sin cansancio indebido y con energía suficiente para disfrutar de las actividades del tiempo libre y para afrontar las emergencias imprevistas que se presenten. Asimismo, la condición física está determinada por varios fenotipos medibles relacionados con la salud, que incluyen principalmente la aptitud cardiorrespiratoria y la función muscular (12).

El consumo de oxígeno máximo ($\dot{V}O_{2max}$) representa la tasa máxima de oxígeno que un organismo puede utilizar para producir energía. El $\dot{V}O_{2max}$ es el producto de multiplicar el gasto cardíaco por la diferencia arteriovenosa de oxígeno, suele expresarse en mililitros de oxígeno consumidos por kilogramo de peso corporal por minuto (mL kg^{-1} min^{-1}), y es aceptado como la medida de referencia de la aptitud cardiorrespiratoria. Existe un claro descenso asociado a la edad en el $\dot{V}O_{2max}$, al que contribuyen la reducción del gasto cardíaco, el descenso en la diferencia arteriovenosa de oxígeno y diversos cambios a nivel del músculo esquelético. Tradicionalmente se describen descensos del $\dot{V}O_{2max}$ entorno a1 10% por década entre los 20-70 años y al 20% por década a partir de los 70-80 años (13), sin embargo un descenso lineal parece posible al observar los records de $\dot{V}O_{2max}$ para cada franja de edad, es decir en individuos con un envejecimiento activo (14). Adicionalmente, para cualquier edad dada, los valores de $\dot{V}O_{2max}$ son mayores en sujetos entrenados que en sus pares inactivos, en otras palabras, aunque el proceso de envejecimiento es complejo y multifactorial, un estilo de vida activo puede atenuarlo (9).

Figura 7. Curvas de percentiles de cohortes cruzadas para fuerza de prensión manual.



Fuente: Figura obtenida del artículo *«Grip Strength across the Life Course: Normative Data from Twelve British Studies»* publicado en 2014 en la revista *«Plos One»* (15). Licencia: Creative Commons (CC-BY 4.0).

El término «sarcopenia» ha evolucionado a lo largo de los años, en la actualidad el *«European Working Group on Sarcopenia in Older People "EWGSOP2"»* define la sarcopenia como una insuficiencia muscular caracterizada por bajos niveles de fuerza y masa muscular. El factor primario de la sarcopenia es el envejecimiento, que interacciona con otros factores secundarios como la enfermedad, la inactividad y la malnutrición; dada su variada etiología, la sarcopenia es común en adultos mayores, pero también puede presentarse en adultos jóvenes (16). También es multifactorial su fisiopatología, entre los principales mecanismos encontramos: desequilibrio en el metabolismo proteico, pérdida de motoneuronas, cambios hormonales, disfunción de las células satélite, inflamación, estrés oxidativo o disfunción mitocondrial entre otros (17). En la ***Figura 7***, podemos observar cómo el pico máximo de fuerza se alcanza al comienzo de la edad adulta, después se estabiliza en una meseta descendente, para descender de forma más acelerada a partir de los 60 años; por ello la sarcopenia debe tratarse a lo largo de toda la vida, ya

que no depende únicamente de la tasa a la que desciende la función muscular durante la senectud, sino también de la dimensión alcanzada por el pico máximo de fuerza en la juventud (15,17).

La realización de ejercicio físico a lo largo de la vida puede ayudar a mantener (o al menos atenuar) muchas de las propiedades (especialmente, masa muscular, fuerza, capacidad funcional, aptitud cardiorrespiratoria) afectadas por el envejecimiento y en particular por el envejecimiento de las personas inactivas (18). Ulteriormente, en el apartado «*1.2.2 Ejercicio físico*» revisaremos los beneficios del ejercicio físico en la vejez.

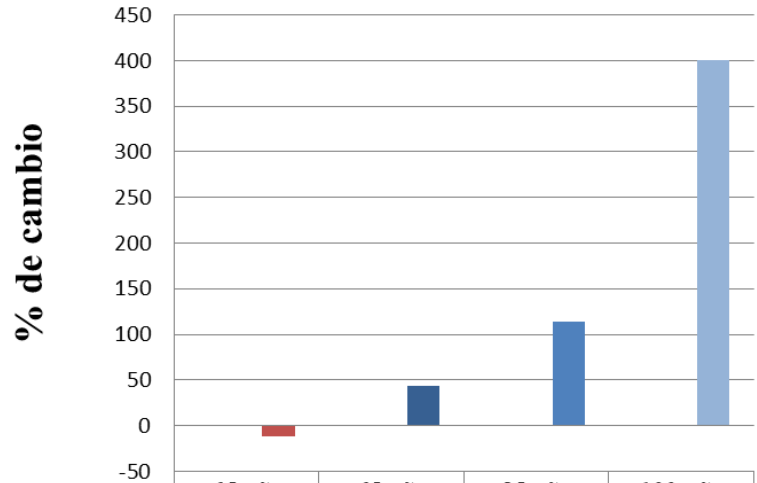
1.1.3 Longevidad excepcional

El ser humano, consciente de la fugacidad de la vida, ha buscado ser perenne a través de piedras filosofales, fuentes y elixires de la juventud. Sin embargo, la vida y la muerte nacieron juntas. Si bien es cierto, algunas personas consiguen esquivar el «*exitus letalis*» hasta 40-50 años más que la media poblacional. Es común que los medios de comunicación se hagan eco de estos cumpleaños, por ejemplo el pasado 2 de enero de 2022 la japonesa Kane Tanaka, persona más longeva con vida, cumplió 119 años. La edad máxima reportada ha sido la de Jeanne Louise Calment que falleció a los 122 años y 164 días en Francia, por su parte, el varón más longevo ha sido Jiroemon Kimura, quien falleció a los 116 años y 54 días en Japón. Las mujeres no se limitan a ostentar los records absolutos de longevidad, sino que también son mayoría numérica, 8 de cada 10 centenarios son mujeres (19).

A pesar de que estos cumpleaños parecen hechos anecdóticos, son la punta de lanza de una realidad subyacente. Del mismo modo que la media de la esperanza de vida ha ido aumentando en los últimos años, los percentiles altos también alcanzan edades mayores, y los centenarios ya no son una excepción. Según las Naciones Unidas, en 2021 había 621.000 centenarios vivos alrededor del mundo (7,9/100.000 habitantes), y las proyecciones estiman 3,2 millones de centenarios en 2050 (32,8/100.000 habitantes) (20).

En el apartado «*1.1.1 Demografía del envejecimiento*» veíamos como a nivel mundial de 2019 a 2050 se doblará el número de personas ≥65 años y se triplicará el número de personas ≥80 años. En zonas desarrolladas y ya envejecidas, como la Europa de los 27, se espera que entre 2019-2050 el número de ≥85 años se doble mientras que el número de ≥100 años se quintuplique, pasando de 96.600 individuos con un siglo de edad en 2019 a 484.000 en 2050 (21). Este crecimiento hasta cerca del medio millón de habitantes hace que los centenarios sean el subgrupo de población que más crece, dentro del subgrupo de población que más crece, ver ***Figura 8***.

Figura 8. Porcentaje de cambio en la población europea (EU-27) por edad: 2019-2050.



	<65 años	≥65 años	≥85 años	≥100 años
2019 (Millones de personas)	356.3	90.5	12.5	0.10
2050 (Millones de personas)	312.1	129.8	26.8	0.48
% de cambio	-12	43	114	401

Fuente: Figura del autor a partir del informe «*Ageing Europe: Looking at the lives of older people in the EU*» publicado en 2020 por la Comisión Europea (21).

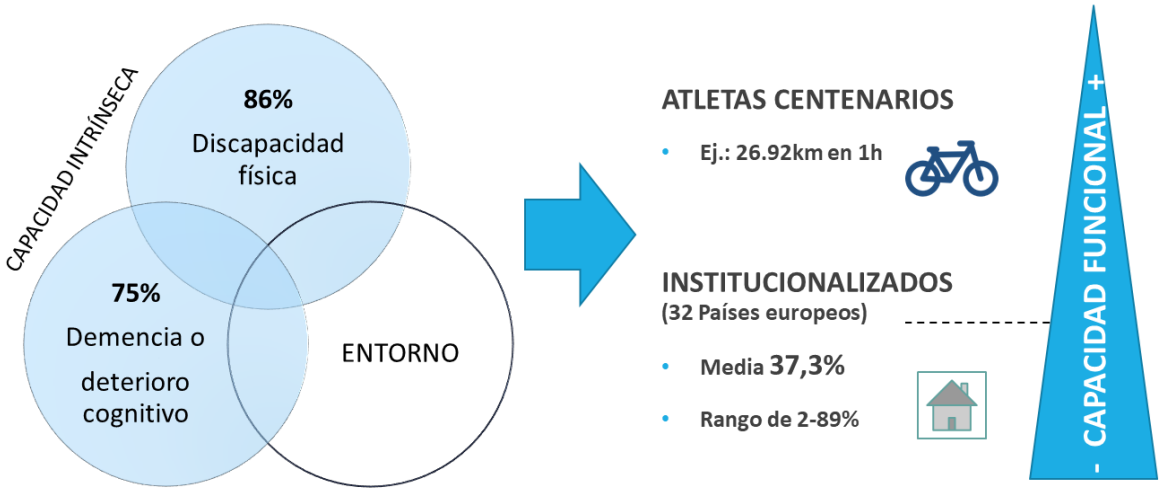
España tiene un gran porcentaje de centenarios respecto de la población total, incluso si la comparamos con Europa. De acuerdo a los datos de 2019 en España había 34,7 centenarios cada 100.000 habitantes (16.303 centenarios en 47.026.208 habitantes), mientras que en EU-27 había 21,6 centenarios cada 100.000 habitantes (96.600 en 446.800.000 habitantes) (2,21). Según las proyecciones de población 2018-2068 del Instituto Nacional de Estadística, la cifra de centenarios también va a crecer rápidamente en los próximos años y en el año 2068 habrá 241.059 españoles centenarios (48.208 hombres y 192.851 mujeres), debido a la llegada de las cohortes del «*baby boom*», lo que supondrán 496,7 centenarios cada 100.000 habitantes (0,5% de la población total estimada para 2068: 48.531.614) (2).

El rápido crecimiento de la población de centenarios en las últimas décadas no es lo único que ha hecho atraer el foco de atención. Y es que además de ser el paradigma de

la longevidad humana también lo son en cuanto a envejecimiento saludable ya que han pospuesto, si no evitado, la mayor parte de enfermedades asociadas a la edad como el cáncer, las enfermedades cardiovasculares o neurodegenerativas (22,23). De hecho, un estudio con 1418 centenarios mostró una mayor compresión de la morbilidad en los centenarios que vivieron más de 100, 105 y 110 años respectivamente (24). Por lo tanto, el estudio de los centenarios constituye una oportunidad fascinante para entender las características que permiten a los individuos alcanzar vidas excepcionalmente largas y sanas.

En lo que respecta a la capacidad intrínseca, según valores normativos del *«Georgia Centenarian Study»*, un 73% de los centenarios y un 86% de las centenarias presentan discapacidad física de acuerdo al test *«Short Physical Performance Battery»* (25), y resultados de estudios transversales indican que aproximadamente un 50% muestran claros síntomas de demencia y 25% deterioro cognitivo, pero estos porcentajes varían a través de los estudios debido a los sesgos de reclutamiento, tamaños muestrales pequeños, distintas cohortes de nacimiento, diferentes instrumentos de medición y dificultades de evaluación intrínsecas a las características de los centenarios (26,27). Consecuentemente los centenarios presentan una amplia variabilidad en su capacidad funcional, algunos de ellos están encamados, mientras que otros son independientes en las tareas de la vida diaria, y unos pocos incluso realizan gestas deportivas, como el francés Robert Marchand, capaz de recorrer en bicicleta 26,9km en una hora (28,29). Todo ello, junto a factores culturales, soporte institucional y recursos familiares resulta en un amplio rango de centenarios institucionalizados en los centros geriátricos de los países europeos (rango: 2-89%, media 37,3%), ver ***Figura 9*** (19).

Figura 9. Diversidad de la capacidad funcional de los centenarios.



1.2 Niveles de actividad física y ejercicio físico

1.2.1 Niveles de actividad física

Ya en el siglo primero de nuestra era, el filósofo Marcus Tullius Cicero afirmaba que «El ejercicio y la templanza pueden preservar algo de nuestra fuerza inicial incluso en la vejez». A día de hoy las sociedades desarrolladas han alcanzado un estilo de vida tan acomodado que la inactividad física causa en el mundo entre 4 y 5 millones de muertes al año (30,31). Diversas investigaciones han mostrado que si eliminámaramos la inactividad física, ya catalogada como pandemia (32), se reduciría: la mortalidad general, el cáncer de colon, cáncer de mama, diabetes tipo II y otras muchas patologías (hasta 26 enfermedades) (33); esto se traduciría en un aumento de la esperanza de vida de 0,68 años a nivel mundial y 0,78 años en el caso de España (30,34) y también en una mejor calidad de vida durante esos años adicionales (35,36). Dada su eficacia en la prevención y manejo de enfermedades, la actividad física ha llegado a denominarse como «la verdadera poli-píldora» (37), que además muestra un efecto dosis-respuesta, es decir mayores niveles de actividad física están asociados con incrementos en los beneficios (38). Sin embargo, el 66,8% de los españoles no cumple con las recomendaciones de actividad física de la Organización Mundial de la Salud (39).

Para conocer el papel de la actividad física en el envejecimiento extremo sería necesario cuantificar objetivamente los niveles de actividad física a lo largo del ciclo vital, en estudios con centenarios esto supondría un estudio longitudinal de un siglo de duración. Otra alternativa sería estimar la actividad física mediante un cuestionario retrospectivo administrado en forma de entrevista que recopile toda la actividad realizada desde la infancia (40), a continuación, puesto que el cuestionario registra tipo, frecuencia y duración de cada actividad, se pueden calcular el gasto energético en MET-h/semana, multiplicando el número de horas/semana en cada actividad por su valor en equivalentes

metabólicos (METs) en base al Compendio de Actividades Físicas (41). Sin embargo, en esta segunda opción, a las limitaciones características de los cuestionarios, se le añade que los centenarios tienen problemas de memoria y deben recordar un periodo largo, 100 años. Debido a la complejidad de ambas alternativas, a día de hoy no existen evidencias sólidas al respecto. La longevidad excepcional es un fenotipo parcialmente heredable, si bien las principales teorías del envejecimiento coinciden en un origen multifactorial, en el que los factores no genéticos (dieta, actividad física, hábitos saludables y factores psicosociales) contribuirían aproximadamente a un 50% de la variabilidad en el esperanza de vida humana (42). Dado que la actividad física es un factor conductual que revierte los principales marcadores del envejecimiento (8), cabría esperar que los centenarios hayan tenido una vida activa como indican algunos estudios que han encontrado un gran número de centenarios en zonas dedicadas a la ganadería, pesca y agricultura (cuando el sector primario todavía no estaba mecanizado) (43). El 75% de los centenarios españoles aseguran haber realizado ejercicio regular a lo largo de la vida (44), del mismo modo centenarios de otras zonas del globo afirman haber seguido estilos de vida saludables: actividad física regular, buenos hábitos dietéticos, no beber y no fumar (45–47), pero la causalidad entre los niveles de actividad física y la longevidad extrema continúa siendo una hipótesis por demostrar.

Los métodos de medición de la actividad física se pueden clasificar en métodos subjetivos y métodos objetivos (48). Los métodos subjetivos (cuestionarios, diarios de actividad y entrevistas) son económicos y fácilmente aplicables, pero su validez es cuestionable debido a la influencia de la interpretación y la memoria del sujeto (49). En cambio, los métodos objetivos proporcionan información de la actividad física del sujeto a través de la cuantificación del movimiento realizado por un dispositivo electrónico, como pueden ser los podómetros o los acelerómetros. Los podómetros se caracterizan por su simplicidad, confort y bajo coste, pero su utilidad es limitada ya que solamente

registran el número de pasos durante actividades ambulatorias (50). Los acelerómetros son sensores de movimiento que cuantifican la dirección y la magnitud de la aceleración corporal mediante un sensor, normalmente piezo-eléctrico, y microprocesadores (51). Su uso en humanos fue propuesto por primera vez en los años 50 (52), y debido a su objetividad, peso y portabilidad se han convertido en una técnica muy popular en la investigación en actividad física, por lo que en la actualidad puede encontrarse una gran variedad de dispositivos. Sin embargo, los acelerómetros no están libres de limitaciones pues no identifican el movimiento de todos los segmentos corporales, no detectan la actividad estática, la pendiente del terreno o si el sujeto trabaja contra una resistencia. Aunque, por otro lado, tienen una gran validez ecológica ya que miden al sujeto durante su vida cotidiana, y han mostrado ser un método de confianza para la medición de los patrones y niveles de actividad física en las diferentes franjas de edad (53,54).

Los acelerómetros registran de forma continua las aceleraciones corporales durante largos periodos de tiempo. El análisis de la frecuencia, intensidad y duración de estas aceleraciones durante las actividades de la vida diaria permite a los investigadores identificar la proporción del día que el sujeto pasa en tiempo sedentario o en actividad física de diferentes niveles de intensidad (Por ejemplo: actividad física ligera, actividad física moderada-vigorosa, o actividad física vigorosa). Sin embargo, los puntos de corte que determinan el paso entre niveles de actividad física están basados en umbrales de METs (ratio entre la energía gastada durante una actividad y la tasa metabólica en reposo), consecuentemente la aceleración que se establece como punto de corte es específica para cada población y para cada protocolo (55,56). Estos escalones de intensidad son ficticios y dificultan las comparaciones entre estudios, por ello se están explorando nuevos enfoques analíticos que no dependan de los puntos de corte, tales como: la aceleración media, el gradiente de intensidad o los «*Mx metrics*» (57). La aceleración media se usa como aproximación del volumen de actividad física y se define

como la media aritmética de la aceleración registrada durante el periodo de medición (58). El gradiente de intensidad refleja la distribución de la intensidad en el perfil de actividad física y se calcula como la pendiente de la regresión lineal entre los logaritmos naturales del tiempo y la intensidad de la aceleración, por lo que la pendiente siempre es negativa (58). Los *«Mx metrics»* evalúan la aceleración por encima de la cual una persona pasa los X minutos no consecutivos más activos del día (por ejemplo M30 se refiere a la intensidad por encima de la cual se han pasado los 30 minutos, no consecutivos, más activos del día), y entre otras utilidades, se pueden usar para ver si una persona cumple con las recomendaciones de actividad física (59).

Es bien sabido que los niveles de actividad física presentan un descenso asociado a la edad, siendo las personas mayores el segmento de la población más inactivo (60). También ha sido descrito que los mayores pasan 8,5-9,6 horas del tiempo que están despiertos en comportamiento sedentario (normalmente definido como el tiempo en actividades a ≤1,5 METs) (61,62). Por añadidura, los «*oldest-old*» parecen ser incluso más sedentarios que sus pares de 65-79 años (63). Hasta donde alcanza nuestro conocimiento no hay datos de acelerometría disponibles en centenarios, previamente a la realización de esta Tesis Doctoral los sujetos más longevos que han sido medidos mediante acelerometría tenían entre 70 y 90 años de edad (64–67). Sí sabemos que algunos centenarios evitan la característica caída de los niveles de actividad física con la edad, y no solo eso, sino que también continúan participando en competiciones deportivas como la maratón o 1500m de natación (68). Estos ejemplos inspiradores parecen indicar que nunca es tarde para realizar ejercicio físico y que el descenso en los niveles de actividad física es parcialmente opcional. Los beneficios de ser más activo se han descrito incluso en personas ≥90 años, donde se observó que los nonagenarios más activos tenían una mejor funcionalidad (distancia recorrida en el test de 6 minutos andando) (64).

1.2.2 Ejercicio físico

En las secciones anteriores hemos visto cómo la actividad física y el ejercicio son una estrategia fundamental en la mejora de la salud, al igual que en la prevención y el manejo de enfermedades (69). Por ende, la actividad física y el ejercicio son herramientas básicas para un envejecimiento saludable y deberían ser una prioridad para la salud pública. El ejercicio físico, entendido como actividad física estructurada y planificada con un fin, es la manera más efectiva de obtener dichos beneficios (le prestaremos atención más adelante). No obstante, a raíz de las contundentes evidencias, las principales organizaciones sanitarias establecen guías con las recomendaciones de actividad física para la salud.

Si echamos un breve vistazo a las Directrices publicadas en 2020 por la Organización Mundial de la Salud sobre actividad física y hábitos sedentarios encontramos un apartado dedicado a las personas de ≥65 años. Según dicha guía, las personas mayores deben acumular a lo largo de la semana un mínimo de 150-300 minutos de actividad física aeróbica de intensidad moderada o bien 75-150 minutos de actividad física aeróbica de intensidad vigorosa, o una combinación equivalente de ambas; además también deben realizar actividades de fortalecimiento muscular al menos dos días por semana e intentar limitar el tiempo dedicado a actividades sedentarias. Estas tres recomendaciones son comunes respecto a la franja de edad de 18-64 años, si bien detallan que los ≥65 años deben ser tan activos como les permita su capacidad funcional y ajustar el esfuerzo a su forma física. Una recomendación específica para el grupo de personas mayores es que realicen al menos 3 días por semana actividades físicas multicomponente variadas que den prioridad al equilibrio funcional y al entrenamiento de fuerza para mejorar su capacidad funcional y evitar caídas. Por último, un importante mensaje de esta guía es que cada movimiento cuenta, cualquier cantidad de actividad física es mejor que ninguna, y cuanta más, mejor (35).

Ningún fármaco puede revertir o frenar el deterioro de la capacidad intrínseca asociado a la edad; pero evidencias científicas recientes soportan los efectos beneficiosos del ejercicio físico, incluso en personas mayores frágiles, institucionalizadas u hospitalizadas (18). El entrenamiento individual basado en la evidencia es el más efectivo, pero requiere una sistematización: I) en primer lugar es necesaria una evaluación que permita diagnosticar la situación inicial, monitorizar los progresos y tomar decisiones; II) cuando conocemos las fortalezas y debilidades características del sujeto hay que establecer los objetivos, para ello se recomienda que sigan el acrónimo del inglés *«SMART»*: específicos, medibles, alcanzables, realistas y oportunos; III) a continuación debemos prescribir el programa de ejercicio (se aconseja describirlo siguiendo el *«Consensus on Exercise Reporting Template -CERT-»)* de acuerdo a los objetivos, características y preferencias individuales (70); IV) por último se vuelve a evaluar, y el proceso comienza de nuevo. Vistos los beneficios multisistémicos del ejercicio en el envejecimiento, quizás, en lugar de perseguir dianas farmacológicas anti-envejecimiento, se deberían de realizar mayores esfuerzos de investigación en busca de la prescripción de ejercicio más óptima, en este caso en función de las características de la persona mayor.

Para lograr un envejecimiento saludable, el ejercicio físico tiene que preservar la función fisiológica de los diferentes sistemas. El tipo y magnitud de los beneficios dependerá del tipo de ejercicio que se lleve a cabo, ver ***Figura 10***. El entrenamiento de fuerza parece ser el tipo de intervención más efectiva en personas mayores (71), y de forma oportuna la *«National Strength and Conditioning Association»* reunió recientemente a un grupo de expertos mundiales para que realizaran un posicionamiento sobre las especificidades del entrenamiento de fuerza en esta población (72). En particular el entrenamiento de fuerza ha mostrado una miríada de beneficios a nivel de: masa muscular, masa ósea, independencia, riesgo de caídas, manejo de enfermedades crónicas, bienestar y calidad de vida (72). Cuando se diseña correctamente y las instrucciones

técnicas son apropiadas el entrenamiento de fuerza es seguro (72), incluso se han descrito intervenciones en población nonagenaria (73–75), pero nunca en individuos que han alcanzado los límites de la esperanza de vida humana.

Figura 10. Visión general de los tipos de ejercicio físico y sus beneficios.

Fuente: Figura del autor a partir de los artículos: «*Exercise in the oldest old*» publicado en 2019 en la revista «*Comprehensive Physiology*» (18) y «*International Exercise Recommendations in Older Adults*» publicado en 2021 en la revista «The journal of nutrition, health & aging» (69). $\dot{V}O_{2pico}$ = Consumo de oxígeno pico.

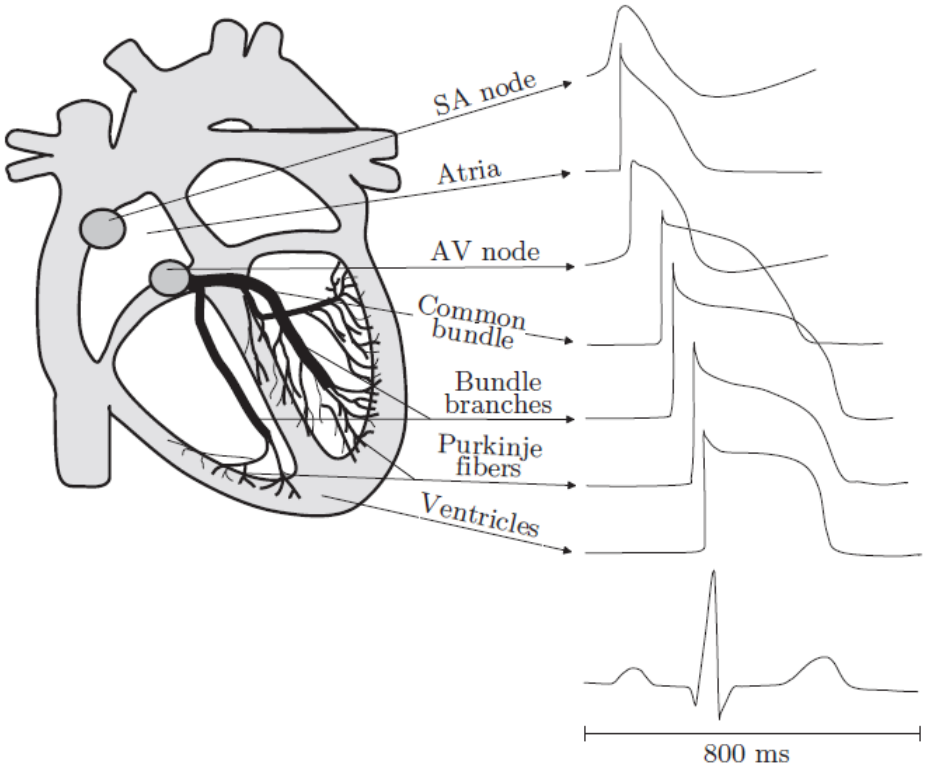
Algunos estudios aseguran que los niveles de actividad física y la condición física están positivamente correlacionados con la salud e independencia de los centenarios (45,46,76). Otras investigaciones subrayan que el mantenimiento de la capacidad funcional y la independencia son las claves para llegar a ser «supercentenario», es decir cumplir ≥110 años (24,77). Según nuestro conocimiento, solamente existen tres estudios de caso que hayan reportado entrenamientos en centenarios. Uno de ellos es el programa de entrenamientos seguido por el ciclista francés Robert Marchand para establecer el record de la hora en velódromo para mayores de cien años (26,92km), considerado el mejor rendimiento alcanzado por un centenario (-50,6% respecto al record absoluto) (68); entre los 101 y los 103 años, Robert mejoró su rendimiento un 11% y su $\dot{V}O_{2max}$ un 13% (29). En segundo lugar, un hombre de 99,5 años con un historial de records mundiales en competiciones atléticas de categoría máster que entrenó durante 16 meses, tras los cuales mejoró la fuerza de tren superior e inferior (+ 0-9,5%) y el equilibrio monopodal (+22%), mientras que empeoró la flexibilidad (-30%) y el salto con contra-movimiento (-4,2%) (78). En tercer y último lugar, los efectos de un entrenamiento de 12 semanas de estimulación eléctrica neuromuscular en una mujer centenaria, quien mejoró un 70% la distancia caminada en el test de 6 minutos andando (64 *vs*. 109 m) y un 300% el número de levantadas en 30 segundos (3 *vs*. 9 repeticiones), y redujo en 8mmHg su tensión arterial sistólica y en 4mmHg su tensión arterial diastólica y tensión arterial media (79). Estos estudios de caso reflejan la plasticidad del cuerpo humano para beneficiarse del ejercicio a cualquier edad, pero únicamente tienen utilidad descriptiva; además dos de los tres estudios se llevaron a cabo en centenarios que competían a nivel mundial, la intervención duró más de un año y estaba pobremente detallada (29,78).

1.3 Electrofisiología cardíaca

1.3.1 Bases neuroanatómicas

El corazón es un órgano, formado principalmente por músculo liso, que contiene cuatro cámaras (dos aurículas superiores y dos ventrículos inferiores) y se encarga de bombear la sangre de los vasos sanguíneos. El ciclo cardíaco está compuesto por la sístole (contracción ventricular, eyección de sangre) y la diástole (relajación y llenado ventricular). En un corazón sano, el nódulo sinoauricular inicia el ciclo cardíaco gracias a la despolarización espontánea de sus células marcapasos, esta despolarización se va propagando hacia el ápex, lo que provoca la contracción sincronizada de las diferentes zonas. Como podemos ver representado en la ***Figura 11***, los sucesivos potenciales de acción generados resultan en la señal típica del electrocardiograma (ECG).

Figura 11. La generación del electrocardiograma.



Fuente: Figura obtenida del libro *«Bioelectrical Signal Processing in Cardiac and Neurological Applications»* publicado en 2005 por la editorial *«Elsevier»* (80).

El nódulo sinoauricular descargaría a una frecuencia de 100 potenciales de acción por minuto, sin embargo la frecuencia cardíaca en reposo es de 75 latidos por minuto, esto se debe a la regulación a nivel nervioso, en este ejemplo mediada por la predominancia del sistema nervioso parasimpático (SNP) durante el reposo. El control nervioso del corazón se coordina desde el centro cardiovascular, localizado en el bulbo raquídeo del tronco encefálico. El centro cardiovascular integra la información: sensitiva (nervios glosofaríngeo y vago), de los propioreceptores (posición), quimiorreceptores (sangre), baroreceptores (presión en seno carotideo y cayado aórtico), sistema límbico (hipotálamo) y corteza cerebral; a partir de todos estos inputs el centro cardiovascular ajusta el sistema nervioso autónomo (SNA), modulando el SNP y el sistema nervioso simpático (SNS). El centro cardiovascular inhibidor, SNP, hace sinapsis con los núcleos del vago (dorsal, solitario y ambiguo), las neuronas eferentes del vago llegan hasta nódulo sinoauricular, nódulo auriculoventricular y músculo auricular, allí liberan acetilcolina, la cual se une a los receptores muscarínicos (M2) y disminuye la frecuencia cardíaca. Por el contrario, el centro cardiovascular acelerador, SNS, hace sinapsis con los ganglios cervicales (superior, medio y estrellado) y ganglios torácicos (1º- 5º), desde ellos salen neuronas simpáticas eferentes que llegan hasta nódulo sinoauricular, nódulo auriculoventricular y miocardio ventricular, allí liberan adrenalina y noradrenalina, las cuales se unen a receptores β-adrenérgicos (β1) y aumenta tanto la frecuencia cardíaca como la contractilidad (81).

La regulación cardíaca no se realiza exclusivamente a nivel nervioso, las hormonas y los iones circulantes también juegan un papel en el control del corazón. De forma somera podemos decir que la adrenalina, noradrenalina, hormonas tiroideas y Ca^{2}+ intracelular aumentan la frecuencia cardíaca y la contractilidad. Mientras que niveles elevados en plasma de K+ y Na+ producen el efecto contrario (81).

1.3.2 El electrocardiograma

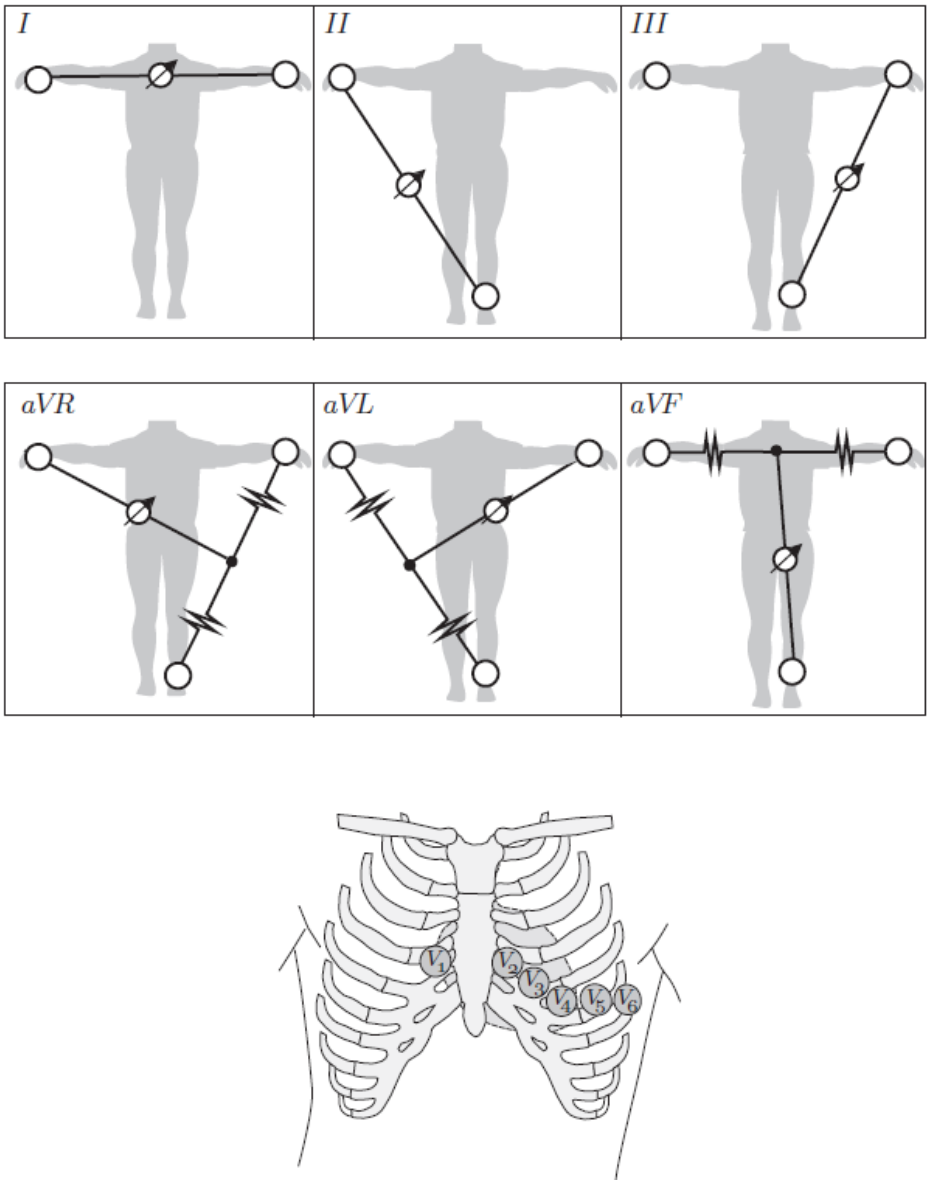
En 1901 el profesor de la Universidad de Leiden, Willem Einthoven registró por primera vez la actividad cardíaca. En la ***Figura 12*** podemos ver las dimensiones del primer ECG funcional, el instrumento contenía un fino filamento de cuarzo bañado en plata que estaba suspendido entre dos imanes, los cambios en la corriente eléctrica cardíaca producían el movimiento del filamento, que se traducía de forma gráfica como una sombra en un papel de fotografía iluminado, dibujando el ECG. La base de este mecanismo es similar a la de los ECG electrónicos que usamos en la actualidad; cuando una onda de despolarización positiva en los cardiomiocitos se dirige hacia un electrodo positivo en la piel, hay una deflexión simultánea (positiva) registrada en el trazo del ECG.

Figura 12. Galvanómetro de cuerda de Willem Einthoven.

Fuente: Fotografía realizada por Alejandro M. Sanz Guillén en el Rijksmuseum Boerhaave.

En el trazo típico del ECG podemos observar diferentes ondas que corresponden a las fases del ciclo cardíaco: la despolarización auricular (onda P), despolarización ventricular (complejo QRS) y la repolarización ventricular (intervalo QT y onda T). Asimismo, el tiempo entre dos ondas R consecutivas se denomina intervalo RR, y es considerado como el tiempo entre dos latidos, se utiliza para caracterizar arritmias y para el estudio de la variabilidad de la frecuencia cardíaca (VFC).

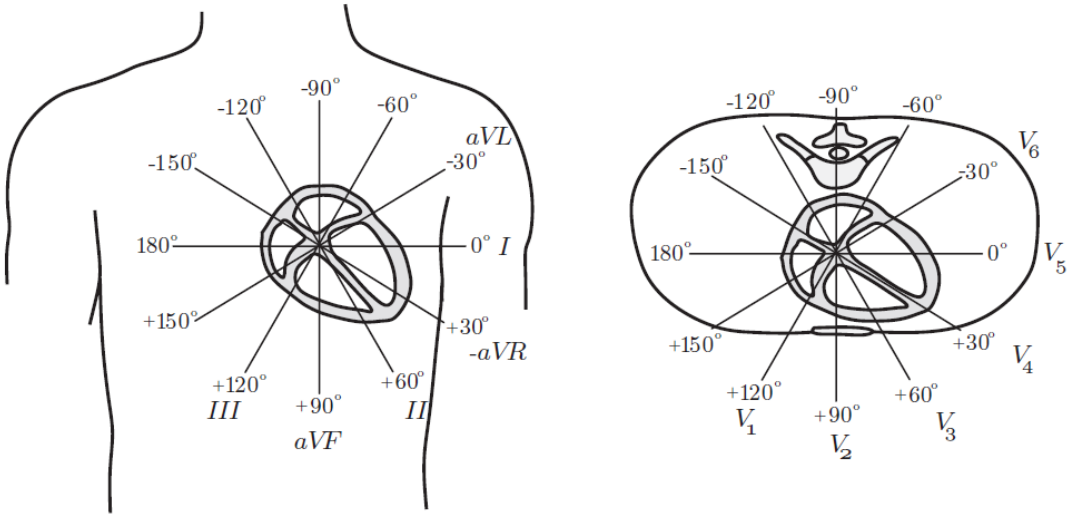
Figura 13. Colocación de los electrodos en un ECG de 12 derivaciones.



Fuente: Figura obtenida del libro «*Bioelectrical Signal Processing in Cardiac and Neurological Applications*» publicado en 2005 por la editorial «*Elsevier*» (80).

En un registro de ECG, una derivación es la diferencia de voltaje entre dos electrodos (derivación bipolar) o entre un único electrodo y un electrodo de referencia con un voltaje constante (derivación unipolar). En la clínica la configuración más empleada es el ECG de 12 derivaciones en el cual los electrodos se sitúan tal y como podemos ver en la ***Figura 13***. Esta configuración registra la actividad eléctrica en el plano coronal a través de las derivaciones bipolares estándar (I, II y III) y de las derivaciones unipolares aumentadas (aVF, aVL y aVR), y en el plano axial a través de las seis derivaciones precordiales unipolares (V_1 a V_6), detallado en ***Figura 14***.

Figura 14. Direcciones angulares registradas por un ECG de 12 derivaciones.



Fuente: Figura obtenida del libro *«Bioelectrical Signal Processing in Cardiac and Neurological Applications»* publicado en 2005 por la editorial *«Elsevier»* (80).

Usando una metáfora ilustrativa, los electrodos del ECG son cámaras de vídeo que graban la actividad eléctrica del corazón desde diferentes ángulos. El ECG convencional de 12 derivaciones, puede transformarse a través de métodos matemáticos a tres ejes ortogonales X, Y y Z; siguiendo la metáfora, estaríamos grabando el corazón desde un plano coronal, sagital y axial. Por lo tanto, podemos determinar la dirección tridimensional del vector de despolarización ventricular (de forma análoga al clásico uso del complejo QRS positivo en derivaciones I y aVF para determinar un «eje cardíaco

normal» según un sistema 2D). Dado que la onda T representa la repolarización ventricular, siguiendo el mismo procedimiento podemos situar en las tres dimensiones del espacio cual es la dirección del vector de repolarización ventricular, como veremos más adelante en el apartado «*1.3.4 DRP y envejecimiento*» gracias a esto es posible calcular las dinámicas de repolarización periódica (DRP).

El ECG de 12 derivaciones es el instrumento de referencia para medir la actividad eléctrica del corazón, su imprescindible papel en el diagnóstico reafirma el premio Nobel recibido por Willem Einthoven en 1924. No obstante, en la actualidad la monitorización cardíaca va más allá de las puertas de los centros sanitarios, un ejemplo son los monitores de frecuencia cardíaca. Estos dispositivos portátiles ya son capaces de registrar ECG de una derivación, además en mediciones más sencillas como el intervalo RR han mostrado una precisión similar a un ECG de laboratorio (82). Dado el bajo coste de los monitores de frecuencia cardíaca su uso se ha expandido al público general, entre las marcas más conocidas encontramos *Polar*, *Garmin* o *Suunto*. Y a pesar del alto ritmo al que las compañías lanzan nuevos modelos al mercado, los sensores más usados suelen estar validados en reposo y ejercicio (83–85). Sin embargo, normalmente los dispositivos se validan en grupos de voluntarios jóvenes, sanos y físicamente activos (83–85), pero son usados por individuos con variadas características fenotípicas, sin tener en cuenta si esto podría influenciar la medición.

El ECG registra la actividad eléctrica del corazón a través de electrodos situados en la superficie corporal. Por una parte encontramos las diferencias inter-sujeto en el espacio que separa el tejido cardíaco del electrodo, por ejemplo la fibrosis miocárdica asociada a la edad, la cantidad de grasa subcutánea o la localización del electrodo (86). Por otra parte el voltaje original está influenciado por el tamaño ventricular, así que también varía entre sujetos, por lo tanto los grupos con mayores voltajes QRS (hombres,

negros, jóvenes, atletas) serán más fácilmente medibles (87). Por último, algunas circunstancias como el ejercicio de alta intensidad añaden ruido al registro y comprometen la medición (88). Cuando coincidan varias circunstancias desfavorables algunos latidos cardíacos podrían no ser detectados, lo cual no afectaría considerablemente a la frecuencia cardíaca media, pero si alteraría notablemente la evaluación de la VFC. Todavía no se ha estudiado si las características fenotípicas de los sujetos podrían afectar las mediciones en reposo y ejercicio registradas por los monitores de frecuencia cardíaca ampliamente usados por la población general.

1.3.3 VFC y envejecimiento

La VFC, *«Heart rate variability –HRV–»* en inglés, se define como la oscilación en los intervalos temporales entre latidos consecutivos, es decir la oscilación en los intervalos RR (89). La VFC es el resultado de la interacción de múltiples mecanismos reguladores que operan en diferentes escalas temporales, incluyendo mecanismos a largo plazo como los ritmos circadianos, la temperatura corporal o el metabolismo, y mecanismos a corto plazo que involucran los sistemas autónomo, cardiovascular y respiratorio (90). La VFC no debe equipararse a la «arritmia sinusal respiratoria» (ASR), fenómeno que ocurre debido a la respiración: durante la inhalación, la presión intratorácica aumenta y el centro cardiovascular acelera el corazón, por el contrario durante la exhalación se restaura la inhibición vagal y el corazón se ralentiza.

El estudio de la VFC es un área de gran interés puesto que se trata de una herramienta no invasiva y de bajo coste que abre una ventana al SNA del corazón. Los índices frecuenciales se derivan al dividir las oscilaciones rítmicas del corazón en bandas de frecuencia, por ejemplo la banda de baja frecuencia, *«low frequency»* (LF), va de 0,04-0,15Hz y proporcionaría información de la actividad barorrefleja, SNS y SNP; por su parte la banda de alta frecuencia, *«high frequency»* (HF), de 0,15 a 0,40Hz, es dirigida por la ASR y proporcionaría información de SNP, sin embargo la interpretación fisiológica de estos índices es controvertida (91,92). Otro tipo de índices son los basados en el dominio temporal del ECG, entre ellos podemos encontrar: *«Standard deviation of all R–R intervals»* (SDNN) que refleja todos los componentes cíclicos responsables de la VFC y podría ser considerado un indicador global de la regulación autónoma; *«Root mean square of successive differences»* (RMSSD), *«Percentage of successive normal sinus RR intervals more than 50 ms»* (pNN50) y *«SD of successive differences»* (SDSD)

son tres índices que correlacionan con la banda HF y se considera que tienen un origen fisiológico vagal (92).

Un corazón sano no es un metrónomo, en general una menor VFC ha sido asociada con peor pronóstico en diferentes condiciones clínicas, mientras que una mayor VFC, especialmente HF, ha sido asociada con una mejor salud. En particular, se han reportado reducciones de la VFC en varias enfermedades cardiovasculares, además la VFC ha sido usada para estratificar el riesgo, confirmando su valor como predictor de mortalidad (93–95). También se han descrito valores bajos de VFC en un amplio rango de enfermedades no cardiovasculares, incluyendo patologías psiquiátricas como la depresión, la ansiedad o la esquizofrenia (96).

Los factores ambientales y comportamentales influyen en la VFC (97). En psicología es común el uso de la VFC, debido a que es modulada por los estados de ánimo, emociones o capacidad cognitiva (96). Asimismo, la VFC es una herramienta muy útil en el campo de las ciencias del deporte dada su sensibilidad a los cambios en la condición física, fatiga y rendimiento (98). Hábitos saludables tales como el ejercicio, una dieta equilibrada, meditación o intervenciones psicológicas han mostrado incrementar los índices de VFC que evalúan la función vagal (96,97). Adicionalmente, factores no modificables como la edad y el sexo también influyen en la regulación autónoma del corazón, la VFC presenta un descenso progresivo asociado a la edad, en cuanto al sexo las mujeres muestran valores de HF más altos y valores de LF más bajos que los hombres (99,100). La mayor actividad vagal de las mujeres podría deberse a factores como los estrógenos, la oxitocina y el control neural (100), sin embargo, estas diferencias entre sexos desaparecerían en los mayores por la restructuración hormonal tras la menopausia (100–102).

En lo que respecta al envejecimiento, el corazón añoso presenta una serie de alteraciones electrofisiológicas asociadas a procesos de remodelado eléctrico y estructural que ocurren progresivamente con la edad. A nivel celular, el potencial de acción cardíaco se ve prolongado debido a la regulación negativa de corrientes de potasio y la regulación positiva de corrientes de calcio, además de otros ajustes en el ciclo de calcio (103). Asimismo, se producen cambios en la masa/volumen de las cavidades cardíacas, pérdida del número de cardiomiocitos así como hipertrofia celular, los cuales se acompañan de un aumento muy notable de la fibrosis y una reducción del acoplamiento entre las distintas células que forman los tejidos cardíacos (104). Todos estos cambios tienen manifestaciones a nivel de la señal ECG, entre las que se incluyen alteraciones en la VFC así como en otros intervalos característicos de la repolarización (105). A estas últimas manifestaciones contribuyen también los efectos que presenta el envejecimiento sobre el SNA, en gran medida relacionados con la desregulación e hiperactividad de su rama simpática, la cual tiene un rol en la fisiopatología de enfermedades como la hipertensión, el infarto de miocardio y la insuficiencia cardíaca (106,107).

Solamente unos pocos estudios han analizado la VFC en centenarios. Piccirillo et al. y Paolisso et al. encontraron que sus centenarios presentaban una mayor HF y una menor LF que una muestra de adultos mayores (81-100 años en Piccirillo et al. y 75-100 años en Paolisso et al.), sugiriendo un incremento del SNP y una reducción del SNS en relación con la edad extrema (108,109). Estos resultados están en línea con los obtenidos por Zulfiqar et al., quienes reclutaron sujetos de hasta 99 años y mostraron que los índices temporales de VFC relacionados con SNP disminuyen con la edad, alcanzando un nadir en la 7ª-8ª década. A partir de la 8ª década de edad, estas mediciones mostraban un aumento, por lo que los autores propusieron que evitar el descenso del SNP podría ser un determinante clave en la longevidad (110). Por el contrario, otro estudio llevado a cabo en

centenarios mostró que entre todas las variables frecuenciales, el ratio LF/HF estaba asociado con la supervivencia a 4 años (111). En resumen, la evidencia disponible sobre VFC en centenarios es escasa y contradictoria.

Hasta ahora hemos hablado de mediciones de VCF en reposo, pero las dinámicas de la VFC durante el ejercicio también han sido extensamente descritas en la literatura. En un test incremental, los índices de VFC muestran un descenso súbito con el comienzo del ejercicio, seguido por un decaimiento curvilíneo en función de la intensidad del esfuerzo y una restitución de los niveles de reposo durante la recuperación. Las variables relacionadas con SNP (Ej. RMSSD y HF) suelen presentar valores cercanos a cero a intensidades moderadas, 50-60% del $\dot{V}O_{2max}$, (probablemente asociado con el primer umbral ventilatorio) (112,113), en ocasiones se observa un incremento de estas variables en intensidades próximas al máximo, aunque dichos aumentos están mediados por el efecto mecánico de la respiración sobre el nódulo sinoauricular (114).

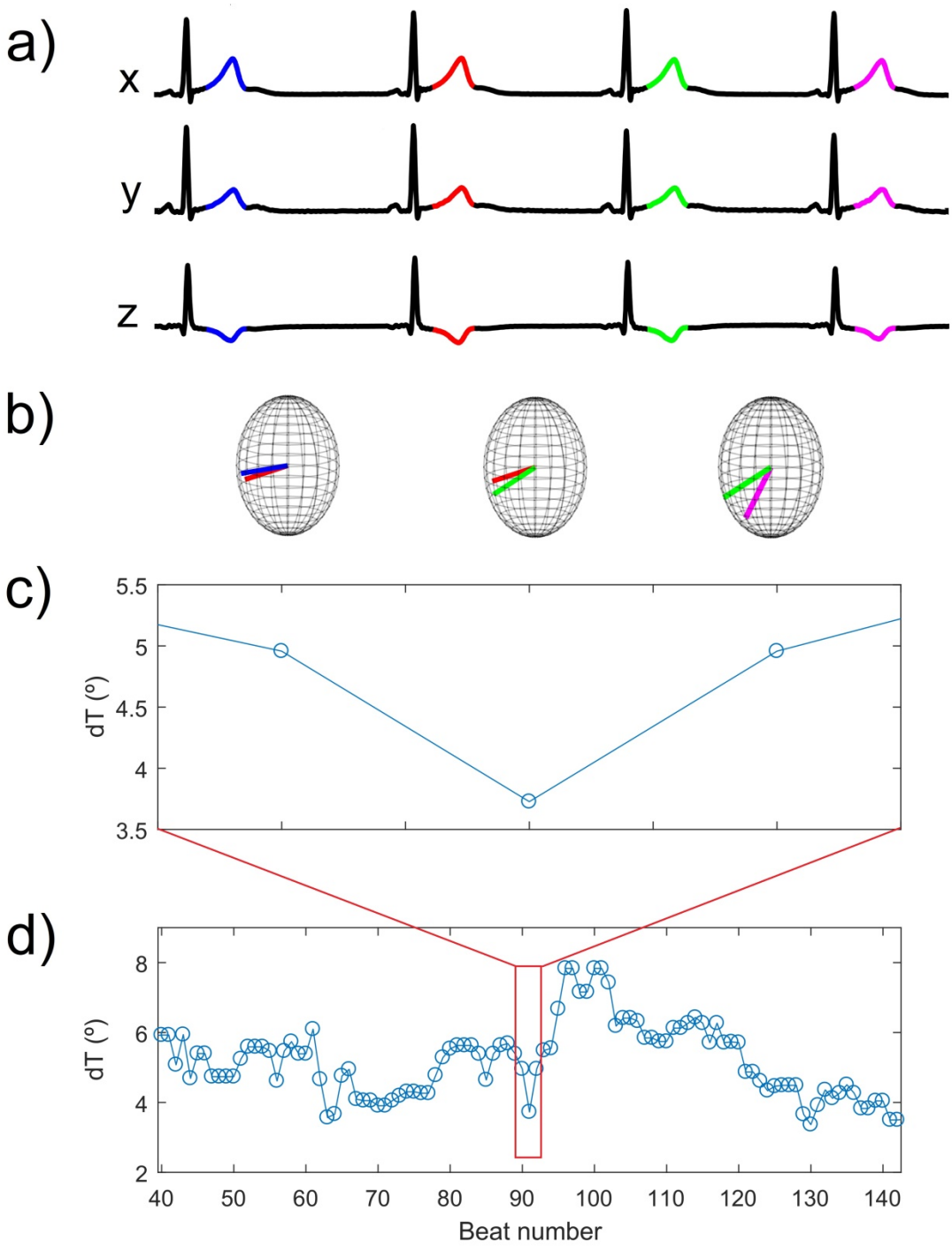
Muchos estudios han mostrado que el ejercicio físico, como tratamiento no farmacológico en pacientes con enfermedad cardiovascular, puede restaurar el equilibrio simpatovagal evaluado mediante VFC en reposo (107). En la población no patológica hasta un 60% de la varianza de la VFC podría estar determinado por factores genéticos y menos del 15% estaría determinado por factores modificables (115,116). Por lo tanto a nivel individual el objetivo de las intervenciones no debería de ser «aumentar la VFC», sino usarla para medir y gestionar los estresores agudos del día a día, adaptando el comportamiento y consecuentemente obtener beneficios en la salud y el rendimiento (116). Por ejemplo, parece que los deportistas de resistencia que siguen un entrenamiento guiado día a día por VFC podrían tener un rendimiento igual o incluso superior al de sus homólogos que siguen una planificación predefinida Ej. Periodización por bloques (117,118).

1.3.4 DRP y envejecimiento

En el apartado anterior nombrábamos que la hiperactividad del SNS tiene una importancia cardinal en la fisiopatología de diferentes enfermedades cardíacas, sin embargo herramientas como la VFC no son capaces de medir el SNS de forma aislada. La hiperactividad del SNS también ha mostrado aumentar la actividad desencadenada y la dispersión de la repolarización ventricular, lo que contribuye a acentuar la probabilidad de sufrir una arritmia ventricular fatal o una muerte súbita cardíaca (119). Recientemente se ha propuesto un marcador basado en ECG que sí reflejaría los efectos del SNS en el miocardio ventricular (120). Este marcador es la DRP, *«Periodic repolarization dynamics –PRD– »* en inglés, y cuantifica la magnitud de las oscilaciones LF en el ángulo dT entre vectores de onda T de latidos consecutivos (120).

La DRP se obtiene a partir de un ECG ortogonal, por lo tanto es necesario registrarlo en la configuración de Frank o bien transformar un ECG de 12 derivaciones mediante la matriz inversa de Dower (121). En la ***Figura 15***, podemos observar los pasos para el cálculo de dT: *a)* Partimos del ECG ortogonal donde se delimita el comienzo y fin de cada onda T, *b)* A continuación se calcula el vector medio de la onda T para cada latido, *c)* Para obtener el ángulo dT entre latidos consecutivos, se realiza el producto escalar de los vectores medios de onda T para cada pareja de latidos consecutivos. Finalmente se filtra la señal dT obtenida (*Figura 15-d*) y se realiza un análisis de ondas, obteniendo DRP como la potencia en la banda LF para cada instante de tiempo (120,122).

Figura 15. Cálculo de dT a partir de un ECG ortogonal.



Fuente: La figura pertenece al artículo *«ECG Ventricular Repolarization Dynamics during Exercise: Temporal Profile, Relation to Heart Rate Variability and Effects of Age and Physical Health»* publicado en 2021 en la revista *«Int J Environ Res Public Health»* (123). Licencia: Creative Commons (CC BY).

Los mecanismos fisiológicos subyacentes a la génesis de DRP todavía no se han descrito por completo, en particular lo concerniente a la intervención de la actividad oscilatoria simpática en la regulación del miocardio ventricular (119). La importancia de las oscilaciones LF a la hora de proporcionar información potencialmente relacionada con el SNS ha sido descrita a través de: diferentes marcadores obtenidos a partir de la onda T, grabaciones de actividad nerviosa simpática, VFC, potenciales de acción o presión arterial sistólica (119). En lo que respecta a la DRP en concreto, existe evidencia clínica y experimental que muestra su aumento ante activación simpática (bien sea inducida mediante una mesa basculante o ejercicio) y su supresión frente al bloqueo adrenérgico mediante fármacos (120,124). Además, las DRP han mostrado ser independiente de la actividad respiratoria en cerdos ventilados de forma mecánica (120), así como en humanos cuando se comparan volúmenes constantes pero con frecuencias respiratorias de 10 *vs*. 20/min (125). Asimismo, DRP ha confirmado no ser un epifenómeno de la frecuencia cardíaca o la VFC, lo que está sostenido por el hecho de que el marcapaseado auricular ejerce un efecto modesto en la DRP, mientras que abole por completo la VFC y varía ampliamente la frecuencia cardíaca (120,126). Esto ha sido respaldado más tarde por estudios que realizan un test de ejercicio incremental, en los cuales se han descrito bajas correlaciones entre DRP y frecuencia cardíaca o VFC (124). Sin embargo, todavía no se conoce bien cómo varía la DRP durante las diferentes fases de una prueba de ejercicio incremental (estímulo simpático creciente). Tampoco se ha cuantificado la fracción de la DRP relacionada y no relacionada con la VFC, ni las dinámicas de estas dos fracciones durante un test incremental. Responder a estas preguntas sería interesante tanto en población general como en subgrupos estratificados según ciertas características fenotípicas o patológicas.

Un meta análisis con 6758 pacientes sugiere que un valor elevado de DRP medido en reposo es un fuerte predictor de mortalidad por todas las causas y mortalidad cardiovascular (127). Estudios retrospectivos en pacientes post-infartados mostraron que un DRP incrementado es predictor de mortalidad por todas las causas, mortalidad cardiovascular y muerte súbita cardíaca (120,128). Lo cual fue confirmado posteriormente en un estudio de diseño prospectivo, en particular un DRP $\geq$5.75 grados^2 fue predictor de mortalidad a 3 años en pacientes post-infartados agudos/crónicos así como en pacientes con fracción de eyección del ventrículo izquierdo reducida/preservada (129). Del mismo modo un estudio prospectivo en pacientes con insuficiencia cardíaca crónica mostró la capacidad de la DRP para predecir riesgo de muerte súbita cardíaca y muerte por fallo de bomba (130). Además la capacidad estratificadora de DRP mostró ser independiente de la de otras variables clínicas y derivadas del ECG, entre las que podemos encontrar: fracción de eyección del ventrículo izquierdo, VFC, diabetes mellitus y puntuación en el *«Global Registry of Acute Coronary Events»* en las poblaciones de post-infartados (129); y ser independiente de la clasificación de la *«New York Heart Association»* en poblaciones con insuficiencia cardíaca crónica (130). Por último, cabe destacar que DRP ha mostrado ser capaz de predecir reducciones de la mortalidad asociadas a implantes profilácticos de desfibriladores en pacientes con cardiomiopatías y por lo tanto podría ayudar a tomar decisiones sobre el tratamiento (131).

Tal y como acabamos de ver, este marcador derivado del ECG tiene un gran poder como estratificador del riesgo, pero no se sabe prácticamente nada sobre cómo cambia en función de la edad, condición física, composición corporal, etcétera. Conocer estas relaciones podría permitir entender cómo la exposición crónica a determinadas variables o comportamientos afecta a la repolarización ventricular mediada por el SNS y cómo esto puede estar relacionado con un riesgo aumentado de mortalidad.

Si atendemos al comportamiento de la DRP durante una prueba de ejercicio incremental, únicamente existen dos estudios que hayan proporcionado una descripción a fondo, y los patrones que explican son contradictorios. Podemos tratar de explicar esta disonancia de resultados si pensamos en que las características de las señales dT y DRP podrían variar en función del protocolo utilizado en el test incremental. Hamm et al. utilizaron un protocolo incremental en escalones y describieron que dT aumentaba de forma concordante a la frecuencia cardíaca hasta alcanzar el umbral anaeróbico de lactato (medido con método de Dickhuth y con umbral fijo de 4mM) y después comenzaba a descender de forma discordante a la frecuencia cardíaca (126). Milagro et al. consideraron un protocolo en rampa más exigente y no observaron dicho valle en la señal dT, pero reportaron un perfil trifásico de las señales dT y DRP durante el esfuerzo incremental. Este perfil trifásico consistió en un rápido incremento desde el reposo y un comportamiento en meseta durante el ejercicio de intensidad ligera, en segundo lugar se mostraba un ligero incremento desde el punto donde la VFC alcanzaba su mínimo (probablemente asociado con el primer umbral ventilatorio), la tercera y última fase se caracterizaba por un incremento súbito tras alcanzar el segundo umbral ventilatorio (124). En resumen, este nuevo índice está comenzando a ser estudiado por las ciencias del deporte, futuros estudios terminarán de desentrañar sus dinámicas durante el ejercicio y su aplicación como método de detección de umbrales (124,126).

2. Hipótesis [Hypothesis]

En lo que respecta a la metodología: i) Los puntos de corte de acelerometría son específicos para cada población, no existen puntos de corte para los muy mayores y el uso de puntos de corte desarrollados con personas de 60-80 años podría presentar un efecto suelo en centenarios; las métricas libres de puntos de corte podrían resolver algunas de las limitaciones de los puntos de corte tradicionales. ii) La edad y otras características fenotípicas también podrían influir en la recogida de datos a través de monitores de frecuencia cardíaca, dado que un menor voltaje QRS o variaciones inter-sujeto en el espacio que separa el tejido cardíaco del electrodo podrían hacer que no se detectaran algunos latidos, lo cual alteraría la evaluación de la variabilidad de la frecuencia cardíaca.

El envejecimiento se caracteriza por el detrimento en la función de todos los sistemas; por lo tanto esperamos un deterioro asociado a la edad en las diferentes variables estudiadas, es decir: descensos en los niveles de actividad física, descensos en la variabilidad de la frecuencia cardíaca y aumentos en las dinámicas de repolarización periódica.

Los niveles de actividad física, la variabilidad de la frecuencia cardíaca y las dinámicas de repolarización periódica ya han mostrado relaciones con diferentes marcadores de salud en otros segmentos poblacionales, por lo que esperamos encontrar resultados similares a pesar de la edad de los sujetos.

Por último, pero no menos importante, la evidencia científica soporta los efectos beneficiosos del ejercicio físico, incluso en personas nonagenarias, frágiles, institucionalizadas u hospitalizadas. Nuestra hipótesis es que los centenarios frágiles institucionalizados no serán una excepción, y el ejercicio de fuerza podría incrementar la fuerza muscular así como otras de las variables del estudio.

2. Hypothesis

Regarding the methodology: i) The accelerometry cut-points are specific for each population, there are no cut-points for the oldest-old and the use of cut-points developed with people aged 60-80 could present a floor effect in centenarians; cut-point-free metrics could overcome some of the limitations of conventional cut-points. ii) Age and other phenotypic characteristics could also influence data collection through heart rate monitors, since a lower QRS voltage or inter-subject variations in the space that separates the cardiac tissue from the electrode could prevent the detection of some heart beats, which would alter the assessment of heart rate variability.

Aging is characterized by the detriment in the function of all systems; therefore we expect an age-related deterioration in the different outcomes studied, in other words: decreases in physical activity levels, decreases in heart rate variability and increases in periodic repolarization dynamics.

Physical activity levels, heart rate variability and periodic repolarization dynamics have already shown relationships with different health markers in other population segments, consequently we expect to find similar results despite the age of the subjects.

Last but not least, scientific evidence supports the beneficial effects of physical exercise, even in nonagenarian, frail, institutionalized or hospitalized people. Our hypothesis is that frail institutionalized centenarians will not be an exception, and resistance training could increase muscle strength as well as other study outcomes.

3. Objetivos [Aims]

El *objetivo general* de la presente Tesis Doctoral es analizar las limitaciones de los métodos de medición de los niveles de actividad física y la electrofisiología cardíaca al utilizarse en población mayor, así como aportar resultados descriptivos en personas muy mayores estudiando su relación con diferentes marcadores de salud, para finalmente examinar los efectos de un programa de ejercicio físico en centenarios.

Los *objetivos específicos* enmarcados en cada uno de los cinco artículos que componen esta Tesis Doctoral son:

Artículo I.

- Evaluar objetivamente la actividad física y el sedentarismo en centenarios y nonagenarios.

Artículo II.

- Describir la actividad física en el límite de esperanza de vida humana a través de métricas de acelerometría libres de puntos de corte.
- Comparar las métricas de acelerometría basadas en puntos de corte *versus* las métricas libres de puntos de corte, en dos niveles:
    - Evaluando el efecto suelo de las diferentes métricas.
    - Explorando las asociaciones de las métricas con un estado positivo en diferentes variables relacionadas con la salud.

Artículo III.

- Examinar los efectos de 12 semanas de entrenamiento supervisado de fuerza sobre la condición física, independencia funcional, fragilidad y calidad de vida relacionada con la salud en centenarios frágiles institucionalizados.

Artículo IV.

- Evaluar la validez del análisis de variabilidad de la frecuencia cardíaca derivado de los intervalos RR registrados por el sensor de frecuencia cardíaca *PolarH7* en reposo y durante el ejercicio y la recuperación en diferentes grupos fenotípicos según la edad, la composición corporal y la condición física.

Artículo V.

- Investigar las posibles diferencias en la variabilidad de la frecuencia cardíaca de las mujeres adultas, octogenarias y centenarias.
- Evaluar si los índices de variabilidad de la frecuencia cardíaca pueden predecir la mortalidad por todas las causas en centenarios que fueron seguidos hasta el momento de la muerte.

Artículo VI.

- Cuantificar de forma continua las dinámicas de repolarización periódica en reposo y durante el ejercicio, evaluando su dependencia de la variabilidad de la frecuencia cardíaca.
- Caracterizar los efectos de la edad, el índice de masa corporal y el nivel de aptitud cardiorrespiratoria sobre las dinámicas de repolarización periódica en reposo y durante el ejercicio.

3. Aims

The *general aim* of the present Thesis is to analyze the limitations of the methods for measuring physical activity levels and cardiac electrophysiology when used in the elderly population, as well as to provide descriptive results in the oldest old studying its relationship with different health markers, to finally examine the effects of a physical exercise program in centenarians.

The *specific aims* of each of the five articles that compose this Thesis are:

Manuscript I.

- To objectively assess physical activity and sedentary behaviour in centenarians and their nonagenarian peers.

Manuscript II.

- To describe physical activity at the end of the human lifespan through cut-point-free accelerometer metrics.
- To compare cut-point-based versus cut-point-free accelerometer metrics at two levels:
    - Evaluating the floor effect in the different metrics.
    - Exploring the associations of the metrics with a positive status in a variety of health outcomes.

Manuscript III.

- To examine the effects of 12-weeks supervised resistance training on physical function, functional independence, frailty and health-related quality of life in frail institutionalized centenarians.

Manuscript IV.

- To evaluate the validity of heart rate variability analysis derived from RR intervals recorded by *PolarH7* heart rate sensor at rest and during exercise and recovery in different phenotype groups based on age, body composition and fitness level.

Manuscript V.

- To investigate potential differences in women's heart rate variability between young adults, octogenarians, and centenarians
- To assess whether heart rate variability variables can predict all-cause mortality in centenarians followed up until the time of death.

Manuscript VI.

- To continuously quantify periodic repolarization dynamics at rest and during exercise, assessing its dependence on heart rate variability
- To characterize the effects of age, body mass index and cardiorespiratory fitness level in periodic repolarization dynamics at rest and during exercise.

4. Resultados y discusión [Results and discussion]

4.1 Artículo I

Publicado en Journal of Aging and Physical Activity, 2019.

Title: Physical activity and sedentary behavior at the end of the human lifespan.

Suggested Running Head: PHYSICAL ACTIVITY IN CENTENARIANS

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ABSTRACT

This study aims to objectively assess physical activity (PA) levels and sedentary behavior (SB) in a cohort of Spanish centenarians and their nonagenarian peers. PA and SB patterns were objectively measured by an ActiGraph GT3X accelerometer in centenarians [n=18; 83% women; 100.8±0.8 (100–103) years] and nonagenarians [n=11; 91% women; 93.3±2.5 (90–98) years]. Centenarians showed less counts per minute (17.6±7.1 vs 46.1±23.7, *P*=0.003, *d*=1.851) and steps per day (455±237 vs 1,249±776, *P*=0.007, *d*=1.587) than nonagenarians. The daily number of sedentary breaks was also lower in the former (5.0±1.5 vs 6.7±2.0, *P*=0.019, *d*=0.971). When observing time distribution the most active day period in both groups was the morning, with a peak between 10:00-11:59. Our data suggest that the decline in PA levels continues to worsen until the end of the human lifespan.

Keywords: longevity, accelerometry, centenarian, sedentary lifestyle, physical activity.

BACKGROUND

The population of centenarians (≥100 years) is steadily increasing globally (Byass, 2008) being the paradigm of human extreme longevity and healthy aging, because they have postponed, if not avoided, major age-related diseases, e.g. cancer, cardiovascular disease or neurodegeneration (Salvioli et al., 2008), as well as the onset of disability (Christensen, McGue, Petersen, Jeune, & Vaupel, 2008; Terry, Sebastiani, Andersen, & Perls, 2008). Despite this global demographic development, very old individuals (i.e. centenarians) remain an understudied and underserved population and little is known about their physical activity (PA) and sedentary behavior (SB) patterns, both factors contributing to healthy aging and longevity (Hall et al., 2014).

It is well-known that PA levels decline with age, being older adults (65+ years) the most physically inactive population (Troiano et al., 2008). It has also been reported that older adults spend 8.5-9.6 hours per waking daytime in SB (usually defined as the length of time spent sitting or lying, i.e., energy expenditure of 1–1.5 metabolic equivalents) (Sedentary Behaviour Research Network, 2013; Wullems, Verschueren, Degens, Morse, & Onambélé, 2016). Further, 'oldest-old' individuals (85+ years), who account for the fastest growing population segment in western societies, seem to be more sedentary than their younger older adults peers (Harvey, Chastin, & Skelton, 2015).

Despite this trend, very old people including centenarians, do not routinely receive counseling by health care professionals on lifestyle habits linked to an improved quality of life and prolonged survival, particularly PA (Berra, Rippe, & Manson, 2015). The current physical activity recommendations of the World Health Organization for adults aged 65 and above, that are the same than those proposed for younger adults from 18 to 64 years old, include to do at least 150 minutes of moderate-intensity aerobic physical activity throughout the week or do at least 75 minutes of vigorous-intensity aerobic physical activity throughout the week or an

equivalent combination of both. However, due to the common health and physical conditions of this older population, the WHO recommends to be as physically active as their abilities and conditions allow. WHO emphasize the importance of adjusting the PA for each individual, based on their exercise capacity and specific health risks or limitations (World Health Organization, 2010). For this reason, PA levels and SB patterns and indicators need to be accurately assessed in the different population subsets with the aim of conducting knowledge based counseling.

Although the exceptional longevity is partially conditioned genetically (Santos-Lozano, Santamarina, et al., 2016), being physically active has demonstrated to delay and even prevent the pathophysiology of several non-communicable disease and alterations associated with aging including Alzheimer´s disease, metabolic syndrome-related disorders, hypertension and cardiovascular disease, some type of cancers, sarcopenia and skeletal muscle dysfunction (Garatachea et al., 2015; Pareja-Galeano, Garatachea, & Lucia, 2015; Alejandro Santos-Lozano, Pareja-Galeano, et al., 2016).

The conventional method of obtaining self-reported PA data through questionnaires is inexpensive and generally well accepted by study participants. However, the validity of such data is questionable (Tucker, Welk, & Beyler, 2011) because of biases arising from different levels of social desirability and the cognitive challenge of quantifying both the intensity and duration of PA (Adams et al., 2005; Hills, Mokhtar, & Byrne, 2014; Welk, 2002). Such biases have prompted an interest in finding a less subjective way of monitoring PA. Accelerometers provide minute-by-minute recordings of PA and have been shown to be a reliable method for assessing both PA and SB in older adults (Gorman et al., 2014; Prince et al., 2008). Although PA and SB have been assessed by accelerometers during free living in older adults from 70-90 years (Chase, Lockhart, Ashe, & Madden, 2014; Johannsen et al., 2008; Simmonds et al., 2014), there is no accelerometry data available, to our knowledge, for centenarians until now.

Therefore, the purpose of this study was to objectively assess PA and SB in Spanish centenarians and their nonagenarian peers.

METHODS

Experimental design

The study participants were of the same Caucasian (Spanish) descent for ≥3 generations and lived in different areas of Spain. Inclusion criteria were to be a woman or a man, aged ≥90 years in the group of nonagenarians and ≥100 years in the case of centenarians. With regard to the exclusion criteria, the bedridden older adults were excluded, however, patients with reduced mobility were included, and also if they were helped by the caregivers, they used walking stick or walker. Likewise, older adults who were going through an acute disease process were excluded; nevertheless, the presence of chronic diseases or mental disorders such as dementia was not considered an exclusion criterion given the high prevalence in the last decades of life. **Table 1** shows the descriptive characteristics of both groups. Almost all were women (83% of centenarians and 91% of nonagenarians). The most frequent chronic diseases were: arterial hypertension, osteoarthritis and cardiopathy. The number of diseases (2.4 *vs.* 5.5) and number of drugs consumed (2.1 *vs.* 9.9) by nonagenarians was greater. However, nonagenarians showed greater independence in the activities of daily living.

Accelerometry was recorded during 7 days. The subjects and their caregivers were required by written and verbal instructions to wear the accelerometer from the moment they woke up in the morning until bedtime at night. Also, they were asked to note in a provided diary the time when they wear the devices, when they were removed at the end of the day, and any time when the devices were removed and reattached during the day.

The study was approved by the Ethical Committee for Clinical Research of Aragón (ID of the approval: PI17/0082) and was conducted adhering to the tenets of the Declaration of Helsinki. Signed informed consent was obtained from all participants or their caregivers.

Measures

PA was objectively measured by an ActiGraph GT3X accelerometer (Actigraph, LLC, Pensacola, FL). The device was mounted on the right hip with an adjusted elastic belt to ensure close contact with the body. Monitors were set to record PA in a 15-second epoch and the "Step Count" mode was selected. This device is lightweight (27 g), compact (3.8 × 3.7 × 1.8 cm) and has a rechargeable lithium polymer battery. The GT3X collects movement in three axes with a range of ~0.05 to 2.5 Gs, then the accelerometer output is digitized, each sample is summed over an "epoch", and the output is given in an arbitrary unit called "counts". The value of the counts will vary based on the frequency and intensity of the raw acceleration. ActiGraph GT3X accelerometer has been validated for the assessment of PA and SB in older adults (Aguilar-Farías, Brown, & Peeters, 2014; A. Santos-Lozano et al., 2013) with a high classification accuracy (ROC-AUC of 0.81, 0.85, and 0.82 for 1-, 15-, and 60-s epochs, respectively) (Aguilar-Farías et al., 2014) and an intra-instrument coefficient of variation of ≤2.5% (Alejandro Santos-Lozano et al., 2012).

Results were processed with Actilife version 6.5.4 software (Actigraph). The classification of wear and non-wear intervals was done using an algorithm based on ≥90 minutes of consecutive counts per minute (CPM) equal to zero, without allowing for interruptions (Peeters, van Gellecum, Ryde, Farías, & Brown, 2013). Also, 10 hours was established as the minimum necessary to be considered a valid day (Matthews, Hagströmer, Pober, & Bowles, 2012), with at least five valid days (Hart, Swartz, Cashin, & Strath, 2011), including one weekend day (Mâsse et al., 2005; Trost, Mciver, & Pate, 2005), to include the subject in the analysis. Moreover, only hours with ≥45 valid wear minutes were included (Sartini et al., 2015). The previously reported cut-points proposed by Aguilar-Farías *et al.* (Aguilar-Farías et al., 2014) for older individuals in free-living environments were used to estimate the time spent in SB (<10 counts/15 seconds for the vertical axis), and active time

(≥10 counts/15 seconds), in this way, the definition achieved when registering in 15 seconds epoch was not lost. Finally, bouts analysis was performed using counts relative to 60 seconds because the Actilife software does not allow different units of one minute, ≥25 CPM at vertical axis was defined for 10-minute activity bouts, and the sedentary bouts were calculated for 30 minutes (<25 CPM at vertical axis) (Aguilar-Farías et al., 2014), these durations are not random, the international physical activity guidelines recommend that aerobic activity should be performed in bouts of at least 10 minutes duration, and 30 minutes is a common used duration in the scientific literature to describe "Prolonged sedentary time" (Thorp et al., 2012; World Health Organization, 2010). Breaks in SB were defined as an activity above 25 CMP after having been more than 30 minutes below this threshold, in other words, the end of a sedentary bout.

Additionally, an analysis of time-of-day effect was also carried out. For this purpose, all outcomes were also calculated during 3 different periods of a day: morning (7:00 to 12:59), afternoon (13:00 to 18:59) and night (19:00 to 6:59) (Davis et al., 2011; Garriguet & Colley, 2012; Sartini et al., 2015).

Statistics

Data are presented as mean±SD values. The normality of data was checked with the Shapiro-Wilk test. Unpaired Student's *t*-test was used to analyze significant differences in variables between groups (or its non-parametric equivalent, the Mann-Whitney *U* test). In order to test if there was an interaction between group and period of the day two-way analysis of variance was carried out. Statistical analyses were performed using IBM SPSS (v.20 for Windows, Chicago, IL, USA) and the significance level was set at $P \leq 0.05$.

RESULTS

Eighteen centenarians [83% women; mean±SD (range): 100.8±0.8 (100–103) years] and eleven nonagenarians [91% women; 93.3±2.5 (90–98) years] (**Table 1**), met inclusion

criteria of the accelerometry analysis (i.e., five valid days with at least 10h recorded, including one weekend day). **Table 2** shows the results obtained by accelerometry. Centenarians had significantly lower CPM values (P=0.003, d=1.851) and steps per day (P=0.007, d=1.587) than their nonagenarian peers. The number of daily sedentary bouts (P=0.018, d=0.914) and breaks (P=0.019, d=0.971) was also lower in the former.

Figure 1 represents the number of steps per day during the last decades of life. Since our study begins in nonagenarians, data from Davis *et al*. is showed from 70-85 years. As can be seen, when the age increases, the decrease in the number of steps was more and more accentuated: -31% (70-74 *vs.* 80-84 years) -61% (75-79 *vs.* ≥85 years), -68% (80-84 *vs.* 90-99 years) and -75% (≥85 *vs.* ≥100 years).

Table 3 shows PA outcomes in different periods of the day. There was a significant interaction effect between the age group and the period of the day, on the PA performed (CPM, activity time and steps) (P≤0.001, η_p^2=0.018), which indicates that centenarians and nonagenarians PA were affected differently by period of the day. As can be seen in **Table 3** and **Figure 2**, nonagenarians had greater activity than centenarians mainly because of the morning activity. Similarly, the younger group had a greater activity at night. On the other hand, the afternoon activity was similar in both groups.

Finally, **Figure 2** shows the mean number of steps per hour throughout the day. There were observed three main peaks where nonagenarians' activity was significantly greater: early morning (7:00 to 10:59) at 14:00 and at the beginning of the night (20:00 to 22:59). Centenarians seem to increase their number of steps at the end of the morning while decrease progressively until night, although its activity was never significantly greater.

DISCUSSION

To our knowledge, this is the first evidence in which PA levels have been objectively assessed by accelerometers in centenarians. Centenarians were less active (CPM and steps per

day, -62% and -64% respectively) and in consequence more sedentary (30' sedentary bouts; +29%) than nonagenarians. This results indicate that even in people aged >90, PA level continue decreasing with age. Davis *et al*. also reported lower values of steps per day (by -61%) in persons aged ≥85 years as compared with younger older adults (75–79.9 years) (1,786 *vs* 4,543 steps/day, respectively), whereas the difference was smaller (-31%) when comparisons were made among younger age ranges, 70–74.9 years (5,661 steps/day) *vs* 80–84.9 years (3,880 steps/day), as can be seen in **Figure 1** (Davis et al., 2011), which represents a greater loss than the 7.5% decline per decade indicated by Doherty *et al.* in subjects from 45 to 79 years (Doherty et al., 2017).

Although rare, there are cases of centenarians who not only avoid the usual fall of PA but continue participating in sports competitions, even improving their sports performance (+11%) and maximal oxygen uptake (VO_2max) (+13%) between 101 and 103 years, which reflects the plasticity and ability of human tissues to favorably adapt to PA at any age (Billat et al., 2017). This could indicate that, although the decrease of PA seems to be higher during the last decades of life, it is never too late to start exercising given that 'oldest-old' adults (≥90 years) who are more active have a greater functionality (indeed, the distance reached in a 6-minute walk test is positively associated with PA levels)(Johannsen et al., 2008). Functional independence is directly dependent on physical fitness, i.e. ''the ability to carry out daily tasks with vigor and alertness, without undue fatigue and with ample energy to enjoy leisure-time pursuits and to meet unforeseen emergencies''(Park, 1989). In turn, physical fitness is determined by several measurable health-related phenotypes, including mainly cardiorespiratory fitness and muscle function.

SB has recently emerged as an independent risk factor for cardiovascular disease, diabetes, mental health problems and some cancers, as well as all-cause cancer and cardiovascular disease mortality (Biswas et al., 2015; de Rezende, Rey-López, Matsudo, & do

Carmo Luiz, 2014; Koster et al., 2012; Thorp, Owen, Neuhaus, & Dunstan, 2011). Some authors have pointed to PA decrement from youth to adulthood, and then PA and SB seem to be stable until retirement age, when another decline occurs (Johannsen et al., 2008). Along this line, centenarians group spent more than 15 hours per day in SB, which is twofold higher than the levels usually found in young older-adults (Wullems et al., 2016). Thus, nonagenarians spent 91% of their waking time in SB, with this value reaching 94% in centenarians, who in addition also showed a more inactive pattern. This indicates that the most rapidly growing subgroup of the population (i.e., oldest-old) is at the same time the most inactive.

On the other side, growing strong epidemiological evidence support that regular PA is an influencing factor for healthy aging and is associated with lower risk of all-cause mortality and major chronic diseases (Hallal et al., 2012). Centenarian group spent only 63 minutes a day actively, while nonagenarians spent 98 minutes. Several studies with mice have shown that voluntary wheel running decreases with age (Garcia-Valles et al., 2013; Holloszy, Smith, Vining, & Adams, 1985; Stolle et al., 2018). In addition, life-long spontaneous exercise improves survival (more mice attain old age) (Holloszy et al., 1985) and also ameliorates healthspan, i.e. the portion of the life span during which function is sufficient to maintain autonomy, control, independence, productivity and well-being (Garcia-Valles et al., 2013). So it could be hypothesized that although centenarians perform less PA now, the protective effect of lifelong PA could be a key factor that allowed them to achieve a long and healthy life. However, it should not be forgotten that exceptional longevity is a complex trait, some authors say that non-genetic factors, including diet, PA, health habits, and psychosocial factors contribute approximately 50% of the variability in human lifespan with another 25% explained by genetic differences (Rea, Dellet, & Mills, 2016).

In our study, both groups were more active during the morning than afternoon or night and nonagenarians were more active than centenarians during morning and even during night

(P<0.05). Results during the afternoon were similar between groups, being the PA during the morning the main contributor to the difference between nonagenarians and centenarians i.e. 7 min *vs*. 5 min of moderate-to-vigorous PA per hour (P≤0.001, η_p^2=0.003) or 124 *vs.* 50 steps per hour (P≤0.001, η_p^2=0.008). In consonance, morning has already been pointed out by other authors as one of the moments of greatest variability in PA levels, and therefore a great moment in order to implement PA enhancement programs (Sartini et al., 2015).

When time-of-day effect was analyzed for number of steps (**Figure 2**), we found similar results to previous studies in older adults (71-91 years), the highest number during the morning (with a peak between 10:00-11:59) and a small increase at 14:00 (Doherty et al., 2017; Sartini et al., 2015). However some slight differences deserve to be discussed. First, nonagenarians' showed another peak at the beginning of the night (20:00pm to 22:59pm) that could be due to cultural differences, since in Spain this period corresponds with dinner time (Eurostat, 2007). Also PA levels during the morning were different between groups. Nonagenarians seems to be more active at early morning (7:00 to 10:59) and centenarians at the end of the morning (9:00 to 13:59), this behavior could be related to the fact that older adults with high fatigability reach their peak of PA later in the morning (Wanigatunga et al., 2018), or it could also be because some participants were institutionalized and their schedules were more defined.

The identification of time patterns of behavior will allow to make some recommendations that could be taken into account when proposing an intervention with the oldest-old adults. Some interventions ideas could be as follow: (i) The morning is the time of greatest variability in PA, whereby is a good time to: try to extend the duration of bouts of PA and/or encourage to increase the intensity of PA in older adults with a level of functionality that allows it. (ii) It is important to try to individualize the intervention: adults with greater fatigability seem to reach their peak of activity at the end of the morning, time patterns may depend on cultural differences as well as whether the older adults are institutionalized or not.

(iii) The afternoon is the time of the day with the highest SB, as recent studies have shown (Nagai et al., 2018) replacing just 30 minutes of SB with light PA decreases the risk for frailty in older adults.

We believe that the main strength of this study comes from the elevated sample size, considering that exceptional longevity is a rare phenotype (≈1/10.000 individuals live to the age of 94–110) (Martin, Bergman, & Barzilai, 2007) and the novelty of the study because it's the first time that PA and SB have been objectively assessed in centenarians. Another important strength of our study is the measurement of PA and SB in everyday life during a whole week, using a device and cut points validated in older adults, so there is a great ecological validity. On the other hand, although most of the long-lived older adults are women and suffer from chronic diseases, one of the main limitations is selection bias since we use a sample of convenience. Other limitation is that all the participants are Spanish and Caucasian and the generalization of the results could be partly limited. In future research, and in order to replicate the results obtained with our cohort, future studies with more representative samples of exceptionally long-lived older adults are necessary.

Up to now it has been shown that the level of PA decreases progressively in the older adults, the present study confirms that this trend continues to worsen until the end of the human lifespan. A progressive decrease in PA and exercise capacity leads to a downward spiral of reduced physical function and health, which translates into increased health costs. Thus, maintaining high levels of PA and reducing SB must be viewed as a main priority for health policy makers.

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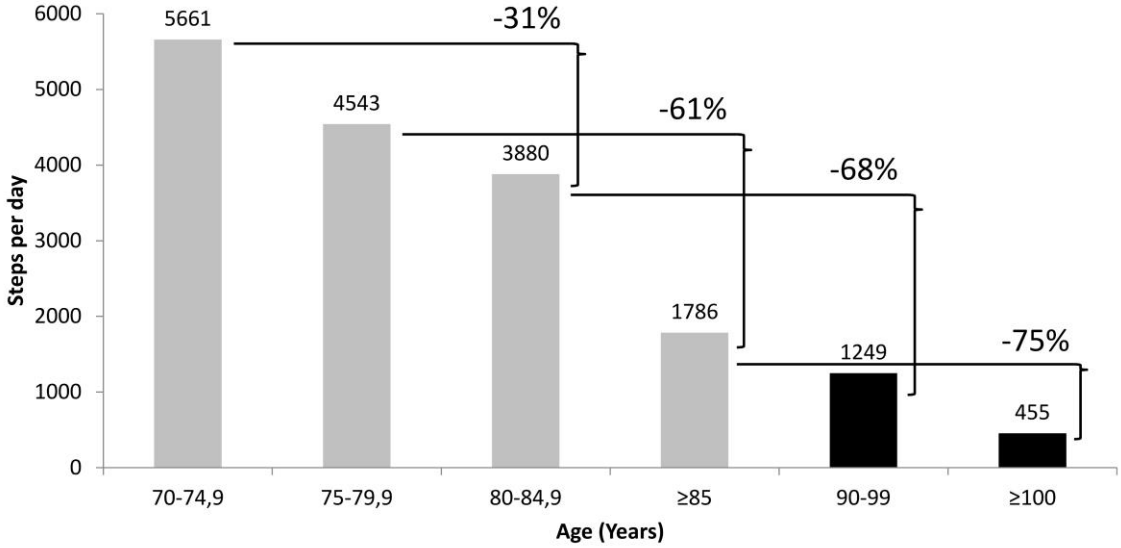

Figure 1: Number of steps per day during the last decades of life.
Columns in gray are data from Davis et al. (2011).

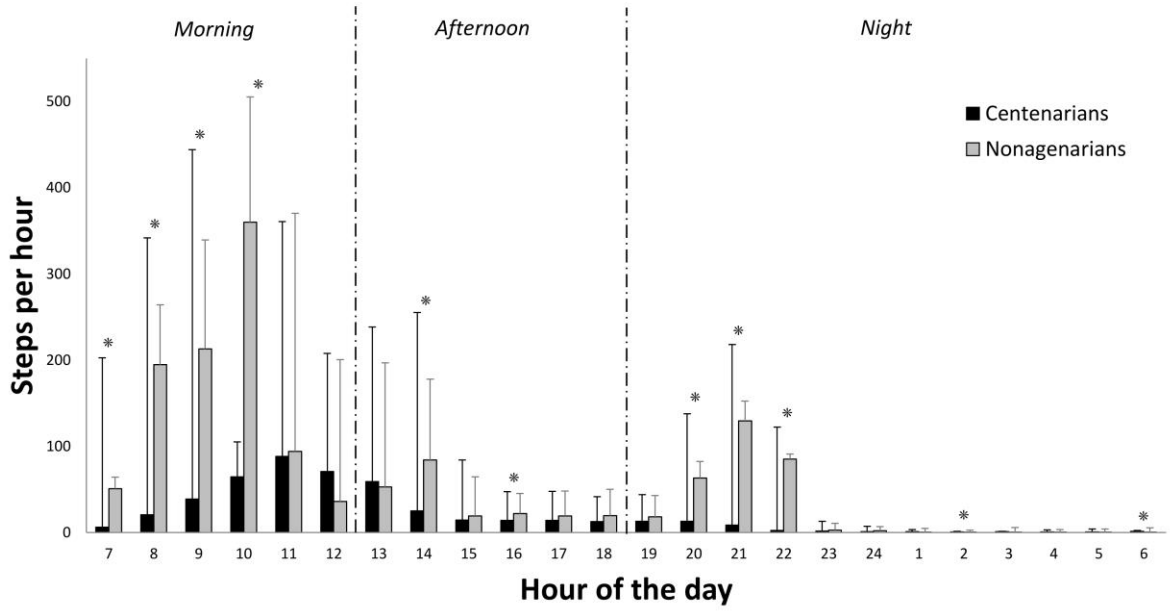

Figure 2: Mean number of steps per hour throughout the day. *= $p<0.05$

Table 1: Descriptive characteristics of centenarians and nonagenerians group.

Outcome		Centenarians	Nonagenarians
Age (years)		*100.8±0.8 (100–103)*	*93.3±2.5 (90–98)*
Sex (%)	Women	83	91
Chronic diseases (%)	Dementia	11	9
	CVD	11	0
	Osteoarthritis	22	64
	Cardiopathy	33	64
	Cancer	22	18
	Diabetes	6	18
	AHT	56	45
	COPD	0	9
	Others	50	82
	Total number	*2.4±1.3 (0-5)*	*5.5±1.7 (3-9)*
Number of drugs		*2.1±1.2 (0-4)*	*9.9±3.5 (2-14)*
Activities of Daily Living (%)			
Dressing	Independent	0	36
	Needs help	21	0
	Dependent	79	64
Bed to chair and back	Independent	7	36
	Needs help	14	18
	Dependent	79	46
Walk more than 50m	Independent	7	36
	Needs help	22	19
	Dependent	71	45
Bath or shower	Independent	0	27
	Needs help	0	0
	Dependent	100	73

Scale values are mean±standard deviation (SD) and (min-max); CVD= Cardiovascular disease; AHT= Arterial hypertension; COPD= Chronic obstructive pulmonary disease

Table 2: Physical activity and sedentary behavior in centenarians and nonagenarians group.

Outcome	Centenarians	Nonagenarians	*P-value**	*d*
Counts per minute for *y*-axis	17.6±7.1	46.1±23.7	**0.003**	1.851
Activity time (min/day)	63±38	98±59	0.061	0.722
Activity bouts (times/day)	0.55±0.71	1.17±1.16	0.130	0.663
Steps/day	455±237	1.249±776	**0.007**	1.587
Sedentary time (min/day)	920±193	1.007±167	0.225	0.483
Sedentary bouts (times/day)	5.2±1.5	6.8±2.0	**0.018**	0.914
Sedentary breaks (times/day)	5.0±1.5	6.7±2.0	**0.019**	0.971

Values are mean±standard deviation (SD). Significant *P*-values are in bold. *unpaired Student's *t* test. *d*= Cohen's d size effect.

Table 3: Physical activity outcomes during different periods of the day in centenarians and nonagenarians group.

Outcome	Centenarians			Nonagenarians		
	Morning	Afternoon	Night	Morning	Afternoon	Night
CPM	38.0±90.3*[a, n]	20.0±57.2 [m, n]	4.6±17.3*[m, a]	113.3±258.7*[a, n]	23.5±72.3 [m]	23.6±66.2* [m]
Activity time (min/h)	4.5±7.3*[n]	4.2±6.6 [n]	1.0±2.8*[m, a]	7.1±12.0*[a, n]	4.9±6.8 [m, n]	2.0±4.8*[m, a]
Steps/ hour	49.5±160.4*[a, n]	23.8±78.7 [m, n]	3.8±12.9* [m, a]	123.8±303.0*[a, n]	36.0±126.1 [m]	25.1±88.8* [m]

CPM= Counts per minute for *y*-axis. Values are mean±standard deviation (SD). * = Differences between centenarians and nonagenarians, same period of the day ($p<0.05$). [m] = Different to morning, same group ($p<0.05$). [a] = Different to afternoon, same group ($p<0.05$). [n] = Different to night, same group ($p<0.05$).

4.2 Artículo II

Bajo revisión en Medicine & Science in Sports & Exercise.

PHYSICAL ACTIVITY IN CENTENARIANS BEYOND CUT-POINT-BASED ACCELEROMETER METRICS

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ABSTRACT

Introduction: Assessment of physical activity (PA) levels can serve as basis for knowledge-based health counseling. Accelerometer-derived PA measures commonly rely on cut-points that are population-specific and would result in a floor effect when applied to centenarians. The aim of this study was to describe and compare PA characteristics at the end of the human lifespan using conventional cut-point-based *versus* novel cut-point-free accelerometer metrics.

Methods: Eighteen institutionalized centenarians (101.5±2.1 years, 72.2% female, 89% frail) wore a GENEActiv accelerometer mounted on the non-dominant wrist 24h/day for 7 consecutive days to objectively measure habitual PA. Conventional metrics, such as time spent in light-intensity PA (LiPA) and moderate-to-vigorous intensity PA (MVPA) were calculated according to standardized cut-points. Furthermore, the following cut-point-free metrics were evaluated: average acceleration (m*g*), intensity gradient and Mx metrics (m*g*), the latter measuring the PA intensity in the X most active minutes (Mx). For both cut-point-based and cut-point-free metrics, Mann–Whitney U tests were used to determine differences between dichotomous groups of various health outcomes (functional, cognitive and physical capacities; health-related quality of life; and 1-year survival).

Results: Depending on the cut-point, centenarians accumulated a median of 15 to 132 min/day of LiPA and 3 to 15 min/day of MVPA. The average acceleration was 9.2m*g* [Q1: 6.7m*g* – Q3: 12.6m*g*] and the intensity gradient was −3.2 [−3.3 - −3.1]. The distribution of Z-values revealed positive skew for MVPA, indicating a potential floor effect, whereas the skew magnitude was attenuated for cut-point-free metrics like intensity gradient or M5. Although both cut-point-based and cut-point-free metrics

presented associations with the studied health outcomes, only the cut-point-free M30 index was significantly associated with 1-year survival.

Conclusions: This is the first time that PA has been described in centenarians using cut-point-free metrics. Our results suggest that new analytical approaches could overcome cut-point limitations when studying the oldest-old. Future studies using these new cut-point-free PA metrics are warranted to provide more complete and comparable information across groups and populations.

INTRODUCTION

The population of “oldest-old”, i.e. people aged 80 and over, is growing faster than any other segment of the population and is projected to triple by 2050, reaching 426.4 million worldwide (1). The increase in total life expectancy is, however, not accompanied by an equivalent increase in healthy life expectancy, with 16-20% of life spent in late-life morbidity (2). Although aging leads to functional decline of all systems and eventually to death, a physically active lifestyle may attenuate the impact of age on morbidity and mortality (3,4). It is well known that physical activity (PA) levels decline with age, being “older adults”, i.e. people aged 65 and over, the most inactive segment of the population (5). This trend continues to worsen until the end of the human lifespan (>100 years) (6), as maintaining a physically active lifestyle becomes more difficult in the aging population due to various socio-environmental barriers and to a progressive lowering of physical functions and capabilities, especially in frail older people (7,8).

Knowledge-based counseling, including the PA recommendations by the World Health Organization, usually rely on epidemiological associations between objective measures and health outcomes (9). For this purpose, PA levels should be accurately assessed in all population segments, but very old individuals (i.e. centenarians) remain an understudied population.

For evaluation of habitual PA, accelerometry is considered as the gold standard since it is an objective method that precisely records bodily accelerations over long periods of time. The analysis of accelerations during the activities of daily living (ADLs) allows researchers to identify the proportion of time spent in sedentary activities or performing PA at different intensities [e.g. light (LiPA), moderate-to-vigorous (MVPA)]. PA patterns and relative intensities depend on physiological factors like age or cardiorespiratory

fitness. Considering that basal metabolism is substantially lower in older adults as compared to the general population, cut-points should be population-specific and protocol-specific (10,11). Despite recent efforts determining cut-points in people above 70 years of age, there are no established cut-points for 100-year-old or frail individuals. The use of cut-points validated in younger older adults would result in a floor effect when applied to centenarians, with the time spent by centenarians in LiPA or MVPA being under-estimated due to the inappropriateness of the “one size fits all” approach (12).

Novel analytical approaches have been recently developed to assess associations between accelerometer-derived PA measures and health parameters in epidemiological studies. These alternatives include the use of Mx metrics measuring the PA intensity in the X most active minutes (13). Also, they involve a deeper examination of the intensity distribution throughout the activity profile, calculating the intensity gradient (IG) in combination with a metric of overall PA volume defined by the average acceleration (14). These cut-point-free measures could help overcome the abovementioned floor effect.

The aim of this study was to describe PA at the end of the human lifespan through cut-point-free accelerometer metrics. The present study also aimed to compare cut-point-based *versus* cut-point-free accelerometer metrics at two levels: evaluating the floor effect in the different metrics, and exploring the associations of the metrics with a positive status in a variety of health outcomes. We hypothesized that cut-point-free accelerometer metrics could solve the limitations of the conventional cut-point-based measures when studying centenarians.

METHODS

Participants

The study population consisted of men and women living in different areas of the region of Aragon in Spain. Only institutionalized individuals reaching at least 100 years of age by the end of the year of the measurements were included. Bedridden centenarians or those going through an acute disease were excluded. Patients with reduced mobility, either helped by their caregivers, using walking stick or walker, and those suffering from chronic diseases or mental disorders such as dementia were included in the study given the high prevalence in the last decades of life. In total, nineteen volunteers (born between 1912 and 1920) were included in the study. After a clear explanation of the potential risks and benefits of the study, all volunteers (or their legally responsible tutor for older adults with cognitive impairments) provided written informed consent to participate in the study. This study was approved by the ethical committee for clinical research of Aragón (ID of the approval: PI18/381). It was conducted by adhering to the Declaration of Helsinki and complying with the European Union General Data Protection Regulation (EU 2016/679).

Protocol

Centenarians were evaluated at their own geriatric nursing home. All the assessments were carried out by the same team of researchers, using the same procedures and equipment. Each participant was evaluated in two sessions. In the first session, volunteers were requested to wear GENEActiv tri-axial accelerometers (ActivInsights Ltd., Cambridgeshire, United Kingdom) 24h/day for 7 consecutive days. The device was mounted on the non-dominant wrist and was set to record accelerations at 10 Hz. GENEActiv accelerometers were initialized and data downloaded in binary format using GENEactiv PC (version 3.2). In the second session, eight days after the first one,

accelerometers were collected and health outcomes were assessed in the following order: Health-Related Quality of Life (HRQoL); Mini-Mental State Examination (MMSE); Short Physical Performance Battery (SPPB); Fried's Frailty Phenotype (FFP); Barthel lndex; and Frailty Trait Scale-short form (FTS-5).

Health outcomes

Frailty was assessed by FFP (15) and FTS-5 (16). FFP classifies a person as frail if 3 or more of the following 5 criteria are met: unintentional weight loss; weak grip strength; self-reported exhaustion; slow walking speed; and low PA. FTS-5 is a shorter version of the Frailty Trait Scale (17), with similar performance in the diagnosis and evolution of frailty. FTS-5 evaluates 5 domains through 5 items: body mass index, Physical Activity Scale for the Elderly, progressive Romberg test, handgrip strength and walking speed. Each item ranges from 0 (best) to 10 (worst). A total score was calculated as the sum of all item scores (0 to 50) and >25 points was used as the cut-off point to identify frailty (16).

Functional independence was measured using the Spanish version of the Barthel Index of independence during ADLs: feeding, bathing, grooming, dressing, bowel control, bladder control, toileting, chair transfer, ambulation and stair climbing (18). The index yields a total score out of 100 and allows to classify elders in 5 levels: total dependence (0-20 points), severe dependence (21-60 points), moderate dependence (61-90 points), slight dependence (91-99 points) and independence (100 points) (19). The sample was dichotomized in 2 groups: "negative outcome" including 6 "totally dependent" centenarians and "positive outcome" including the remaining 13 participants with a score >20 points.

Cognitive capacity was assessed by the Spanish version of the MMSE (from 0-30 points), which is used worldwide to assess global cognitive functioning through the examination of different domains such as orientation to time, orientation to place, registration, attention and calculation, recall, language, repetition and ability to follow commands (20). The conventional cut-off score for cognitive impairment screening was used to classify subjects according to a dichotomous variable: subjects with ≤23 points were classified in the "negative outcome" group and subjects with 24 points or more were classified in the "positive outcome" group.

Physical capacity was measured using the SPPB test scores (from 1-12 points), depending on performance in: hierarchical standing balance test, gait speed over 4m and 5-sit-to-stand test (21). Centenarians were classified in four stages: dependent (1-3 points), frail (4–6 points), pre-frail (7–9 points) and robust (10-12 points) (22). According to these criteria, 12 centenarians were classified as "dependent" and were included in the "negative outcome" group, while the remaining 7 centenarians with a score >3 points were included in the "positive outcome" group.

HRQoL was assessed with the visual analog scale (VAS) of the Spanish EuroQoL-5 Dimension (EQ-5D) questionnaire (23). The EQ-5D is a standardized HRQoL questionnaire widely used throughout the world. In particular, the VAS is a vertical scale ranging from 0 "worst imaginable health state" to 100 "best imaginable health state" and participants are asked to tick the level they think their current health corresponds to. VAS cut-off values were extracted from the available normative data (Spanish values), corresponding to 66.7 for older males (≥75y) and 59.4 in the case of older females (≥75y) (24). Participants who had a VAS score below their cut-off value were classified in the "negative outcome" group, while participants who showed a HRQoL above the cut-off value were classified in the "positive outcome" group.

Date of birth and date of death were obtained from the Spanish National Dead Index (Ministry of Health, Consumer Affairs and Social Welfare). The study population was followed up for 1.5 years from baseline. Early mortality was defined as all-cause mortality within 1 year following the measurements. Centenarians in the early mortality group (≤1 year) where classified as "negative outcome" and those in the survival group where classified as "positive outcome".

Accelerometer processing

The analysis of accelerometry data was carried out using the GGIR 2.3-0 (25) package of the statistical programming language R v.3.5.1 (26). Non-wear time detection and minimum valid time requirements for each accelerometry register were evaluated using GGIR's default settings to facilitate comparability with previous studies. ***Supplementary Table 1*** includes the relationship between the nomenclature used throughout this paper and the variable names from GGIR output.

Time spent in LiPA (18-60m*g*) and MVPA (>60m*g*) were calculated using the sensitivity optimized cut-points proposed by Migueles et al. (10). These cut-points were established based on a population of older adults (≥70 years old) but not the oldest elders. Moreover, data were also analyzed using other previously reported cut-points based on Euclidean Norm Minus One *G* (ENMO), such as the cut-points for adults published by Hildebrand et al. (LiPA: 45.8-93.2m*g*; MVPA: >45.8m*g*) (27,28) and the cut-points for older adults published by Sanders et al. (LiPA: 57-104m*g*; MVPA: >104m*g*) (29).

In addition to these traditionally used metrics, recently proposed approaches were evaluated (30), including the average acceleration, the IG or the Mx metrics (14). Average acceleration (14) reflects the average acceleration throughout the entire measurement period and can be used as a proxy for total daily PA-related energy

expenditure (30) or PA volume. IG, calculated as the slope (negative) of the linear regression between natural logs of time and acceleration intensity, assesses PA intensity across all levels.

Mx metrics (13) evaluate the most active X minutes from a participant's daily activity (e.g. M30 refers to the acceleration above which the most active 30 minutes were spent), which can in turn be used to describe the distribution of intensities across different time frames and to establish a direct comparison with health-related PA guidelines. Here, the intensity levels corresponding to the most active 1, 5, 15, 30, 60, 120 and 480 minutes were recorded.

Statistical analysis

Statistical analyses were carried out using the statistical software Jamovi v.2.2.5. The statistical significance was set at an alpha value of 0.05. Descriptive values of all previously defined variables were obtained and the skewness and kurtosis of the variable distributions were quantified to detect a potential floor effect. Standardized values were calculated for each variable to allow comparability among them.

Given that the normality assumption was violated, as checked with the Shapiro-Wilk normality test, non-parametric tests were selected. After dividing the sample into dichotomous groups according to the abovementioned health and functional outcomes, Mann–Whitney U tests and its associated effect sizes (31) were used to identify differences in LiPA, MVPA, average acceleration, IG and Mx metrics between groups.

RESULTS

Participants

Eighteen out of nineteen centenarians had accelerometry registers that met the inclusion criteria for the analysis. Only one subject was excluded due to issues with the accelerometer during the recording. ***Table 1*** shows the descriptive characteristics of the centenarians: 72.2% of them were women and almost all were frail, i.e. 83.3% according to FFP and 88.9% according to FTS-5 (15,16).

Table 1. Outcome measures (mean and SD) for the overall group and for each dichotomized group according to health outcomes.

Outcome	Overall (*N* = 18)		Negative outcome			Positive outcome		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD
Age (years)	101.5	2.1	-	-	-	-	-	-
FFP (5-0)	3.3	0.9	-	-	-	-	-	-
FTS-5 (50-0)	33.1	6.1	-	-	-	-	-	-
Barthel (0-100)	39.7	23.5	6	17.5	2.7	12	50.8	21.2
MMSE (0-30)	22.1	5.9	9	17.6	5.1	9	26.6	1.4
SPPB (0-12)	2.9	2.8	11	1.3	1.3	7	5.6	2.4
VAS (0-100)	60.3	33.5	7	24.3	19.0	11	83.2	14.5
1-year survival	-	-	6	-	-	12	-	-

The scoring ranges for the outcomes are expressed as (worst score - best score). FFP = Fried's Frailty Phenotype; FTS-5 = Frailty Trait Scale-short form; Barthel = Barthel index of independence during activities of daily living; MMSE = Mini-Mental State Examination; SPPB = Short Physical Performance Battery; VAS = Visual Analog Scale of health-related quality of life. For each health outcome the sample was dichotomized into "negative outcome" and "positive outcome" groups. SD = Standard deviation.

Descriptive accelerometry results

Table 2 shows the descriptive results obtained by accelerometry. Depending on the cut-point, centenarians accumulated a median of 132 min/day [Interquartile range (IQR): 129.5 min/day] to 14.6 min/day [IQR: 32 min/day] of LiPA and 15.5 min/day [IQR: 36.7 min/day] to 3.3 min/day [IQR: 7.9 min/day] of MVPA. The cut-point-free measures for average acceleration (proxy for PA volume) and IG (proxy for PA intensity) were 9.2m*g* [IQR: 5.9 m*g*] and −3.2 [IQR: 0.2], respectively.

Table 2. Descriptive results of physical activity in centenarians.

Outcome	Minimum	Q1	Median	Q3	Maximum	Skewness	Kurtosis
LiPA 18m*g* (min/day)	11.2	89.5	132.0	219.0	313.0	0.321	-0.735
LiPA 45.8m*g* (min/day)	1.8	13.1	27.4	52.1	118.0	1.080	0.032
LiPA 57m*g* (min/day)	1.2	6.6	14.6	38.6	85.3	1.180	0.279
MVPA 60m*g* (min/day)	1.6	6.7	15.5	43.4	105.0	1.130	-0.135
MVPA 93.2m*g* (min/day)	0.6	1.7	4.4	13.1	60.8	2.100	4.370
MVPA 104m*g* (min/day)	0.4	1.3	3.3	9.2	53.5	2.520	6.900
Avg. Accel. (m*g*)	5.3	6.7	9.2	12.6	17.9	0.696	-0.786
IG	-3.6	-3.3	-3.2	-3.1	-2.5	1.260	3.410
Mx metrics (m*g*)							
M480	5.7	7.1	9.6	13.3	21.1	0.989	-0.038
M120	10.3	13.2	20.5	27.9	45.2	0.758	-0.566
M60	13.3	16.3	24.8	34.7	57.3	0.792	-0.372
M30	15.8	20.0	29.0	40.9	68.4	0.858	-0.142
M15	18.8	25.1	33.6	47.9	80.2	0.949	0.193
M5	25.0	35.0	40.9	59.9	97.7	1.010	0.476
M1	33.0	46.4	58.8	78.8	132.0	1.270	2.090

LiPA = Light-intensity physical activity; MVPA = Moderate-to-vigorous physical activity; Avg.Accel. = Average acceleration; IG = Intensity gradient; Mx metrics = acceleration above which a person's most active X minutes (Mx) are accumulated.

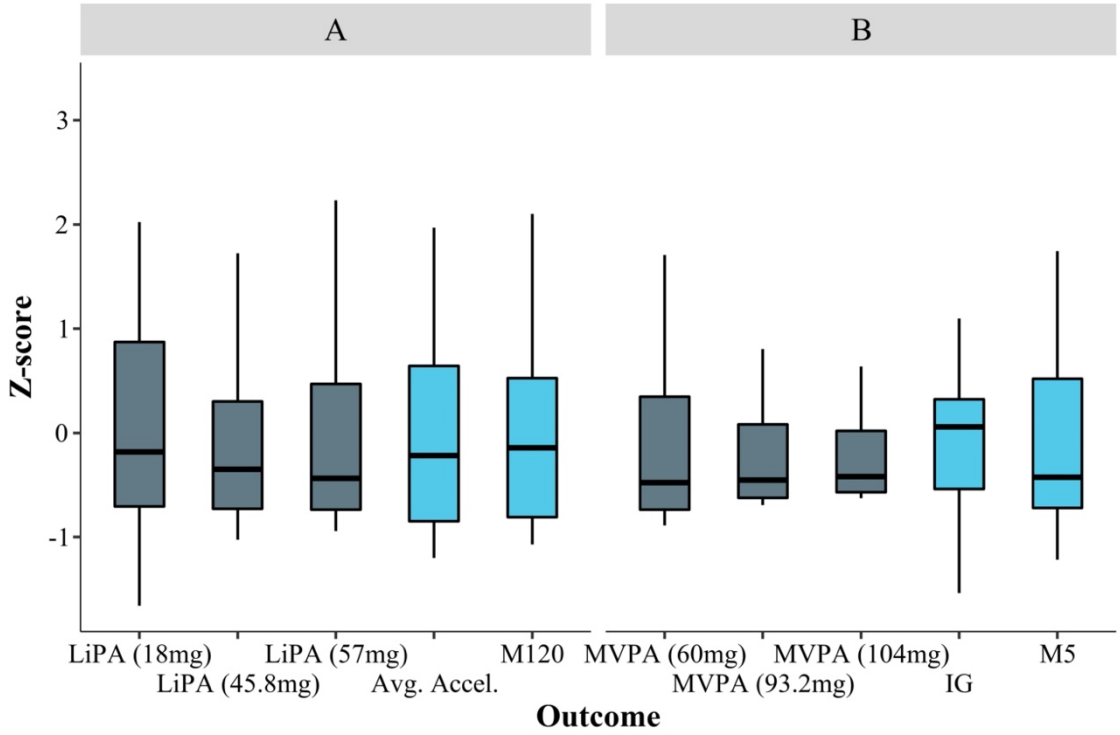
Comparison of cut-point-based and cut-point-free approaches

Figure 1 shows box plots representing the distribution of the Z-values for the conventional and cut-point-free PA accelerometer metrics. Regarding PA volume variables, see ***Figure 1.A***, no remarkable differences were observed in the floor effect between cut-point-based (LiPA) and cut-point-free metrics (i.e. Avg. Accel. and M120). ***Table 2*** shows a positive skew for MVPA (Skewness 1.13 to 2.52), indicating a potential floor effect for the conventional cut-points as hypothesized. The magnitude of the skew and kurtosis was attenuated for the cut-point-free metrics. Similar observations can be made from ***Figure 1.B***, presenting PA intensity variables.

Differences between dichotomous groups of health outcomes according to conventional and cut-point-free PA accelerometer metrics are presented in ***Table 3***. Both cut-point-based and cut-point-free metrics presented significant associations with variables like Barthel Index, MMSE or SPPB. Only the cut-point-free index M30 was significantly associated with 1-year survival.

Descriptive results for the dichotomized groups presenting significant differences are displayed in ***Table 4***. Centenarians who survived more than 1-year presented a median of 36.8 m*g* [IQR: 19.8 m*g*] for M30, while those who died in the year following the measurements reported a median of 19.6 m*g* [IQR: 7.7 m*g*] for M30.

Figure 1. Box plots representing the distribution of Z-values for the conventional (grey) and cut-point-free (blue) physical activity accelerometer metrics, corresponding to physical activity volume (panel A) and intensity (panel B).



LiPA = Light-intensity physical activity; Avg.Accel. = Average acceleration; M120 = acceleration above which the most active 120 minutes of the day are accumulated. MVPA = Moderate-to-vigorous physical activity; IG = Intensity gradient; M5 = acceleration above which the most active 5 minutes of the day are accumulated.

Table 3. Differences in physical activity variables between dichotomous groups for different health outcomes.

PA variable / Outcome	Barthel		MMSE		SPPB		VAS		1-year survival	
	U	**ES**	**U**	**ES**	**U**	**ES**	**U**	**ES**	**U**	**ES**
LiPA 18m*g*	13*	0.639	7*	0.827	12*	0.688	34	0.117	18	0.500
LiPA 45.8m*g*	7*	0.806	9*	0.778	6*	0.844	36	0.065	19	0.472
LiPA 57m*g*	7*	0.806	11*	0.728	5*	0.870	35	0.091	20	0.444
MVPA 60m*g*	7*	0.806	14*	0.654	5*	0.870	34	0.117	19	0.472
MVPA 93.2m*g*	6*	0.833	15*	0.630	5*	0.870	32	0.169	20	0.444
MVPA 104m*g*	4*	0.889	15*	0.630	5*	0.870	30	0.221	22	0.389
Avg. Accel.	9*	0.750	13*	0.679	7*	0.818	33	0.143	15	0.583
IG	9*	0.750	15*	0.630	11*	0.714	31	0.195	26	0.278
Mx metrics										
M480	10*	0.722	11*	0.728	7*	0.818	33	0.143	17	0.528
M120	8*	0.778	14*	0.654	5*	0.870	33	0.143	16	0.556
M60	9*	0.750	14*	0.654	5*	0.870	34	0.117	15	0.583
M30	10*	0.722	15*	0.630	6*	0.844	33	0.143	14*	0.611
M15	11*	0.694	17*	0.580	7*	0.818	34	0.117	16	0.556
M5	9*	0.750	17*	0.580	5*	0.870	34	0.117	18	0.500
M1	10*	0.722	16*	0.605	6*	0.844	35	0.091	21	0.417

PA= physical activity; LiPA = Light-intensity physical activity; MVPA = Moderate-to-vigorous physical activity; Avg.Accel. = Average acceleration; IG = Intensity gradient; Mx metrics = acceleration above which a person's most active X minutes (Mx) are accumulated. * = Significant differences between "negative outcome" and "positive outcome" groups ($p \leq 0.05$, Mann-Whitney *U*-test). U = U Statistic. ES = Effect size. Barthel = Barthel index of independence during activities of daily living; MMSE = Mini-Mental State Examination; SPPB = Short Physical Performance Battery; VAS = Visual Analog Scale of health-related quality of life.

Table 4. Descriptive results of physical activity in the dichotomized groups of centenarians (median and interquartile range) for the health outcomes with statistical differences.

PA variable / Outcome	Barthel		MMSE		SPPB	
	Negative (N=6)	**Positive (N=12)**	**Negative (N=9)**	**Positive (N=9)**	**Negative (N=11)**	**Positive (N=7)**
LiPA 18m*g*	81.5 (53.8)	169 (115)	89.1 (40.6)	209 (85.0)	92.8 (68.9)	231 (83.0)
LiPA 45.8m*g*	10.3 (8.0)	51.0 (54.2)	14.0 (11.2)	52.3 (73.1)	14.0 (19.5)	65.3 (58.6)
LiPA 57m*g*	5.4 (3.8)	32.1 (36.5)	7.1 (7.6)	34.5 (53.5)	7.1 (10.4)	41.3 (37.2)
MVPA 60m*g*	5.7 (3.5)	35.0 (65.8)	6.9 (8.1)	39.0 (61.9)	6.9 (10.7)	79.0 (46.9)
MVPA 93.2m*g*	1.5 (0.8)	10.4 (19.7)	1.7 (2.8)	12.5 (19.4)	1.7 (2.9)	23.8 (21.3)
MVPA 104m*g*	1.1 (0.5)	7.2 (13.4)	1.4 (1.9)	8.7 (13.0)	1.4 (2.2)	16.3 (15.8)
Avg. Accel.	6.7 (1.4)	11.2 (6.8)	6.5 (1.5)	10.5 (5.3)	7.2 (3.0)	15.1 (4.6)
IG	-3.4 (0.2)	-3.2 (0.1)	-3.4 (0.2)	-3.1 (0.1)	-3.3 (0.3)	-3.1 (0.2)
Mx metrics						
M480	7.3 (1.8)	12.2 (6.5)	6.8 (1.9)	11.1 (7.8)	7.9 (3.3)	14.6 (7.5)
M120	12.1 (3.3)	24.8 (17.7)	13.2 (4.4)	25.9 (13.3)	13.2 (9.2)	35.3 (11.9)
M60	15.8 (3.8)	32.3 (20.5)	16.4 (4.3)	33.8 (13.1)	16.4 (9.7)	42.4 (13.5)
M30	20.2 (4.6)	39.2 (22.9)	20.8 (4.2)	40.7 (13.4)	20.8 (9.5)	49.5 (15.6)
M15	24.7 (6.0)	45.6 (24.2)	25.6 (6.5)	46.0 (12.3)	25.6 (9.8)	55.4 (18.2)
M5	33.8 (6.7)	57.2 (26.9)	35.8 (6.1)	59.0 (14.8)	35.8 (9.3)	67.0 (18.3)
M1	44.8 (4.0)	70.5 (26.0)	46.8 (10.4)	74.9 (15.3)	46.8 (16.0)	81.4 (17.2)

PA= physical activity; LiPA = Light-intensity physical activity; MVPA = Moderate-to-vigorous physical activity; Avg.Accel. = Average acceleration; IG = Intensity gradient; Mx metrics = acceleration above which a person's most active X minutes (Mx) are accumulated. For each health outcome the sample was dichotomized into negative = "negative outcome" and positive = "positive outcome" groups. Barthel = Barthel index of independence during activities of daily living; MMSE = Mini-Mental State Examination; SPPB = Short Physical Performance Battery.

DISCUSSION

The daily time spent by a centenarian in LiPA and MVPA varies greatly depending on the cut-point used for the calculation. Our centenarians showed a potential floor effect in MVPA, being intensified in the most exigent cut-points. Regarding cut-point-free metrics, decreased PA volume and intensity could be observed in centenarians as compared to values reported in younger populations. This age-related decrease in PA volume and intensity up to the limit of human lifespan should be confirmed in futures studies providing more extensive characterization in the 80-100-year-old population. The advantages and disadvantages of cut-point-free metrics with respect to cut-point-based metrics are discussed below.

Descriptive accelerometry results

To the best of our knowledge, there is only one published study that assessed PA levels in centenarians using accelerometry (6). That study evaluated conventional metrics and, thus, just a few of the reported results can be directly compared. In addition, it should be taken into account that the cut-points, brand and location of the accelerometer were different between studies. The centenarians in (6) accumulated a mean of 63 min/day of active time (LiPA and MVPA), with the centenarians in the present study accumulating 17.9 to 147.5 min/day depending on the cut-point used. One of the main conclusions from (6) was that the decline in PA levels continues to worsen until the end of the human lifespan (6), which should be interpreted with caution, as it depends on the cut-point used in the evaluation. As an example, if the lowest cut-point reported in (10) were used, our centenarians would perform a median of 147.5 min/day of active time, being higher than the 98 min/day reported in nonagenarians (6), or the 117.6 min/day measured in subjects aged 85 years or older (32).

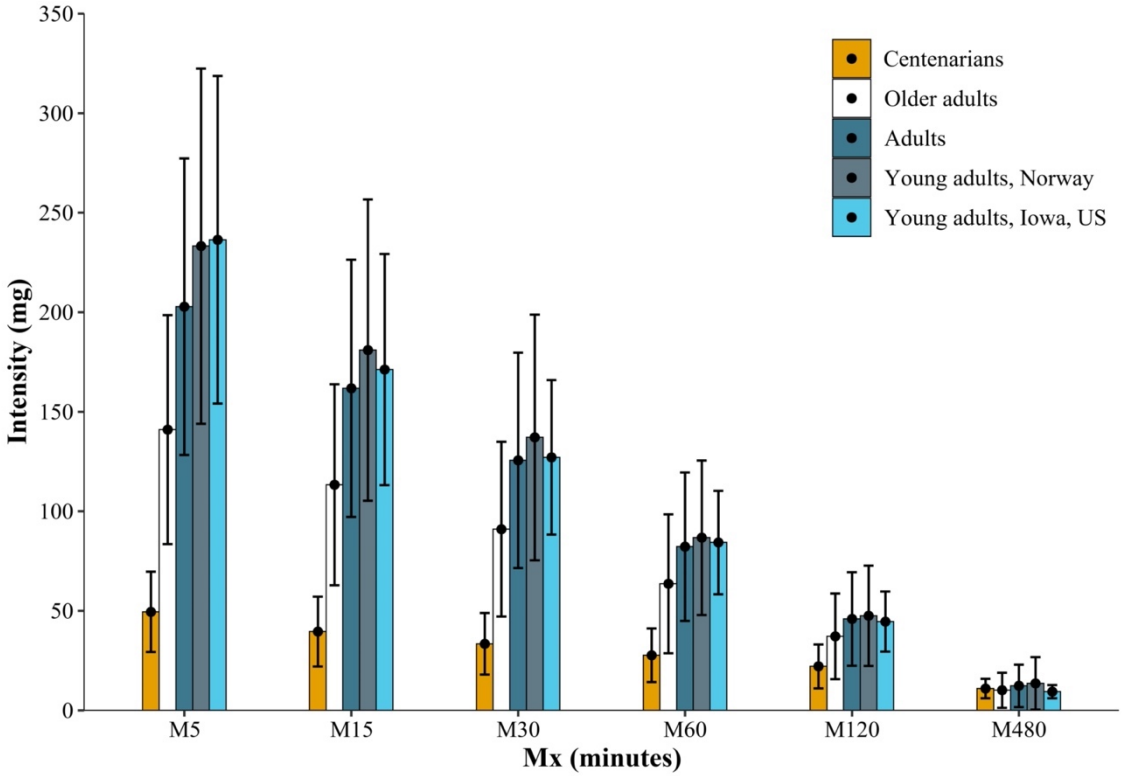
The selection of a cut-point is avoided when using cut-point-free metrics, since they are not based on intensity thresholds. There are currently no available cut-point-free data for octogenarians and nonagenarians. In this study, average acceleration in centenarians was 9.2m*g*. Previous studies have reported values of 27.1m*g* and 34.3m*g* in populations of postmenopausal women and 13-14yr adolescent girls, respectively (33). Considering that more than 7.2% of all-cause deaths and up to 8% of non-communicable diseases are attributable to physical inactivity (34), it is worrying that the most rapidly growing subgroup of the population (i.e. oldest-old) is highly inactive. The physical inactivity of the oldest-old should be viewed as an urgent priority for policy makers, given its implications for HRQoL and the associated healthcare cost (35).

For another cut-point-free metric like IG, a decline with age has been reported: -1.96 (Sample mean age: 9.6yr), -2.19 (12.3yr), -2.28 (13.6yr), -2.55 (41.2yr), -2.66 (46.2yr), -2.74 (59.0yr), -2.74 (64.2yr) (33). In concordance with this, our centenarians rendered an IG value of -3.2 [IQR: 0.2]. These results can be interpreted in light of the independent positive association between IG and physical function (14) and the fact that aging leads to functional decline of all systems (4).

Regarding Mx metrics, an age-related decrease can be observed for all Mx durations, being more pronounced for short-duration Mx, see ***Figure 2***. According to these results, centenarians perform all their efforts at a more similar intensity, while young people can reach high intensities in short-duration efforts. Nevertheless, our results should be interpreted with caution, since our sample is just composed of institutionalized centenarians. A broad variation in functional capacity can be observed in centenarian populations, with some centenarians performing all ADLs and others being bedridden (36), resulting in a wide range (2-89%, mean 37.3%) of centenarians living in geriatric nursing hommes across European countries (37). Due to the aforementioned selection

bias, certain centenarian populations could show equal or even improved results than those of older adults. In fact, there are some inspirational examples of centenarian athletes who continue participating in sport competitions, including marathon or 1500m swimming (38).

Figure 2. Mx metrics during the lifespan.



Mx metrics = acceleration above which a person's most active X minutes are accumulated (i.e. M120 refers to the intensity at which the most active 120 minutes of the day were spent). Young adults, Adults and Older adults are normative data from Norway by HS Rosfjord (39) and Young adults from US are data from Rowlands et al. (40).

Comparison of cut-point-based and cut-point-free approaches

The limitations of cut-points are well known and lead to some problems that we have noticed throughout this article (41). First, cut-points are protocol- (e.g. accelerometer placement) and population- (e.g. age group) specific, therefore: i) results are not comparable across studies; ii) the time spent by a centenarian in LiPA and MVPA can vary greatly depending on the cut-point used; iii) scientists have to select one among the many available cut-points for a population e.g. older adults, with no cut-points available for specific age segments like centenarians (10,29,42). Cut-point-free metrics emerged as a solid alternative since they are population-independent, although are wear-site specific and may differ between some brands of monitors (13).

A second limitation of cut-points is that two participants score very different if one has activity falling just above the cut-point and one has activity falling just below the cut-point (41). These crude boundaries between intensity levels do not exist in human physiology. Third and last, many participants fail to reach any activity above cut-points (particularly in the vigorous range) (41). In particular, our centenarians showed a potential floor effect in MVPA, being intensified in the most exigent cut-points. This is avoided when using cut-point-free metrics, with no participant scoring near zero values. We expected a more attenuated right-skewed distribution for the variables that cover the intensity spectrum continuously (i.e. Average acceleration and IG) than for Mx metrics, which was confirmed for average acceleration while IG skewness was similar to that of the Mx metrics (13).

With the Mx approach, data is not collapsed into categories but the continuous nature of the data is maintained and post-hoc interpretations can be made in relation to any cut-point (e.g. in order to see the prevalence of meeting PA guidelines) and/or accelerations

indicative of typical activities, facilitating the development of public-health friendly recommendations (13). Moreover it should be highlighted that in the Mx metrics, minutes can be accumulated in any way across the day, with no need for the activity to be in bouts, being coherent with the "*every move counts*" perspective from the World Health Organization PA recommendations (9). As an example Rowlands et al. (41), estimated MVPA thresholds representative of a brisk walk (170m*g*) or a fast walk (250m*g*) for adults, and our centenarians do not reach those accelerations even in M_1. However, the descriptive data presented in this manuscript can be compared in the future with as many alternative cut-points as needed, for example if the VO_{2net} age-equivalent cut-points were expanded to the whole human lifespan (43). In the same vein, research in the area of cut-point-based metrics is moving towards post-data collection approaches such as personalized accelerometer cut-points using machine learning (12).

Previous studies stated that: "*Future research should assess how the PA profile is related with health outcomes by age and disease categories with a view to informing acccelerometer-driven PA prescriptions and recommendations"* (44). The present study responds to this demands, providing an insight into the PA profile of people who have lived 20-30 years longer than the average westerner. The dose-response associations between PA and health are so strong (45) that, despite its limitations, conventional cut-point-based metrics also found differences between groups with "negative" *vs.* "positive" functional independence, cognitive capacity and physical capacity. Therefore, conventional cut-point-based metrics preserve certain clinical utility since they are capable of identifying health status in our sample.

Currently the World Health Organization PA guidelines recommend that older adults should be as physically active as their functional ability allows (9). The present study provides PA profile descriptive results for a specific population, i.e. institutionalized

centenarians, as well as for subgroups associated with “positive outcome” in different health variables, see ***Table 4***. As an example, if the proposed 70m*g* threshold representative of a slow walking for adults is applied (40), descriptive results show that the “slow walking” intensity can be mantained during 1 minute (i.e. M1) by those centenarians with “positive outcome” in functional independence, cognitive capacity and physical capacity. This information may result in evidence-based PA guidelines for institutionalized centenarians or as an objective for maintaining these specific health outcomes until the end of our lives. Moreover, the applicability of accelerometers is not restricted to evaluation of PA outcomes. When accelerometer-driven PA guidelines are available for the oldest old, accelerometers could be used to motivate them to reach evidence-based goals on PA intensity, duration, timing or type (14).

Strengths and limitations

The present study has several main strengths. One of them is the exceptionality of the sample, particularly considering that being centenarian is a rare phenotype 7.9/100000 world inhabitants, 31.2/100000 U.S.A (46), 21.6/100000 Europe (47). The percentage of women in our sample was similar to the overall centenarian population in Europe (83% women) (37). This study is novel and represents the first study in centenarians that assessed PA using cut-point-free metrics. Another important strength of our study is the measurement of PA in everyday life during a whole week using a validated device and calculating different cut-point-based variables. In addition, several health outcomes were measured, with centenarians being followed up during a year for early mortality. Therefore the study is not merely descriptive, but also explores the relationship between the PA profile and health outcomes. Last but not least, the study makes a comparison of cut-point-based and cut-point-free approaches, and discusses possible future application of accelerometers in centenarian populations.

On the other hand, the main limitation to be acknowledged is the specificity of the sample. All the participants are Spanish, white and institutionalized. Consequently, generalization of our results could be partly limited. In future research, samples including non-institutionalized centenarians would allow to complete the image, expanding and confirming the results obtained by this study.

CONCLUSIONS

This is the first time that PA has been described in centenarians using cut-point-free metrics. In line with literature reports describing that cut-point-free metrics presents an age-related reduction in PA volume and intensity; our centenarians had the lowest values in all the variables. This is in contrast to cut-point-based metrics like MVPA, which presented a floor effect, suggesting that cut-point-free approaches could overcome cut-point-based metrics limitations when studying the oldest-old. Both cut-point-based and cut-point-free measures were related to health states, but the cut-point-free M30 was the only one related to early mortality. Future studies are warranted to confirm the value of the cut-point-free PA metrics in centenarians.

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Supplementary Table 1. Correspondence between the nomenclature of the present paper and variable names from GGIR outputs.

Nomenclature in this paper	**Variable names in GGIR**	**Results file in GGIR**
LiPA	dur_day_total_LIG_min_pla	Part 5
MVPA	dur_day_total_MOD_min_pla	Part 5
Average acceleration	AD_mean_ENMO_mg_0-24hr	Part 2
Intensity gradient	AD_ig_gradient_ENMO_0-24hr	Part 2
M1	p99.93_ENMO_mg_0-24h_fullRecording	Part 2
M5	p99.65_ENMO_mg_0-24h_fullRecording	Part 2
M15	p98.96_ENMO_mg_0-24h_fullRecording	Part 2
M30	p97.92_ENMO_mg_0-24h_fullRecording	Part 2
M60	p95.83_ENMO_mg_0-24h_fullRecording	Part 2
M120	p91.67_ENMO_mg_0-24h_fullRecording	Part 2
M480	p66.67_ENMO_mg_0-24h_fullRecording	Part 2

4.3 Artículo III

Borrador presentando resultados preliminares.

EFFECTS OF 12-WEEK RESISTANCE EXERCISE TRAINING IN FRAIL INSTITUTIONALIZED CENTENARIANS: A RANDOMIZED CONTROLLED TRIAL.

KEYPOINTS

Question: Can frail institutionalized people over 100 years old benefit from exercise training?

Findings: Significant group-by-time interaction was observed and no adverse effects of exercise training were reported. Physical function, functional independence, frailty and health-related quality of life were improved in the intervention group after 12 weeks of resistance training.

Meaning: No one is too old or too unfit to benefit from resistance training, being a safe intervention despite the frailty of the centenarians.

ABSTRACT

Importance: The population aged 80 and over is growing faster than any other age groups in the world, with the number of centenarians being increasing too. The oldest-old are at high risk of intrinsic capacity deterioration with subsequent loss of functional independence, which translates into increased personal vulnerability and health cost.

Objective: To determine whether a resistance training intervention can improve physical function, functional independence, frailty and health-related quality of life in frail institutionalized elderly over 100 years old.

Design: Multicenter, pilot randomized controlled trial.

Setting: Conducted at 11 geriatric nursing homes in Spain.

Participants: A total of 12 frail institutionalized centenarians (101.33±2.06 yrs, 83.3% female) were included between June 11, 2019, and March 15, 2020.

Intervention: Centenarians were randomized to an intervention (n = 6) or a control group (n = 6). The intervention group performed supervised resistance training (1-3 sets of 8-10 repetitions as fast as possible, at 50-70% of the estimated one-repetition maximum; 8 exercises) at 2 sessions/week for 12 weeks.

Main Outcome(s) and Measure(s): Physical function [Short Physical Performance Battery (SPPB), Physical Performance Mobility Examination (PPME), isometric knee extension strength (IKE) and 30-second Sit-to-stand], functional independence [Barthel index], frailty [Fried's Phenotype and FTS-5] and health-related quality of life [EuroQoL-5D index (EQ-5D) and Visual Analog Scale (VAS)] were evaluated in both groups before and after the intervention.

Results: Repeated measures ANOVA revealed significant group-by-time interaction ($p \leq 0.05$) for all the measured outcomes. After the 12-week training period, the intervention group significantly improved all the variables except for FTS-5: PPME (Baseline: 3.8±2.6points *vs.* Post-training: 6.5±3.3points), IKE (9.7±4.3kg *vs.* 12.5±3.9kg), Barthel index (32.50±18.64points *vs.* 50.00±19.24points), Fried's Phenotype (3.83±0.75 *vs.* 3.00±0.63) and EQ-5D (0.112±0.118 *vs.* 0.233±0.090) among others (all $p<0.05$).

Conclusions: To our knowledge this is the first time that the effects of 12-week resistance training have been described in centenarians. The results suggest that no one is too old to benefit from resistance training, delay the age-related loss of functionality and improve the quality of life. Additionally, no major adverse effects were noted over the intervention period despite the frailty of the centenarians.

INTRODUCTION

The population of “oldest-old” (i.e. people aged ≥80 years) is growing faster than any other age segment, and is projected to reach 426.4 million individuals in the world by 2050 (1). Within this group the number of individuals aged 100 and over has risen exponentially, with currently 621,000 centenarians (7.9/100,000 worldwide, 31.2/100,000 U.S.A), and the United Nations projections suggest there will be 3.2 million centenarians across the globe in 2050 (32.8/100,000 worldwide, 112.3/100,000 U.S.A) (1).

The oldest-old are at high risk of intrinsic capacity decline (i.e. physical and cognitive impairment) with subsequent loss of functional independence (2). No single drug can reverse the age-related deterioration in intrinsic capacity. However, emerging evidence supports the health benefits of exercise training, also in frail, institutionalized or hospitalized older adults (2). Even small increases in physical activity levels have been associated with a 2-year improvement in life expectancy for the oldest-old (3). Resistance training induces multiple health benefits among older adults, including improvements in: muscle mass, bone mass, muscular strength, independence, risk of falls, chronic disease management, well-being and quality of life (4).

The benefits of exercise training have not been assessed at the limits of human lifespan (5,6). Therefore, the aim of this randomized controlled trial was to examine the effects of 12-weeks supervised resistance training on physical function, functional independence, frailty and health-related quality of life in frail institutionalized centenarians. We hypothesized that exercise training would significantly increase muscle strength which in turn could improve some of the other study outcomes such as functional independence.

METHODS

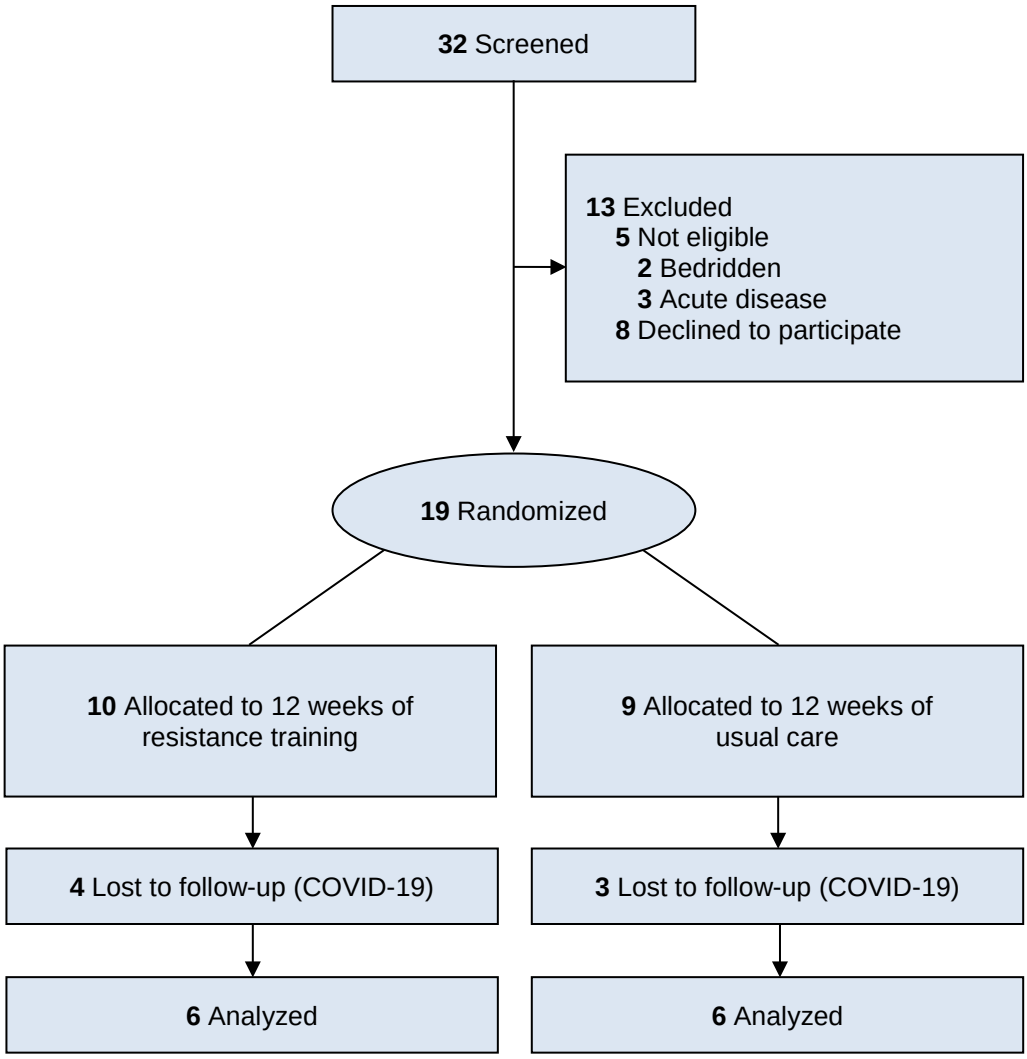
Study Design and Settings

This was a multicenter pilot randomized control trial performed from June 2019 to March 2020. Centenarians were randomized in a 1:1 fashion to either a control group of usual care or an intervention group of 12-week resistance training plus usual care. Participants were evaluated at baseline and at the end of the 12-week period. After a clear explanation of the potential risks and benefits of the study, all the participants (or their legally responsible for subjects with cognitive problems) provided written informed consent to participate in the study. The study was approved by the ethical committee for clinical research of Aragón, Spain (#PI18/381), was conducted by adhering to the Declaration of Helsinki and complying with the European Union General Data Protection Regulation (EU 2016/679).

Participants

Women and men aged 100 years and over living in 11 geriatric nursing homes were screened. Participants were excluded if they were bedridden, going through an acute disease or presented any clinical condition contraindicating physical exercise as outlined in the American College of Sports Medicine's Guidelines. Although 19 centenarians were finally enrolled, only 12 completed the study, since 7 participants could not finish the entire protocol due to the COVID-19 lockdown (***Figure 1***).

Figure 1. CONSORT Flow Diagram Depicting the Study Design



Resistance Training Program

The intervention group performed twice per week, nonconsecutive, resistance training sessions over 12 weeks (24 sessions in total). All the sessions were face-to-face, in the gym of the geriatric nursing home, one person at a time and supervised by an experienced (5 years) strength and conditioning trainer (MSc in Sports Science). After the baseline assessment, participants enrolled in the program conducted the corresponding resistance exercise routine (Type 0-2) with 8 different exercises according to their Functional Ambulation Classification (FAC) level (***Supplement 1***) (7,8).

Each session lasted 40-60 minutes, including a 10-minute warm-up and 30-50 minutes of resistance training. Warm-up was composed of 1 set of 8 single-joint seated exercises without resistance, 10 repetitions, 30-second rest between exercises. Resistance training consisted of 1-3 sets of the 8-exercise routine adjusted to FAC (8-10 repetitions as fast as possible, at 50-70% of the estimated one-repetition maximum) with resting periods of 1 minute between exercises and 3-5 minutes between sets (4). Load, number of sets and type of exercise routine were adjusted to the new physical capacity level every two weeks.

Outcomes

The primary outcome was physical function measured by: Short Physical Performance Battery (SPPB) (9), Physical Performance Mobility Examination (PPME) (10), isometric knee extension (IKE) strength (Lafayette Manual Muscle Testing Systems, Lafayette, IN, USA), and 30-second sit-to-stand. Secondary outcomes included: functional independence (Barthel index of independence during activities of daily living (ADLs)) (11); frailty [Fried's Frailty Phenotype and Frailty trait scale-short form (FTS-5)] (12,13); and health-related quality of life with the EuroQoL-5 Dimension questionnaire [EuroQoL-5D index (EQ-5D) and Visual Analog Scale (VAS)] (14).

Statistical analysis

Exploratory data analysis and the Kolmogorov-Smirnov test demonstrated the normality of the data. Variables are expressed as mean±standard deviation (SD). A repeated measure ANOVA was performed to analyze group-by-time interaction. Paired T-test was calculated for within-group comparisons from baseline to the end of the 12-

week period. Independent samples T-test was used for comparisons between groups at baseline and after the 12-week period. All analyses were performed using IBM SPSS (version 25; Chicago, IL, USA). The significance level was set at $p \leq 0.05$.

RESULTS

12 institutionalized centenarians (101.33±2.06 years, 83.3% female) completed the study. ***Table 1*** shows the descriptive characteristics of the two study groups at baseline, with no statistical differences found between groups. All the centenarians were frail according to Fried's Frailty Phenotype and FTS-5.

Table 1. Descriptive characteristics of the intervention and control groups at baseline

Outcome	Intervention (N= 6)	Control (N= 6)
Age (years)	101.08 ± 2.74	101.58 ± 1.29
Sex (% female)	66.7%	100%
Body mass (kg)	49.50 ± 9.74	59.15 ± 19.57
Height (m)	1.53 ± 0.03	1.54 ± 0.06
Body mass index ($kg \cdot m^{-2}$)	21.06 ± 3.96	24.74 ± 7.65
FAC = 0; 1; 2 (%)	33%; 33%; 33%	50%; 33%; 17%
SPPB (range 0-12)	2.33 ± 2.07	1.00 ± 1.10
PPME (range 0-12)	3.83 ± 2.64	3.67 ± 1.97
Isometric knee extension (kg)	9.67 ± 4.31	7.40 ± 3.87
30-second sit-to-stand (Nº) ₸	8.33 ± 1.53	5.00 ± 3.00
Barthel (range 0-100)	32.50 ± 18.64	23.33 ± 7.53
Fried (range 5-0)	3.83 ± 0.75	3.50 ± 0.84
FTS-5 (range 50-0)	34.08 ± 4.04	38.25 ± 2.14
EQ-5D (−0.224 - 1)	0.11 ± 0.12	0.09 ± 0.10
VAS (range 0-100)	39.17 ± 35.83	56.67 ± 32.04

Values are expressed as mean ± standard deviation (SD) or %. The scoring ranges for the test are expressed as (worst score – best score). FAC= Functional Ambulation Classification; FAC 0= cannot ambulate; FAC 1= continuous manual contact; FAC 2= light assistance. SPPB= Short Physical Performance Battery. PPME= Physical Performance Mobility Examination. ₸= only centenarians with a Functional Ambulation Classification of 1-2 were able to perform the test. Barthel= Barthel index of independence during activities of daily living. Fried= Fried's frailty phenotype. FTS-5= Frailty trait scale-short form. EQ-5D= EuroQol-5D index. VAS= Visual Analog Scale.

Repeated measures ANOVA revealed significant group-by-time interaction ($p \leq 0.05$) for all the measured outcomes (see ***Table 2***).

Table 2. ANOVA group-by-time interaction

Outcome	*p*	η^2 Effect size (eta^2)
SPPB (range 0-12)	0.006 *	0.544
PPME (range 0-12)	<0.001*	0.875
Isometric knee extension (kg)	0.013 *	0.515
30-second sit-to-stand (Nº) ₸	0.007 *	0.866
Barthel (range 0-100)	<0.001*	0.771
Fried (range 5-0)	0.001 *	0.653
FTS-5 (range 50-0)	0.027 *	0.399
EQ-5D (−0.224 - 1)	<0.001*	0.776
VAS (range 0-100)	0.001 *	0.680

*= $p \leq 0.05$. The scoring ranges for the test are expressed as (worst score – best score). SPPB= Short Physical Performance Battery. PPME= Physical Performance Mobility Examination. ₸= only centenarians with a Functional Ambulation Classification of 1-2 were able to perform the test; Intervention (Baseline N=4; Follow-up N=5); Control (Baseline N=3; Follow-up N=2). Barthel= Barthel index of independence during activities of daily living. Fried= Fried's frailty phenotype. FTS-5= Frailty trait scale-short form. EQ-5D= EuroQol-5D index. VAS= Visual Analog Scale.

When studying within-group comparisons from baseline to the end of the 12-week period, the intervention group significantly improved all the variables, except for FTS-5 (see ***Table 3***). Regarding the control group, a tendency to deterioration of all outcomes was observed after the 12-week period, although the only variables that showed significant differences were: PPME, Barthel index of independence and VAS.

Figure 2 depicts the changes in some of the measured variables after the 12-week period. It should be highlighted that after 12 weeks, one centenarian from the intervention group (FAC=2 at baseline) managed to leave the frailty condition (Fried's Phenotype = 2 points and FTS-5 = 19 points). At the end of the intervention all variables, except for Fried's Phenotype, were different between groups.

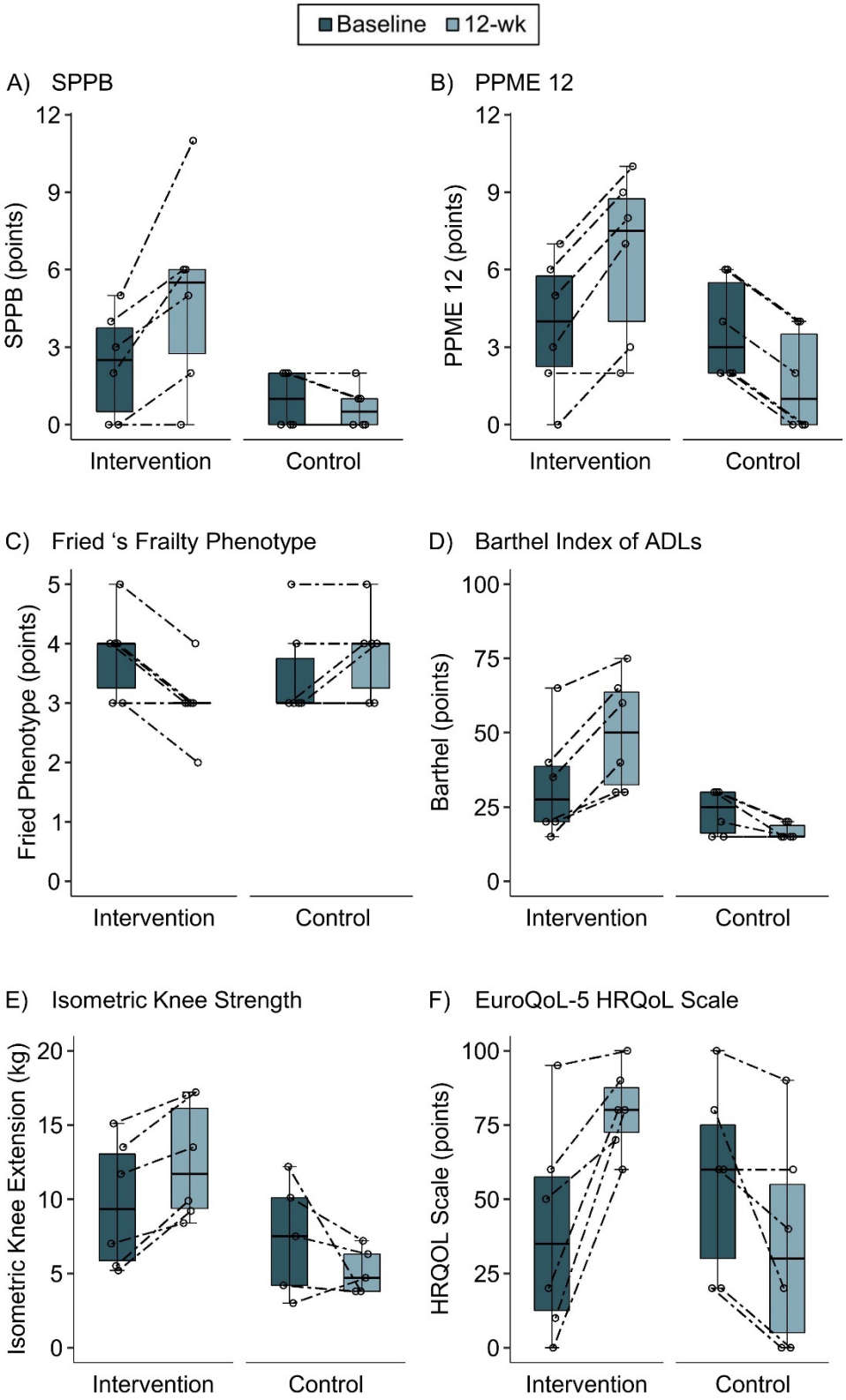
After approximately 150 hours of resistance training, the only adverse effects occasionally reported by some participants in the intervention group were mild musculoskeletal discomfort and delayed onset muscle soreness.

Table 3. Within-group comparisons from baseline to the end of the 12-week period

Outcome	Intervention (N= 6)			Control (N= 6)		
	Baseline	**Follow-up**	***p***	**Baseline**	**Follow-up**	***p***
SPPB (range 0-12)	2.33 ± 2.07	5.00 ± 3.79	0.025*	1.00 ± 1.10	0.67 ± 0.82	0.175
PPME (range 0-12)	3.83 ± 2.64	6.50 ± 3.27	0.005*	3.67 ± 1.97	1.67 ± 1.97	0.001*
Isometric knee extension (kg)	9.67 ± 4.31	12.53 ± 3.94	0.003*	7.40 ± 3.87	5.16 ± 1.53	0.260
30-second sit-to-stand (Nº) Ŧ	8.33 ± 1.53	12.00 ± 2.00	0.008*	5.00 ± 3.00	2.33 ± 1.15	0.157
Barthel (range 0-100)	32.50 ± 18.64	50.00 ± 19.24	0.003*	23.33 ± 7.53	16.67 ± 2.58	0.043*
Fried (range 5-0)	3.83 ± 0.75	3.00 ± 0.63	0.004*	3.50 ± 0.84	3.83 ± 0.75	0.175
FTS-5 (range 50-0)	34.08 ± 4.04	30.75 ± 6.23	0.064	38.25 ± 2.14	38.58 ± 2.50	0.175
EQ-5D (−0.224 - 1)	0.11 ± 0.12	0.23 ± 0.09	0.001*	0.09 ± 0.10	0.04 ± 0.09	0.070
VAS (range 0-100)	39.17 ± 35.83	80.00 ± 14.14	0.012*	56.67 ± 32.04	35.00 ± 35.64	0.048*

Values are expressed as mean ± standard deviation (SD). The scoring ranges for the test are expressed as (worst score – best score). SPPB= Short Physical Performance Battery. PPME= Physical Performance Mobility Examination. Ŧ= only centenarians with a Functional Ambulation Classification of 1-2 were able to perform the test; Intervention (Baseline N=4; Follow-up N=5); Control (Baseline N=3; Follow-up N=2). Barthel= Barthel index of independence during activities of daily living. Fried= Fried's frailty phenotype. FTS-5= Frailty trait scale-short form. EQ-5D= EuroQol-5D index. *= $p \leq 0.05$ in the paired T-test by group. VAS= Visual Analog Scale.

Figure 2. Box plots showing the changes in each group after the 12-week period.



Each point represents a value for a centenarian. Dashed lines connect the baseline value with the final value of the same participant.

DISCUSSION

Centenarians display a broad variation in functional independence. Some of them perform all activities of daily living while others are bedridden (15), which together with cultural factors, institutional support and family resources results in a wide range (2-89%, mean 37.3%) of centenarians living in nursing homes across European countries (16). Regarding their intrinsic capacity, normative data from the Georgia Centenarian Study identified 73.0% of centenarian men and 86.0% of centenarian women as severely disabled (0-3 points) according to the SPPB (17), but these numbers vary across studies because of recruitment bias, small sample sizes, birth year cohort, different test instruments and difficulties in the assessment of centenarians (18,19). At baseline, our sample of institutionalized centenarians showed a similar percentage (83.3%) of severely disabled individuals as the normative results from the USA (17).

To the best of our knowledge, only three case studies training centenarians have been reported: one of them was the training program that Robert Marchand followed to set the 1h cycling record (26.92km), considered to be the best performance achieved by a centenarian (-50.6% compared with the all-age world record) (20,21); secondly, a study of a 99.5-year-old male with a history of world records in master athletic competitions (22); third and last, a study of the effects of a functional electrical stimulation training program in a centenarian woman (23). As there is no control group, these case studies only have descriptive utility. In addition, two of these studies were in elite athletes, the intervention lasted more than a year and was poorly detailed (20,22). Therefore, our study is the first study of its kind, including a control group, and the first study assessing the effects of exercise training in centenarians who are not athletes.

Aging, especially in frail older people, leads to a progressive lowering of physical functions and capabilities (24). Consequently, the tendency to deterioration of all outcomes that we observed in the control group after the 12-week period was expected. Importantly, repeated measures ANOVA revealed significant group-by time interaction for all the measured outcomes, extending the known benefits of physical exercise in the oldest-old to the limit of the human lifespan (2). Moreover, it should be outlined that the intervention group not only eluded the age-related decline, but also significantly improved their physical function, functional independence, frailty and health-related quality of life. Last but not least, the National Strength and Conditioning Association emphasizes that injuries in older adults who perform resistance training are mainly related to incorrect technique and exercise selection (4). In concordance with it, no major adverse effects were noted in our sample of centenarians, in the same line as previous studies with older adults (25).

CONCLUSIONS

This is the first published randomized control trial study investigating the effects of 12-week resistance training in centenarians. The results suggest that no one is too old to benefit from resistance training, delay the age-related loss of functionality and improve their quality of life. Additionally, no major adverse effects were noted over the intervention period despite the frailty of the centenarians.

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SUPPLEMENT 1

Detailed description of exercises for each resistance exercise routine adjusted to Functional Ambulation Classification.

	Exercise name	Equipment *	Exercise description [Position 1 (P1) – Position 2 (P2) – Position 1 (P1) = 1 repetition]
Common warm-up	1. Biceps curl	-	*P1:* Sitting upright with the arms extended down in the sides. *P2:* Move the hands towards the shoulders, bending the elbows and keeping them close to the ribcage.
	2. Shoulder abduction/ adduction	-	*P1:* Sitting upright with the arms extended down in the sides. *P2:* Raise the arms outward and upward to shoulder height (like a bird flapping its wings).
	3. Shoulder horizontal abduction/ adduction	-	*P1:* Sitting upright with the arms lifted in front of you at the height of the chest. Elbows straight. *P2:* Open the arms out to the sides, maintaining chest height (like a big hug / clap).
	4. Leg extension and hip rotation	-	*P1:* Sitting upright, with one leg extended horizontally, perpendicular to the floor. *P2:* Turn inward and outward the toes of the raised leg. Repeat with the other leg.
	5. Ankle extension/ flexion	-	*P1:* Sitting upright, with the heels on the floor, and the tip of the feet pointing to the ceiling. *P2:* Lift the heels by rolling the sole until you are on tiptoe (like the pedal of an old sewing machine).
	6. Knee extension/ flexion	-	*P1:* Sitting upright. *P2:* Horizontally extend one leg trying to keep it as straight as possible. Repeat with the other leg.
	7. Trunk extension/ flexion	-	*P1:* Sitting at the edge of the chair, leaning forward from the hips, while keeping the upper back straight. *P2:* Use the armrest and rock back until reaching the back of the chair. Without lifting the feet.
	8. Upper-body twist	-	*P1:* Sitting upright. Place the right hand on the left knee and hold the chair with the left hand. *P2:* Turn the upper body and head to the left. Repeat on the opposite side.
Type 0	1. Hip abduction	RB	*P1:* Sitting upright, place the elastic band centered over the knees. *P2:* Separate the knees gradually until you can´t more.
	2. Biceps curl	D	*P1:* Sitting upright with the arms extended down in the sides, with a weight in each hand. *P2:* Move the hands towards the shoulders, bending the elbows and keeping them close to the ribcage.
	3. Calf muscles	AW	*P1:* Sitting upright, with the feet flat on the floor, and wearing a ballasted ankle brace in each ankle. *P2:* Get on your tiptoes until you are as high as possible, and remain in that position for 3 seconds.
	4. Hip adduction	Ball	*P1:* Sitting upright, place a ball between the thighs, just over the knees. *P2:* Squeeze the ball tightly during 3 seconds.
	5. Hip flexion	AW	*P1:* Sitting upright, with the feet flat on the floor and wearing a ballasted ankle brace in the exercising leg. *P2:* Lift one leg vertically, with the knee bent, as far as is comfortable. Repeat with the other leg.
	6. Knee extension	AW	*P1:* Sitting upright, wearing a ballasted ankle brace in the exercising leg. *P2:* Horizontally extend one leg trying to keep it as straight as possible. Repeat with the other leg.
	7. Low row	RB	*P1:* Sitting upright with the arms extended in front of you, grasping a stretched RB anchored to the wall rack. *P2:* Pull the elbows back behind you. Maintain the elbows close to the ribcage and scapular retraction.
	8. Flexed configuration after lift-off (wall rack)	WV	*P1:* Sitting upright with the hands on the wall rack. Wear the weighted vest to achieve the required load. *P2:* Rise from the seat using the wall rack, without rising to a full standing position (half squat).

Detailed description of exercises for each resistance exercise routine adjusted to Functional Ambulation Classification. (Continued)

	Exercise name	Equipment *	Exercise description [Position 1 (P1) – Position 2 (P2) – Position 1 (P1) = 1 repetition]
Type 1	1. 3-meters walk	AW	*P1:* Stand at the beginning of the parallel walking bars. *P2:* Walk 3 meters using the handrails. [Note that only 2 repetitions were performed per set]
	2. Biceps curl	D	*P1:* Sitting upright with the arms extended down in the sides, with a weight in each hand. *P2:* Move the hands towards the shoulders, bending the elbows and keeping them close to the ribcage.
	3. Balance on 2 legs	-	*P1:* Stand up from the chair. *P2:* Once you are up, try to stand for 10 seconds before sitting down again. [Only 2 repetitions per set]
	4. Flexed configuration after lift-off	WV	*P1:* Sitting upright. Wear the weighted vest to achieve the required load. *P2:* Get up from the seat without rising to a full standing position (half squat). Use armrest if needed.
	5. Half squat	WV	*P1:* Stand with the hands on the wall rack. Wear the weighted vest to achieve the required load. *P2:* Bend over, flexing the hips and knees; then, before sitting on the chair, return to the initial position.
	6. Knee extension	AW	*P1:* Sitting upright, wearing a ballasted ankle brace in the exercising leg. *P2:* Horizontally extend one leg trying to keep it as straight as possible. Repeat with the other leg.
	7. Low row	RB	*P1:* Sitting upright with the arms extended in front of you, grasping a stretched RB anchored to the wall rack. *P2:* Pull the elbows back behind you. Maintain the elbows close to the ribcage and scapular retraction.
	8. Squats (wall rack)	WV	*P1:* Sitting upright with the hands on the wall rack. Wear the weighted vest to achieve the required load. *P2:* Stand up using the wall rack. Once you are up, stand for 2 seconds before sitting down again.
Type 2	1. Stand up hip abduction	AW	*P1:* Stand up, wearing a ballasted ankle brace in the exercising leg. Use the wall rack if needed. *P2:* Raise one leg outward and upward. Keep the back straight and feet forward. Repeat with the other leg.
	2. Biceps curl	D	*P1:* Sitting upright with the arms extended down in the sides, with a weight in each hand. *P2:* Move the hands towards the shoulders, bending the elbows and keeping them close to the ribcage.
	3. Stand up knee flexion	AW	*P1:* Stand up, wearing a ballasted ankle brace in the exercising leg. Use the wall rack if needed. *P2:* Flex the knee keeping the foot back. Maintain the back straight. Repeat with the other leg.
	4. Flexed configuration after lift-off	WV	*P1:* Sitting upright. Wear the weighted vest to achieve the required load. *P2:* Get up from the seat without rising to a full standing position (half squat). Use armrest if needed.
	5. Half squat	WV	*P1:* Stand with the hands on the wall rack. Wear the weighted vest to achieve the required load. *P2:* Bend over, flexing the hips and knees; then, before sitting on the chair, return to the initial position.
	6. Knee extension	AW	*P1:* Sitting upright, wearing a ballasted ankle brace in the exercising leg. *P2:* Horizontally extend one leg trying to keep it as straight as possible. Repeat with the other leg.
	7. Low row	RB	*P1:* Sitting upright with the arms extended in front of you, grasping a stretched RB anchored to the wall rack. *P2:* Pull the elbows back behind you. Maintain the elbows close to the ribcage and scapular retraction.
	8. Squats	WV	*P1:* Sitting upright. Wear the weighted vest to achieve the required load. *P2:* Stand up. Once you are up, stand for 2 seconds before sitting down again. Use armrest if needed.

P1= Position 1; P2= Position 2. *= The required load was obtained through overloads such as: WV= Weighted vest; D= Handheld dumbbells; RB= Sports rubber band; AW; Ankle weights; or through the assistance of the strength and conditioning trainer if body weight exceeded the load. The motivational strategy consisted of reinforcement techniques, with the strength and conditioning coach emphasizing the improvements, giving positive feedback and commending the older adults for their efforts.

4.4 Artículo IV

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MDPI

Article

Validity of the Polar H7 Heart Rate Sensor for Heart Rate Variability Analysis during Exercise in Different Age, Body Composition and Fitness Level Groups

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Abstract: This work aims to validate the Polar H7 heart rate (HR) sensor for heart rate variability (HRV) analysis at rest and during various exercise intensities in a cohort of male volunteers with different age, body composition and fitness level. Cluster analysis was carried out to evaluate how these phenotypic characteristics influenced HR and HRV measurements. For this purpose, sixty-seven volunteers performed a test consisting of the following consecutive segments: sitting rest, three submaximal exercise intensities in cycle-ergometer and sitting recovery. The agreement between HRV indices derived from Polar H7 and a simultaneous electrocardiogram (ECG) was assessed using concordance correlation coefficient (CCC). The percentage of subjects not reaching excellent agreement (CCC > 0.90) was higher for high-frequency power (P_{HF}) than for low-frequency power (P_{LF}) of HRV and increased with exercise intensity. A cluster of unfit and not young volunteers with high trunk fat percentage showed the highest error in HRV indices. This study indicates that Polar H7 and ECG were interchangeable at rest. During exercise, HR and P_{LF} showed excellent agreement between devices. However, during the highest exercise intensity, CCC for P_{HF} was lower than 0.90 in as many as 60% of the volunteers. During recovery, HR but not HRV measurements were accurate. As a conclusion, phenotypic differences between subjects can represent one of the causes for disagreement between HR sensors and ECG devices, which should be considered specifically when using Polar H7 and, generally, in the validation of any HR sensor for HRV analysis.

Keywords: electrocardiography; wearable devices; HRV analysis; cluster analysis; exercise test

1. Introduction

Heart rate (HR) variability (HRV) is the oscillation in the intervals between consecutive heartbeats (RR intervals) [1]. In the last decades, the use of HRV has been popularized, since it allows assessing cardiac autonomic modulation using simple and non-invasive techniques. In general, lower HRV has been associated with poorer prognosis in different clinical conditions, while higher HRV, especially regarding high-frequency oscillations, has been associated with better health. In particular, reduced HRV has been reported in several cardiovascular diseases and has been used for risk stratification, confirming its value as a

predictor of total and cardiac mortality [2–4]. Also, low HRV has been described in a wide range of non-cardiovascular diseases, including psychiatric disorders such as depression, anxiety or schizophrenia [5].

Environmental and behavioral factors influence HRV [6]. In psychology, HRV is commonly used due to its modulation by mood states, emotions or cognitive capacity [5]. Likewise, HRV is a useful measurement in the sports field since it is sensitive to changes in fitness, fatigue and performance [7]. Healthy habits like exercise, balanced diet, mindfulness or psychological interventions have been shown to increase HRV measures indexing vagal function [5,6]. Additionally, non-modifiable factors, such as age or sex, also influence the autonomic regulation of the heart, with HRV having been reported to progressively decline with age and to present enhanced high-frequency oscillations and attenuated low-frequency oscillations in females as compared to males [8]. Because of these high inter- and intra-individual variations in HRV, it is key to use wearable devices validated for HRV analysis that can allow for precise interpretation of cardiac responses to different autonomic states.

A recent systematic review and meta-analysis showed that HRV measurements derived from portable devices are generally accurate when compared to lab-based electrocardiogram (ECG) [9]. Given the low cost of HR monitors it is not surprising that they are widely used by practitioners and researchers. Particularly, Polar Electro Oy (Kempele, Finland) is one of the most well-established brands in HR monitoring, with Polar H7/H10 HR sensors having been validated both at rest and during exercise [10–12]. Nevertheless, previous Polar validation studies have been carried out in small groups of young, lean, healthy and physically fit volunteers [10–12]. However, the device is commonly used by individuals with various phenotypic characteristics, regardless of how these may affect the accuracy of the measurements [13,14]. In the meta-analysis described in [9], the absolute error of portable devices was found to vary with the evaluated HRV metric, tilt/recovery position and the percentage of women in the study sample. The characteristics of the subjects and their influence on the measurements provided by portable devices have not been analyzed yet.

The ECG records the electrical activity of the heart using electrodes placed on the surface of the body. Therefore, differences between measurements from HR sensors and ECG could vary depending on the characteristics of the population under study. To start with, the age-related myocardial fibrosis present in the cardiac tissue, the amount of subcutaneous fat or the electrode placement are expected to affect the voltage tracings [15]. Additionally, voltage will be influenced by ventricular size or mass, as observed when comparing trained athletes with non-athletes [16]. Accordingly, some groups of subjects such as men, athletes or black/African have been reported to have higher QRS voltage and, consequently, RR intervals become easier to be detected [16]. On the other hand, obesity, older age and sedentary lifestyle may cause lower voltage, which may result in lower accuracy of portable devices. In particular, under such circumstances, some heart beats can be misdetected and, while this may not considerably affect mean HR, it may notably hamper HRV assessment. For these reasons and in light of previous studies, we hypothesized that when the quality of the Polar H7 ECG is compromised by high noise during intense exercise and/or by specific phenotypic characteristics of the subjects, HRV measures can be distorted, particularly those related to high-frequency power [17].

The purpose of the present study was to evaluate the validity of HRV analysis derived from RR intervals recorded by Polar H7 HR sensor at rest and during exercise and recovery in different phenotype groups based on age, body composition and fitness level.

2. Materials and Methods

2.1. Subjects

A total of sixty-seven males agreed to participate in the study. The sample consisted of three groups of volunteers: 22 young adults (20–30 years old), 22 middle-aged adults (40–50 years old) and 23 older adults (60–70 years old). Only subjects within the prede-

fined age ranges were included in the study. Subjects were excluded from the study if they were going through an acute disease, were suffering from heart diseases (e.g., heart failure or atrial fibrillation), were on cardiac medication or presented any clinical condition contraindicating physical exercise. However, subjects who were overweight, sedentary or suffering from chronic diseases such as hypertension, diabetes or hypercholesterolemia were included in the study, because of their high prevalence in the society. Table 1 shows the descriptive characteristics of the three age groups. The study was approved by the ethical committee for clinical research of Aragón (ID of the approval: PI17/0409), and was conducted by adhering to the Declaration of Helsinki. After a clear explanation of the potential risks of the study, all volunteers provided written informed consent.

Table 1. Descriptive characteristics of the three age groups.

Outcome	Young Adults (n = 22)	Middle-Aged Adults (n = 22)	Older Adults (n = 23)
Age (years)	25.46 ± 2.85	43.17 ± 3.32	63.97 ± 2.79
Height (m)	1.75 ± 0.06	1.77 ± 0.06	1.71 ± 0.05
Weight (kg)	72.01 ± 11.92	78.19 ± 10.30	76.31 ± 7.76
BMI (m/kg^2)	23.43 ± 2.95	25.02 ± 2.83	26.21 ± 2.84
Body fat (%)	15.25 ± 5.59	19.69 ± 5.65	23.38 ± 5.17
Trunk fat (%)	16.31 ± 6.32	21.29 ± 6.38	25.69 ± 6.42
$PWC_{80\%}$ (W/kg)	2.00 ± 0.64	2.01 ± 0.58	1.73 ± 0.65

Values are expressed as mean ± standard deviation (SD). BMI = Body mass index; $PWC_{80\%}$ = Physical Work Capacity at 80% of maximum HR (208 − 0.7 ∗ age in years) in watts/kg bodyweight.

2.2. Procedure

All subjects completed one test session. Prior to the test, they were asked to adhere to the following instructions [18]: (1) avoid exercise or strenuous physical activity the day before the test; (2) drink plenty of fluids over the 24-h period preceding the test; (3) get an adequate amount of sleep (6–8 h) the night before the test; (4) avoid substances such as tobacco, alcohol or stimulants (caffeine, theine, taurine, etc.) in the 8 h before the test; (5) avoid food intake for 3 h prior to performing the test; and (6) wear comfortable, loose-fitting clothing. Subjects' skin was prepared by using a razor to remove any hair from the electrode sites, cleaning the skin with alcohol and drying it with a gauze. A 12-lead high-resolution Holter ECG was acquired, with the 10 electrodes placed as indicated by the manufacturer (H12+, Mortara Instrument, Milwaukee, WI, USA), ensuring that they did not interfere with the HR sensor strap (Polar H7, Polar Electro Oy).

The test was conducted in an environmentally controlled room (22–23 °C), between 16:00–20:00, and was divided into 3 consecutive segments: resting (S_{REST}), cycling (S_{CY}) and recovery (S_{REC}). During S_{REST}, volunteers were monitored while seated at rest for 5 min, without any movement or talking. A period of 2–3 min was established to change from the chair to the cycle-ergometer, namely from S_{REST} to S_{CY}, during which the subject rode the electrically braked cycle-ergometer (Ergoselect 200 K, Ergoline; Bitz, Germany) at 50 W workload and chose a cadence which was maintained during the entire test according to the workload and cadence displayed in the cycle-ergometer screen. S_{CY} was a submaximal cycle-ergometer test divided into three stages lasting 5 min each. In order to avoid a maximal exercise test, the maximum heart rate (HRmax) was estimated for each subject by using the formula defined by Tanaka et al. HRmax = 208 − 0.7 ∗ age (years) [19]. Workload was adjusted during each stage to 60, 70 and 80% of HRmax, with these stages denoted as S_{CY60}, S_{CY70} and S_{CY80}, respectively. Finally, during S_{REC}, volunteers remained seated again for 5 min without any movement or talking. Figure 1 shows an example of the temporal evolution of RR intervals from a subject throughout the entire test.

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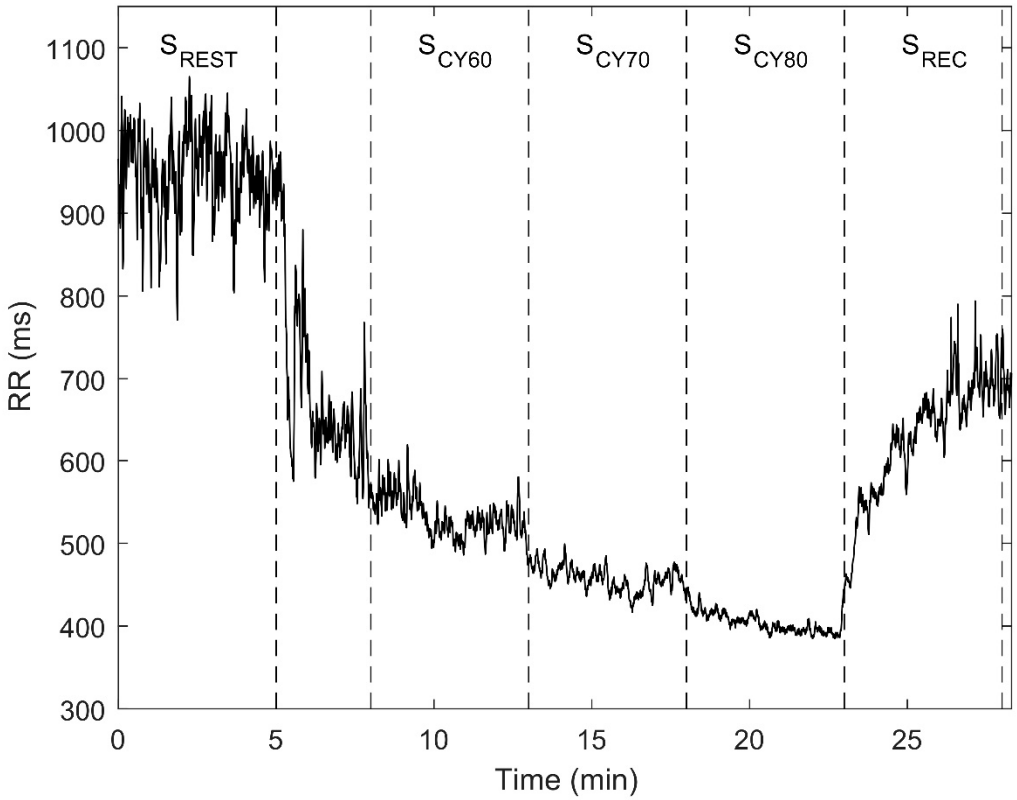

Figure 1. Example of the RR intervals for one subject throughout the entire test. Dotted lines separate the different test segments: resting (S_{REST}), cycling (S_{CY}) and recovery (S_{REC}). S_{CY} was divided into three consecutive stages corresponding to 60, 70 and 80% of HRmax, denoted as S_{CY60}, S_{CY70} and S_{CY80}, respectively.

2.3. Data Recording

Subjects self-reported their birth date, current diseases and medication. The anthropometric characteristics of the subjects were assessed. Stature was measured to the nearest 0.001 m using a portable stadiometer (SECA 225, Hamburg, Germany), with subjects standing with their scapula, buttocks and heels resting against a wall, the feet with the heels touching, forming a 45° angle and the head in the Frankfort's plane. A portable body composition analyzer (TANITA BC-418MA; Tanita Corp., Tokyo, Japan) was used to measure the body mass to the nearest 0.1 kg, with underwear and after urination. TANITA BC-418MA was also used to estimate the percentage of body fat and trunk fat ($r = 0.87$, $p < 0.001$ vs. dual-energy X-ray absorptiometry) [20]. Body mass index (BMI) was calculated dividing weight in kilograms by height in squared meters.

Beat-to-beat RR intervals with 1-ms resolution were obtained using a Polar V800 HR monitor simultaneously with a Polar H7 chest Soft Strap (Polar Electro Oy, henceforth referred to as PolarH7). Concomitantly, a 12-lead ECG was recorded at a sampling rate of 1000 Hz using a high-resolution Holter device (H12+, Mortara Instrument, henceforth referred to as ECG and used here as a reference).

VO_2max can be estimated from submaximal exercise tests, a safe and feasible method showing good validity against maximal tests (correlation coefficients: 0.69 to 0.98) [21]. Rather than commonly used tests with stages of short or variable duration, an ad-hoc test with 5-min stages was defined to allow reliable estimation of the low-frequency power of HRV. This enabled assessment of HRV response to increased sympathetic activity with each cycling stage [22]. Cardiorespiratory fitness was assessed using the approach of "Physical Work Capacity" (PWC). PWC in watts was measured during S_{CY80} of the submaximal cycle-ergometer test and was subsequently divided by the subject's body weight ($PWC_{80\%}$ in W/kg). Alternatively to the use of fixed HR thresholds, this method incorporates the

age-dependent decline of HRmax [23,24] and has been previously used as an objective assessment of cardiorespiratory fitness [25,26].

2.4. Data Analysis and Processing

Raw RR interval time series, RR_P (i), recorded by PolarH7 were downloaded from the "Polar Flow" web platform. RR interval time series from the ECG, RR_E (i), were extracted using a multi-lead approach by a wavelet-based detector [27] with optimized parameters for noisy environments as described in [28]. Each beat detection was manually verified by an operator with a dedicated interface.

The delay between the RR interval series RR_P (i) and RR_E (i) was estimated as the time lag maximizing their cross-correlation over the first 3 min of the test when the subject is relaxed. Then, both series were synchronized by compensating for this delay. Since the two RR interval series can have different lengths, due to, e.g., wrong or missed beat detections in the Polar data, an algorithm was developed to match the RR intervals from both series, thus allowing characterization of the agreement between the paired series RR_P (ip) and RR_E (ip), where ip refers to the indices of beats that are matched in the two series.

2.5. Heart Rate Variability

HRV indices were obtained by algorithms specifically developed and previously published by our research group using MATLAB version R2017a (MATLAB, MathWorks Inc., Natick, MA, USA) [17,27–30].

2.5.1. Temporal Domain

The following temporal HRV indices were studied [1]: mean HR (MHR), standard deviation of normal-to-normal RR intervals (SDNN) and root mean square of successive differences of adjacent normal-to-normal RR intervals (RMSSD). MHR was obtained as the inverse of the mean of the RR intervals. SDNN is considered a measure of the total power of HRV and was calculated from the standard deviation of the NN intervals, i.e., normal RR intervals after correcting for ectopic beats [29]. RMSSD is a measure of short-term variability and was computed by the root mean square of successive differences between adjacent NN intervals. These indices were obtained from RR_P (i) and RR_E (i) in each segment of the test.

2.5.2. Frequency Domain

The instantaneous HR signal, $d_{HR}(n)$, was derived from both RR_P (i) and RR_E (i) and sampled at 4 Hz. The integral pulse frequency modulation (IPFM) model was used while dealing with the presence of ectopic beats [29]. This signal was high-pass-filtered (0.03 Hz) to remove the very low-frequency components, $d_{MHR}(n)$, and it was also corrected by it: $m(n) = (d_{HR}(n) - d_{MHR}(n))/d_{MHR}(n)$ [30].

The smoothed pseudo Wigner–Ville distribution (SPWVD) was applied to $m(n)$ to estimate its time-varying spectrum. Time and frequency smoothing windows were chosen as described in [17]. The instantaneous power in the low-frequency band, P_{LF} (n), was extracted integrating the SPWVD from 0.04 to 0.15 Hz for each time instant. The instantaneous power in the high-frequency band, P_{HF} (n), was computed in a band centered on the respiratory frequency with a bandwidth of 0.25 Hz. Figure 2 shows an example of $d_{HR}(n)$, P_{LF} (n) and P_{HF} (n) obtained from RR_E. In some analyses, mean P_{LF} and P_{HF} were calculated from P_{LF} (n) and P_{HF} (n) for each segment of the test.

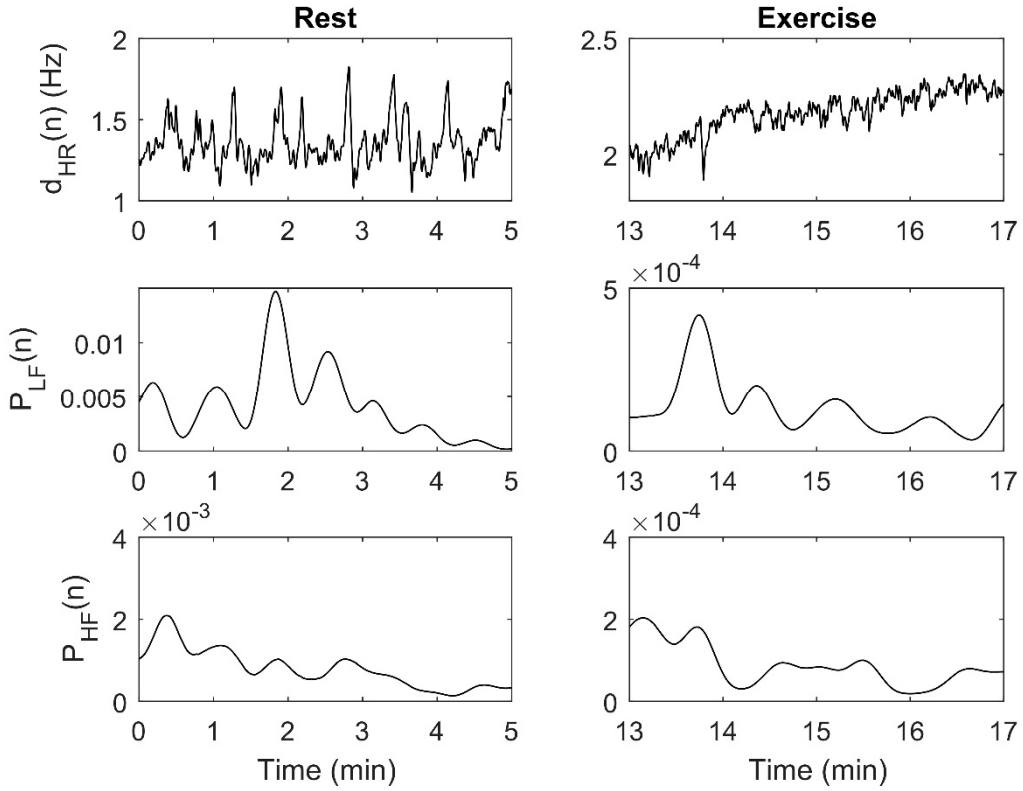

Figure 2. Example of $d_{HR}(n)$, P_{LF} (n) and P_{HF} (n) obtained from RR_E for one subject: Resting segment (**left**) and cycling segment (**right**). Note that the axes have different scales. $d_{HR}(n)$ = instantaneous HR signal; P_{LF} (n) = Instantaneous low-frequency power; P_{HF} (n) = Instantaneous high-frequency power; RR_E = RR intervals series from the ECG.

2.6. Statistical Analysis

The normality of data was checked with the Kolmogorov-Smirnov test. Since the data distribution violated the assumption of normality of the parametric tests, and such a condition was not achieved by commonly employed transformations, a non-parametric analysis was performed. Descriptive values are presented as mean ± standard deviation (SD) and HRV values are reported as median and interquartile range. Statistical analyses were performed using IBM SPSS (version 25; Chicago, IL, USA). The significance level was set at $p \leq 0.05$.

Wilcoxon test for paired samples, the non-parametric equivalent of the paired samples *t*-test, was used to determine differences between the temporal domain HRV data obtained from PolarH7 and from ECG. The magnitude of the differences was calculated by determining the effect size (ES): $ES = Z/\sqrt{n}$ where Z represents the Z-score for the Wilcoxon statistic and n is the total number of observations [31]. Differences were considered small when ES < 0.2, small to medium when ES = 0.2–0.5, medium to large when ES= 0.5–0.8 and large when ES > 0.8 [32].

Lin's concordance correlation coefficient (CCC) was used to study the agreement between the following PolarH7-derived and ECG-derived signals: RR (ip), P_{HF} (n) and P_{LF} (n). CCC determines how much the observed data deviate from the perfect concordance line at 45° on a square axis scatter plot [33]. CCC was evaluated in each segment (S_{REST}, S_{CY60}, S_{CY70}, S_{CY80} and S_{REC}). A CCC value greater than 0.90 was considered "excellent" [34] and the percentage of subjects with CCC values below this threshold was reported for each segment.

Cluster analysis was performed to identify groups of subjects with similar characteristics in terms of the following three variables of interest: age, body composition (trunk fat percentage) and fitness level ($PWC_{80\%}$). Trunk fat percentage was selected among all body composition variables, since it is the most specific to the electrode placement area.

Following the methodology described in previous studies [35,36], two types of cluster analyses were combined: hierarchical clustering (Ward's method) and k-means clustering. First, individual and multivariate outliers (according to Mahalanobis distance) were detected to reduce the sensitivity of the Ward's method to outliers. Second, hierarchical cluster analysis was used, as the number of clusters in the data were unknown beforehand. Examination of dendrograms showed that a four-cluster solution produced good differentiation between groups. Finally, k-means cluster was performed with four possible solutions. Compared to hierarchical methods, k-means cluster analysis is considered less sensitive to outliers and has been found to result in greater within-cluster homogeneity and between-cluster heterogeneity [35].

To assess differences in the percentage of error for each HRV index between the four cluster groups, a Kruskal-Wallis test (non-parametric equivalent of one-way analysis of variance, ANOVA) with Bonferroni correction was performed. The Dunn-Bonferroni post hoc method was used for pairwise comparisons. The relative error in HRV indices was calculated as the absolute error of the PolarH7 with respect to the ECG measurement divided by the reference ECG measurement, e.g., $(SDNN_{ECG} - SDNN_{PolarH7})/SDNN_{ECG}$, which was then multiplied by 100 to obtain the percentage of error (%Error). In the case of the frequency HRV variables, %Error was calculated from the mean value for each segment of the test. To evaluate the magnitude of the differences, ES was calculated as: $ES = H/((n^2 - 1)/(n + 1))$, where H stands for the Kruskal-Wallis test statistic and n is the total number of observations [31].

3. Results

Table 2 shows the descriptive characteristics of the 4 cluster groups, which were described as CLUSTER A (High $PWC_{80\%}$), CLUSTER B (Low $PWC_{80\%}$ and low age), CLUSTER C (Low $PWC_{80\%}$, high age and medium trunk fat percentage) and CLUSTER D (Low $PWC_{80\%}$, high age and high trunk fat percentage).

Table 2. Descriptive characteristics of the four cluster groups.

Outcome	CLUSTER A (n = 19)	CLUSTER B (n = 13)	CLUSTER C (n = 18)	CLUSTER D (n = 17)	Main Effect	
					p	Effect Size
Age (years)	38.99 ± 13.31 ^{B,D}	24.35 ± 2.20 ^{A,C,D}	52.61 ± 10.20 ^{B}	57.47 ± 12.23 ^{A,B}	<0.001 *	0.586
Height (m)	1.77 ± 0.05 ^{D}	1.72 ± 0.06	1.74 ± 0.06	1.72 ± 0.07 ^{A}	0.020	0.149
Weight (kg)	72.65 ± 9.13	69.18 ± 12.27 ^{D}	76.32 ± 7.13	82.72 ± 8.78 ^{B}	0.007	0.182
BMI (m/kg^2)	23.00 ± 2.24 ^{D}	23.23 ± 2.77 ^{D}	25.17 ± 1.40	28.04 ± 2.79 ^{A,B}	<0.001 *	0.430
Body fat (%)	14.09 ± 4.11 ^{C,D}	15.45 ± 4.54 ^{D}	20.40 ± 2.36 ^{A,D}	27.67 ± 2.42 ^{A,B,C}	<0.001 *	0.743
Trunk fat (%)	14.76 ± 5.11 ^{C,D}	16.64 ± 5.03 ^{D}	22.19 ± 2.86 ^{A,D}	30.69 ± 2.12 ^{A,B,C}	<0.001 *	0.725
$PWC_{80\%}$ (W/kg)	2.73 ± 0.39 ^{B,C,D}	1.61 ± 0.37 ^{A}	1.79 ± 0.28 ^{A,D}	1.35 ± 0.22 ^{A,C}	<0.001 *	0.704

Values are expressed as mean ± standard deviation (SD). BMI = Body mass index; $PWC_{80\%}$ = Physical Work Capacity at 80% of HRmax ($208 - 0.7 * age$ in years) in watts/kg bodyweight. Clusters were based on: age, body composition (trunk fat percentage) and fitness level ($PWC_{80\%}$). * = Significant differences between clusters ($p \leq 0.05$, Kruskal-Wallis test). ^{A} = Different to CLUSTER A; ^{B} = Different to CLUSTER B; ^{C} = Different to CLUSTER C; ^{D} = Different to CLUSTER D.

Table 3 shows the values of HRV indices obtained from PolarH7 and ECG. Mean P_{LF} and P_{HF} were calculated from P_{LF} (n) and P_{HF} (n) for each segment (differently from Table 4, where the instantaneous series were used). Wilcoxon test for paired samples revealed that P_{HF} and temporal domain HRV indices (MHR, SDNN and RMSSD) were lower at all cycling stages (S_{CY60}, S_{CY70} and S_{CY80}) when measured by PolarH7, with P_{LF} being lower at the highest intensity (S_{CY80}) when measured by PolarH7. The magnitude of all these differences was small to medium, i.e., 0.2–0.5 according to the effect sizes.

Table 3. HRV indices obtained from PolarH7 and ECG data (n = 67).

		PolarH7	ECG	p	ES
S_{REST}	P_{LF} (e^{-4})	9.78 (3.88 to 24.74)	9.76 (3.85 to 24.78)	0.074	0.155
	P_{HF} (e^{-4})	5.77 (2.30 to 10.91)	5.74 (2.22 to 10.84)	0.067	0.159
	MHR (bpm)	62.55 (53.25 to 71.95)	62.84 (53.36 to 72.00)	<0.001 *	0.378
	SDNN (ms)	60.27 (40.50 to 75.22)	60.28 (40.33 to 75.26)	0.570	0.049
	RMSSD (ms)	39.48 (22.48 to 60.04)	39.26 (22.39 to 60.66)	0.336	0.083
S_{CY60}	P_{LF} (e^{-4})	1.17 (0.62 to 2.01)	1.17 (0.65 to 2.23)	0.112	0.137
	P_{HF} (e^{-4})	0.51 (0.26 to 1.29)	0.86 (0.44 to 2.07)	<0.001 *	0.419
	MHR (bpm)	106.00 (100.45 to 112.91)	106.66 (100.75 to 113.17)	<0.001 *	0.433
	SDNN (ms)	15.94 (12.06 to 20.77)	16.21 (12.85 to 20.32)	0.010 *	0.222
	RMSSD (ms)	6.48 (4.34 to 8.51)	8.38 (5.67 to 10.65)	<0.001 *	0.482
S_{CY70}	P_{LF} (e^{-4})	0.45 (0.21 to 0.87)	0.46 (0.22 to 0.88)	0.851	0.016
	P_{HF} (e^{-4})	0.30 (0.15 to 0.55)	0.41 (0.24 to 0.75)	<0.001 *	0.377
	MHR (bpm)	124.24 (115.77 to 130.89)	124.32 (115.76 to 130.92)	<0.001 *	0.482
	SDNN (ms)	10.22 (8.19 to 12.93)	10.48 (8.40 to 13.10)	0.001 *	0.280
	RMSSD (ms)	3.74 (2.96 to 4.91)	4.37 (3.64 to 6.68)	<0.001 *	0.443
S_{CY80}	P_{LF} (e^{-4})	0.13 (0.09 to 0.23)	0.18 (0.10 to 0.26)	<0.001 *	0.364
	P_{HF} (e^{-4})	0.23 (0.14 to 0.36)	0.37 (0.25 to 0.66)	<0.001 *	0.362
	MHR (bpm)	141.01 (130.62 to 148.81)	141.09 (130.69 to 149.05)	<0.001 *	0.451
	SDNN (ms)	8.11 (6.20 to 9.92)	8.10 (6.34 to 10.65)	<0.001 *	0.378
	RMSSD (ms)	2.90 (2.32 to 3.90)	3.75 (3.16 to 5.52)	<0.001 *	0.405
S_{REC}	P_{LF} (e^{-4})	4.75 (1.83 to 10.25)	4.59 (1.88 to 9.97)	0.881	0.015
	P_{HF} (e^{-4})	2.06 (0.65 to 4.71)	2.12 (0.82 to 4.70)	0.308	0.102
	MHR (bpm)	99.76 (90.76 to 112.15)	98.39 (90.12 to 111.35)	0.002 *	0.268
	SDNN (ms)	33.11 (23.27 to 58.30)	33.67 (23.04 to 57.40)	0.094	0.147
	RMSSD (ms)	12.87 (7.65 to 23.78)	12.90 (7.99 to 23.32)	0.603	0.046

Values are expressed as median and interquartile range. Segments are based on the test phases: resting (S_{REST}), cycling (S_{CY}) and recovery (S_{REC}). S_{CY} was divided in three stages at 60, 70 and 80% of HRmax, denoted as S_{CY60}, S_{CY70} and S_{CY80}, respectively. P_{LF} = low-frequency power; P_{HF} = high-frequency power; MHR = mean HR; SDNN = SD of the NN intervals; RMSSD = root mean square of successive differences between NN intervals. ES = Effect size. * = Significant differences between devices ($p \leq 0.05$, Wilcoxon test for paired samples).

Table 4. Agreement between devices in: RR (ip), PLF (n) and PHF (n). CCC mean and percentage of subjects not reaching excellent agreement for each segment.

		S_{REST}	S_{CY60}	S_{CY70}	S_{CY80}	S_{REC}
Whole sample (n = 67)	RR (ip)	0.9929 (1%)	0.9560 (6%)	0.9467 (13%)	0.9319 (16%)	0.9612 (14%)
	P_{LF} (n)	0.9885 (1%)	0.9713 (4%)	0.9677 (9%)	0.9106 (19%)	0.8251 (30%)
	P_{HF} (n)	0.9813 (3%)	0.9494 (13%)	0.8858 (27%)	0.6661 (60%)	0.5262 (75%)
CLUSTER A (n = 19)	RR (ip)	0.9970 (0%)	0.9844 (0%)	0.9472 (21%)	0.9243 (21%)	0.9778 (6%)
	P_{LF} (n)	0.9999 (0%)	0.9990 (0%)	0.9911 (5%)	0.9169 (16%)	0.7440 (47%)
	P_{HF} (n)	0.9982 (0%)	0.9645 (16%)	0.9316 (11%)	0.7970 (37%)	0.4859 (88%)
CLUSTER B (n = 13)	RR (ip)	0.9828 (8%)	0.8996 (15%)	0.8690 (23%)	0.9258 (23%)	0.9473 (15%)
	P_{LF} (n)	0.9423 (8%)	0.8544 (23%)	0.9757 (8%)	0.8601 (15%)	0.8838 (15%)
	P_{HF} (n)	0.9284 (8%)	0.8710 (23%)	0.9112 (23%)	0.7455 (62%)	0.7402 (54%)
CLUSTER C (n = 18)	RR (ip)	0.9943 (0%)	0.9363 (11%)	0.9665 (11%)	0.9035 (22%)	0.9615 (12%)
	P_{LF} (n)	0.9990 (0%)	0.9998 (0%)	0.9792 (6%)	0.9164 (28%)	0.7791 (31%)
	P_{HF} (n)	0.9909 (6%)	0.9670 (6%)	0.8906 (28%)	0.5914 (67%)	0.3843 (88%)
CLUSTER D (n = 17)	RR (ip)	0.9945 (0%)	0.9884 (0%)	0.9844 (0%)	0.9751 (0%)	0.9541 (24%)
	P_{LF} (n)	0.9999 (0%)	0.9996 (0%)	0.9233 (18%)	0.9361 (18%)	0.9045 (24%)
	P_{HF} (n)	0.9929 (0%)	0.9739 (12%)	0.8100 (47%)	0.5381 (76%)	0.5366 (65%)

Values are expressed as CCC mean and (percentage of subjects under 0.9 threshold). Segments are based on the test phases: resting (S_{REST}), cycling (S_{CY}) and recovery (S_{REC}). S_{CY} was divided in three stages at 60, 70 and 80% of HRmax, denoted as S_{CY60}, S_{CY70} and S_{CY80} respectively. The characteristics of each cluster were the following: CLUSTER A = high fitness; CLUSTER B = low fitness and low age; CLUSTER C = low fitness, high age and medium trunk fat percentage; CLUSTER D = low fitness, high age and high trunk fat percentage. RR (ip) = paired RR interval series; P_{LF} (n) = instantaneous low-frequency power; P_{HF} (n) = instantaneous high-frequency power.

Table 4 shows CCC values for RR (ip), P_{LF} (n) and P_{HF} (n) and outlines the percentage of subjects not reaching excellent agreement (CCC > 0.90) for each segment of the test. The number of subjects not reaching excellent agreement was clearly higher for P_{HF} (n) than for

P_{LF} (n) (χ^2(degrees of freedom); $\chi^2(1) = 45.52$; $p < 0.001$), it increased with exercise intensity ($\chi^2(2) = 38.47$; $p < 0.001$) and was lower during exercise than during S_{REC} ($\chi^2(1) = 42.31$; $p < 0.001$). When performing the analysis separately for each identified cluster, CLUSTER A obtained the highest CCC values, with CLUSTER D being the group with less subjects showing optimal agreement between devices in P_{HF} (n). Due to the presence of noise in the RR_P (i) series during S_{REC}, the instantaneous power could not be properly extracted in 4 volunteers and the final sample for S_{REC} was N = 63.

Table 5 shows %Error for each HRV index. Kruskal-Wallis test demonstrated significant differences between clusters in P_{HF} at S_{REST} and during exercise (S_{CY70} and S_{CY80}). With regards to temporal domain HRV indices, SDNN showed significant differences between groups at S_{REST} and during exercise (S_{CY60} and S_{CY80}) and RMSSD showed significant differences at the highest intensities (S_{CY70} and S_{CY80}). Both for P_{HF} and for temporal domain HRV indices, CLUSTER D was the group with the highest %Error. The magnitude of all these differences was small, i.e., <0.2 according to the effect sizes.

Table 5. Percentage of error (%) for each HRV index and comparison between clusters.

		CLUSTER A (*n* = 19)	CLUSTER B (*n* = 13)	CLUSTER C (*n* = 18)	CLUSTER D (*n* = 17)	Main Effect	
						p	Effect Size
S_{REST}	P_{LF}	0.0 (−0.2 to 0.3)	0.2 (−0.3 to 0.6)	0.1 (−0.2 to 0.2)	0.3 (−0.1 to 0.4)	0.534	0.034
	P_{HF}	−0.5 (−2.3 to 0.2)	−0.2 (−0.7 to 0.4)	−0.5 (−1.4 to 0.4)	0.5 (−0.3 to 2.0)	0.049 *	0.121
	SDNN	0.0 (−0.2 to 0.5) [B]	−0.1 (−0.8 to 0.0) [A,D]	0.0 (−0.2 to 0.2)	0.1 (0.0 to 0.2) [B]	0.021 *	0.147
	RMSSD	−0.2 (−1.0 to 0.3)	−0.1 (−2.3 to 0.4)	−0.1 (−1.3 to 0.4)	0.2 (−0.2 to 2.3)	0.150	0.081
S_{CY60}	P_{LF}	0.8 (−0.7 to 18.7)	0.0 (−0.8 to 1.3)	0.4 (−4.0 to 3.0)	0.8 (−0.5 to 4.4)	0.444	0.041
	P_{HF}	25.7 (10.3 to 48.3)	2.1 (−12.5 to 17.7)	21.7 (−2.4 to 44.6)	27.0 (10.9 to 65.6)	0.164	0.077
	SDNN	0.1 (−2.5 to 0.9) [D]	−0.2 (−1.2 to 1.4)	1.2 (−0.1 to 4.2)	2.2 (1.1 to 8.5) [A]	0.018 *	0.153
	RMSSD	14.4 (4.6 to 32.4)	1.8 (−3.2 to 14.6)	21.7 (4.2 to 29.8)	15.8 (7.9 to 44.1)	0.266	0.060
S_{CY70}	P_{LF}	1.6 (−0.5 to 7.5)	−0.2 (−2.8 to 0.9)	−0.4 (−1.7 to 2.1)	−0.6 (−7.8 to 2.8)	0.125	0.087
	P_{HF}	9.2 (−6.2 to 50.8)	−0.9 (−42.6 to 25.6) [C,D]	25.3 (17.5 to 57.2) [B]	51.8 (14.5 to 71.7) [B]	0.007 *	0.186
	SDNN	0.6 (−0.5 to 2.8)	0.2 (−1.4 to 1.6)	1.5 (−1.2 to 7.0)	2.3 (0.3 to 8.0)	0.104	0.093
	RMSSD	15.4 (−2.0 to 29.5)	0.6 (−27.9 to 19.3) [D]	15.6 (14.2 to 36.5)	27.1 (14.2 to 56.0) [B]	0.010 *	0.172
S_{CY80}	P_{LF}	1.5 (−2.6 to 32.0)	2.8 (−1.0 to 3.6)	2.7 (−1.1 to 12.6)	5.9 (0.3 to 16.1)	0.596	0.029
	P_{HF}	31.6 (−27.4 to 87.7)	−9.0 (−113.1 to 56.4) [D]	28.0 (8.5 to 45.2)	44.7 (37.2 to 77.7) [B]	0.047 *	0.121
	SDNN	4.0 (−0.8 to 13.7)	−0.4 (−2.9 to 6.1)	1.1 (−0.3 to 4.1)	5.7 (1.6 to 14.3)	0.050 *	0.119
	RMSSD	19.2 (−9.9 to 67.0)	8.4 (−43.0 to 26.1) [D]	19.0 (4.7 to 32.7)	32.9 (22.1 to 58.2) [B]	0.028 *	0.137
S_{REC}	P_{LF}	−0.4 (−33.8 to 0.1)	0.5 (−0.5 to 6.2)	0.2 (−17.6 to 3.9)	0.2 (−4.9 to 12.7)	0.161	0.105
	P_{HF}	−1.6 (−8.2 to 5.8)	1.6 (0.1 to 5.9)	−1.2 (−8.3 to 10.1)	9.3 (−0.2 to 37.2)	0.053	0.157
	SDNN	−0.1 (−0.9 to 0.1)	0.1 (−1.5 to 0.3)	−0.1 (−1.0 to 0.4)	0.0 (−1.8 to 0.7)	0.933	0.007
	RMSSD	−0.9 (−2.3 to 2.0)	0.1 (−9.8 to 3.0)	1.5 (−1.4 to 5.5)	2.2 (−2.9 to 11.5)	0.226	0.068

Percentage error (%) values are expressed as median and interquartile range. The characteristics of each cluster were the following: CLUSTER A = high fitness; CLUSTER B = low fitness and low age; CLUSTER C = low fitness, high age and medium trunk fat percentage; CLUSTER D = low fitness, high age and high trunk fat percentage. Segments are based on the test phases: resting (S_{REST}), cycling (S_{CY}) and recovery (S_{REC}). S_{CY} was divided in three stages at 60, 70 and 80% of HRmax, denoted as S_{CY60}, S_{CY70} and S_{CY80} respectively. P_{LF} = low-frequency power; P_{HF} = high-frequency power; SDNN= SD of the RR intervals; RMSSD = root mean square of successive differences between NN intervals. * = Significant differences between clusters ($p \leq 0.05$, Kruskal-Wallis test). [A] = Different to CLUSTER A; [B] = Different to CLUSTER B; [C] = Different to CLUSTER C; [D] = Different to CLUSTER D.

4. Discussion

In this study, HRV analysis from RR intervals provided by PolarH7 at rest and during various exercise intensities has been validated against the same analysis from a simultaneous ECG recording. Wilcoxon test showed a large number of significant differences between devices in HRV indices during exercise. However, the effect size was small to medium and of little practical relevance in the case of MHR. When observing RR (ip), P_{LF} (n) and P_{HF} (n) signals, the percentage of subjects not reaching excellent agreement between devices (CCC > 0.90) increased with exercise intensity and was higher for P_{HF} (n) than for P_{LF} (n). Cluster analysis revealed that phenotypic characteristics like age, body composition and fitness level influenced HRV measurements as well as the differences between PolarH7 and ECG. In particular, CLUSTER D, composed of subjects with low fitness level, high age and high trunk fat percentage, was the group with the lowest number

of subjects obtaining excellent agreement between devices for P_{HF} (n) and with the highest %Error for time- and frequency-domain HRV indices.

The large number of significant differences in the temporal domain HRV indices (MHR, SDNN and RMSSD) between PolarH7 and ECG at all cycling stages could be due to a small but consistent difference between devices. Specifically, PolarH7 values were usually slightly lower than those measured by the ECG, which is supported by the obtained small to medium effect sizes. In the case of MHR, the values measured by the two devices for individual subjects were the same up to the 2nd-3rd decimal figure. Even if significant, such differences between devices may not be meaningful in practice, as differences of less than one beat per minute are below what has been reported as the smallest worthwhile change in previous studies [37].

Regarding analysis of the full paired series of RR intervals, excellent agreement between devices was found at rest, in accordance with previous validation studies of PolarH7/H10 HR sensors [10,12]. Also, our results confirmed that the agreement between devices decreased with the intensity of exercise, as previously reported [11,12]. Despite this reduction, Gilgen-Ammann et al. proposed Polar H10 as the gold standard for RR interval assessment during intense activities for HR and HRV evaluation [12]. It should be noted, however, that a reduced set of ten healthy, lean and physically fit volunteers was considered in [12], whereas here we investigated a larger set of volunteers with a broader range of ages, body compositions and fitness levels. This may explain why we found a more noticeable reduction in the agreement between devices during exercise.

Frequency-domain HRV indices, including P_{LF} and P_{HF}, were not usually investigated in previous studies validating PolarH7/H10 HR sensors. Nevertheless, these frequency-domain signals were evaluated in the previous Polar RS800 model, reporting that differences between devices increased with exercise intensity and were higher for P_{HF} than for P_{LF} [17]. In the present study, P_{LF} showed excellent agreement at rest and during the whole exercise test, meaning that PolarH7 can follow HR oscillations up to 0.15 Hz. Still, the percentage of subjects reaching excellent agreement for P_{LF} at the highest intensities (81% with CCC > 0.9 at 80% of HRmax) was lower than in [17] (96% with Pearson correlation coefficient > 0.8 at 80–100% of VO_2max), possibly due to the greater heterogeneity of the present population sample. In the case of P_{HF}, we found that PolarH7 and ECG showed disagreement at the highest intensities, in accordance with results reported for Polar RS800, possibly due to a multifactorial etiology, including the higher respiratory frequency and higher noise level during exercise, the processing performed by Polar when a beat cannot be detected and the effect of the body characteristics of the subjects [17]. Since both P_{HF}, reflecting vagal modulation of cardiac activity, and its highly correlated time-domain HRV measures, such as RMSSD [38], are commonly used to monitor the autonomic status before, during and after exercise [39], a note of caution on the interpretation of results obtained from PolarH7 is suggested, especially at exercise intensities greater than 70% of HRmax.

Our results from clustering analysis confirmed the hypothesis that the phenotypic characteristics of the subjects are one of the causes for the observed differences between PolarH7 and ECG devices [17]. As initially postulated, CLUSTER D, containing subjects with low fitness, high age and high trunk fat percentage, was the one showing the highest %Error for HRV indices, reaching 50% error in P_{HF} and 30% error in RMSSD for intensities greater than 70% of HRmax. Nevertheless, even in CLUSTER D, some subjects presented excellent agreement between devices, confirming that the characteristics of the subjects are not the only cause of disagreement. Future studies should clarify other possible reasons underlying the observed differences between devices.

The recovery from exercise was the time period when the lowest CCC values were measured, especially for P_{LF} and P_{HF} signals. To our knowledge, this is the first time that PolarH7 validity has been analyzed during sitting recovery. The lack of agreement between devices could be due to the noisy signal recorded by PolarH7 in some volunteers, as illustrated in Figure 3. Consequently, despite the correction algorithms, 10% of P_{LF} signals and 27% of P_{HF} signals presented very low agreement (CCC < 0.1). Based on these

results, assessment of HRV, particularly P_{LF} and P_{HF}, during the recovery period may not be reliable if PolarH7 is used. This is in line with Schneider et al., who recommended evaluation of HR recovery rather than evaluating post-exercise HRV [7].

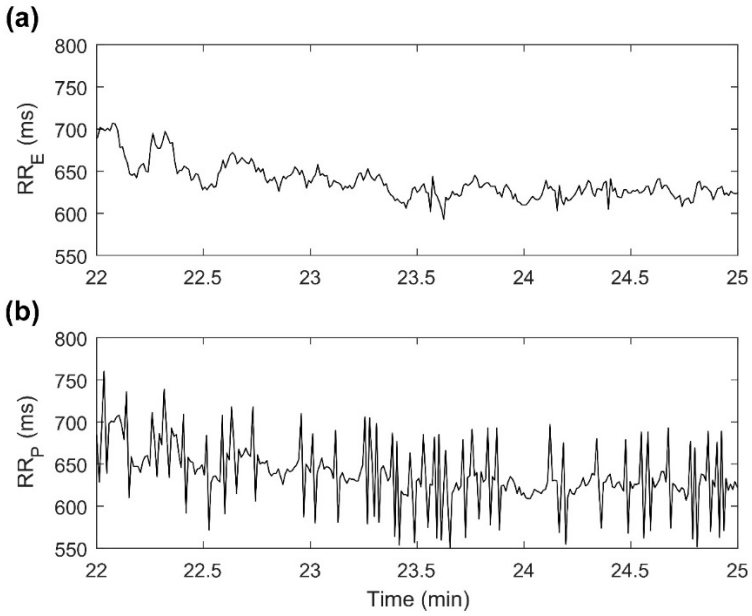

Figure 3. Example of RR intervals recorded by both devices during the recovery segment for one subject. (**a**) RR_E = RR intervals series recorded by ECG; (**b**) RR_P = RR intervals series recorded by PolarH7.

Strengths and Limitations

The present study has several main strengths. One of them is the phenotypic variety of the 67 volunteers. HR sensor validation studies are often carried out in groups of 20, or even fewer, young, lean, healthy and physically fit volunteers [10–12], and in these conditions it may be easier to detect RR intervals. Therefore, our larger sample of 20- to 70-year-old subjects with varied physical conditions is much more heterogeneous and representative of HR sensor users. Secondly, our assessment of PolarH7 validity as a function of phenotypic characteristics, including age, is particularly relevant considering that the older population is growing all around the world and more so in Europe [40], with advanced age being associated with changes in body composition and reduced cardiorespiratory fitness [41,42]. Taking into account these associations, cluster analysis was used to evaluate how the concurrence of these characteristics in the volunteers could affect HRV measurements obtained from PolarH7. In third place, cardiorespiratory fitness and the excess of body fat are strong predictors of mortality and risk of cardiovascular disease, being age the main risk factor for multimorbidity [40,42]. Accordingly, it is of special interest to evaluate the validity of these devices in subjects with these phenotypic characteristics. As discussed in the introduction of the study, the applications of HRV in the evaluation and management of a wide range of diseases are growing. The use of these inexpensive and simple to use devices could be a very useful tool for E-health in primary care. HRV measures that can be reliably assessed by HR sensors like PolarH7 need to be established so that interpretations can be safely made. Last but not least, all our body measurements and signal recordings were performed in the laboratory, under homogeneous conditions, enabling the control of confounding factors and the reproducibility of the study.

On the other hand, some limitations need to be acknowledged. According to the meta-analysis by Dobbs et al., the degree of absolute error between portable devices and ECG measurements was larger among studies involving a greater number of female subjects [9]. Here, only men were studied so that sex was not a confounding variable. Further research over other populations including not only women but also black/African would allow

confirming the results obtained by this study in wider populations. Another potential limitation is that PolarH7 has been superseded by the Polar H10 band. Even so, millions of users still wear a PolarH7 band and the performance of both bands seems to be similar during stationary exercise [43]. Future studies could extend the research here presented to the analysis of other HR sensors.

5. Conclusions

Three major findings have emerged from the present study. First, assessment of HR and HRV in a relatively large and heterogeneous sample has confirmed that PolarH7 can accurately measure mean HR and low-frequency oscillations (up to 0.15 Hz) of HR at rest and during exercise. However, disagreement between PolarH7 and ECG exists when evaluating high-frequency HR oscillations during moderate-to-high intensity exercise. Second, the validity of PolarH7 measurements during sitting recovery has been studied for the first time. The results of the present research support the notion that PolarH7 is appropriate to study HR recovery rather than post-exercise HRV. Third, clustering analysis shows that the agreement between PolarH7 and ECG devices varies depending on the characteristics of the subjects regarding age, body composition and fitness level. Our results point to the need of ensuring phenotypic variety in any validation studies of HR sensors.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the ethical committee for clinical research of Aragón (ID of the approval: PI17/0409; date: 17/1/2018).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The datasets analyzed during the current study are available from the corresponding author on reasonable request.

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4.5 Artículo V

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Heart Rate Variability and Exceptional Longevity

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Centenarians are the paradigm of human extreme longevity and healthy aging, because they have postponed, if not avoided, mayor age-related diseases. The purpose of this study was to investigate potential differences in resting heart rate variability (HRV) between young adults, octogenarians, and centenarians and assess whether HRV variables are predictors of all-cause mortality in centenarians. To this end, three groups of participants: young adults (N = 20; 20.6 ± 2.3 years), octogenarians (N = 18; 84.1 ± 2.6 years), and centenarians (N = 17; 101.9 ± 1.9 years) were monitored for 15 min at rest (seated, without moving or talking) to measure RR intervals, from which HRV was evaluated. Our results showed a clear decrease with age in the main parasympathetic HRV variables, as well as in the standard deviation (SD) of the RR series [SD of normal-to-normal interval (SDNN)] and in low frequency (LF) heart rate (HR) oscillations, although differences between octogenarians and centenarians did not reach statistical significance. In 14 centenarians followed until death, only SDNN showed significant correlation (ρ = 0.536; p = 0.048) with survival prognosis. Additionally, SDNN <19 ms was associated with early mortality (≤1 year) in centenarians (Hazard Ratio = 5.72). In conclusion, HRV indices reflecting parasympathetic outflow as well as SDNN and LF all present an age-related reduction, which could be representative of a natural exhaustion of allostatic systems related to age. Moreover, low SDNN values (<19 ms) could be associated with early mortality in centenarians. HRV seems to play a role in exceptional longevity, which could be accounted for by centenarians' exposome.

Keywords: electrocardiography, autonomic nervous system, parasympathetic nervous system, heart rate, heart rate variability, mortality, centenarians, aging

INTRODUCTION

Heart rate variability (HRV) is defined as "the oscillation in the interval between consecutive heart beats" (Malik et al., 1996). HRV is the result of the interaction of multiple regulatory mechanisms that operate at different time scales, including long-term mechanisms like circadian rhythms, core body temperature, or metabolism and short-term mechanisms involving the autonomic, cardiovascular, and respiratory systems (Shaffer and Venner, 2013). Short-term spectral analysis of HRV usually reveals at least two frequency components, a low frequency (LF) component (0.04–0.15 Hz) and a high frequency (HF) component (>0.15 Hz; Malik et al., 1996). These components have been widely used to measure sympathetic and parasympathetic nervous systems, although their underlying physiological mechanisms are still unclear and a matter of debate (Billman, 2013).

In the last decades, several studies have reported that HRV decreases with age, suggesting an age-dependent decline in autonomic nervous system (ANS) activity in geriatric patients (Craft and Schwartz, 1995; Piccirillo et al., 1995). The majority of these studies have been mainly performed in older adults up to 80–85 years old, whereas older adults over age 85 have not received much attention. Centenarians represent the survival tail of the population (with a lifespan at least 15–20 years longer than the average westerner) and a model of healthy aging (Christensen et al., 2008). Indeed, centenarians escaped the diseases of the pre-antibiotic era and have postponed/avoided aging-related diseases as well as their fatal consequences (Salvioli et al., 2008).

The study of centenarians constitutes a fascinating research into the characteristics that allow individuals to attain an exceptionally long lifespan. Few works have studied HRV in centenarians. Piccirillo et al. (1998) and Paolisso et al. (1999) found that centenarians present higher power in HF heart rate (HR) oscillations and lower power in LF than old adults (75–100 years old in Paolisso et al. and 81–100 years old in Piccirillo et al.), suggesting age-related increase in parasympathetic activity and reduction in sympathetic activity. These results are in line with those obtained by Zulfiqar (2010) who enrolled subjects up to 99 years old and demonstrated that parasympathetic time-domain HRV measures decrease with age, reaching a nadir in the 7th–8th decade. From the 8th decade, these HRV measures are shown to rise, with the authors proposing this reversal of the decrease in parasympathetic function as a key determinant of longevity. In contrast, another study conducted in centenarians linked HRV with mortality during 4-year follow-up, showing that among all frequency-domain variables only higher LF/HF ratio was associated with survival (Shimizu et al., 2002).

Eight out of 10 centenarians are women (Teixeira et al., 2017). In 2016, Koenig and Thayer (2016) published a meta-analysis with 63,612 participants (31,970 females), revealing that: although adult women showed greater mean HR (MHR) than adult male, the female heart is characterized by a dominance of vagal activity (greater HF) and lower standard deviation (SD) of normal-to-normal intervals (SDNN). However, these sex differences may disappear in older adults (Voss et al., 2015; Koenig and Thayer, 2016), as a consequence of a variety of age-related changes such as: endocrine, brain structure, brain perfusion, or behavioral differences.

Due to the lack of current evidence and the discrepancies in the reported outcomes, the present study aimed at investigating potential differences in women's HRV between young adults, octogenarians, and centenarians and assess whether HRV variables can predict all-cause mortality in centenarians followed up until the time of death.

MATERIALS AND METHODS

Participants

Women aged 18–26 years in the group of young adults, 80–90 years in the group of octogenarians, and ≥100 years in the case of centenarians were included in the study. Due to the low number of centenarians, four men were additionally included in this group. In total, the young adults, octogenarians, and centenarians groups contained 20, 18, and 17 subjects, respectively. Exclusion criteria included the following: subjects going through an acute disease, suffering from heart diseases (e.g., heart failure or atrial fibrillation), or being on cardiac medication. Subjects who had a stroke or were suffering from chronic diseases such as diabetes, hypertension, chronic obstructive pulmonary disease, osteoarthritis, dementia, Parkinson's, or thyroid diseases were included in the study because of their high prevalence in the last decades of life. The study was approved by the Clinical Research Ethics Committee of the University Hospital of Alcorcón (ID of the approval: 16/50) and was conducted adhering to the Declaration of Helsinki. After a clear explanation of the potential risks of the study, all volunteers (or their legally responsible for older adults with cognitive problems) provided written informed consent to participate in the study.

Experimental Design

All the subjects completed one test session. Prior to the test session, subjects were asked to adhere to the following instructions: (1) avoid exercise or strenuous physical activity the day before the test; (2) drink plenty of fluids over the 24 h period preceding the test; (3) get an adequate amount of sleep (6–8 h) the night before the test; (4) avoid substances such as tobacco, alcohol, or stimulants (caffeine, theine, taurine, etc.) in the 8 h before the test; (5) avoid food for 3 h prior to taking the test; and (6) wear comfortable, loose-fitting clothing. All the subjects were tested in an environmentally controlled room (22–23°C) between 9:00 and 13:00 h. They were monitored for 15 min at rest (seated, without any movement or talking) to measure RR intervals. RR intervals were recorded on a beat-to-beat basis by using an HR monitor (RS800, Polar Electro Oy, Kempele, Finland) with a sampling frequency of 1,000 Hz, thus providing an accuracy of 1 ms for each RR period. This device has been recently validated, showing to provide comparable performance with respect to the electrocardiogram when analyzing HRV at rest (de Rezende Barbosa et al., 2016; Hernando et al., 2016).

HRV Variables

HRV variables have commonly used to assess sympathetic and parasympathetic nervous systems. The LF component of HRV is assumed to provide information on cardiac sympathetic and parasympathetic neural activity, together with other regulatory mechanisms and baroreflex (Eckberg, 1997). The HF component, on the other hand, is assumed to be vagally mediated and driven by respiration, measuring the so-called respiratory sinus arrhythmia (RSA; Berntson et al., 1993). Based on these assumptions, the ratio of LF to HF (LF/HF) has been proposed to quantify the relationship between sympathetic and parasympathetic activities (i.e., the sympatho-vagal balance; Malik et al., 1996).

However, although these spectral indices are well-standardized, their physiological interpretation has been criticized. This especially applies to the relationship between LF power and cardiac sympathetic regulation (Eckberg, 1997; Billman, 2013; Reyes del Paso et al., 2013), with LF power decreasing during situations expected to increase sympathetic activity, such as exercise or myocardial ischemia, and lack of correlation between direct recording of sympathetic nerve activity and LF power in either healthy subjects or patients with heart failure. The interpretation of HF power has been also challenged, especially when the respiratory rate does not fall within the HF band (0.15–0.4 Hz; Laborde et al., 2017). Different approaches have been proposed to overcome this limitation by redefining the HF band (Bailón et al., 2007; Varon et al., 2018). It has also been suggested that sympathetic neural activity may modulate the HF component (Billman, 2013). Therefore, the physiological interpretation of the LF/HF ratio is unclear and likely underestimates the complex interactions between the sympathetic and parasympathetic regulation of HR (Billman, 2013).

Normalized LF power (LFn) represents the proportional contribution of sympathetic modulation (Malik et al., 1996), in the same way and with the same limitations as LF/HF ratio represents sympatho-vagal balance. A mathematical relationship exists between LFn and LF/HF ratio: $LFn = (1 + (LF/HF) - 1) - 1$, so individual LFn values contain no more information than individual LF/HF ratio values (Heathers, 2014). However, statistical results on them might differ due to the volatility of the LF/HF ratio when HF power approaches zero (Billman, 2013; Heathers, 2014).

Regarding time-domain variables, SDNN reflects all the cyclic components responsible for HRV (Laborde et al., 2017). Lastly, the root mean square of successive differences (RMSSD), the percentage of RR intervals which exceed 50 ms from the previous one (pNN50), and the SD of successive differences (SDSD) are correlated with the HF band, so vagal activity is considered to be in the physiological origin of these three variables. Of these, RMSSD is normally preferred since it is less influenced by respiration (Laborde et al., 2017). Despite the former caveats in their interpretation, the study of HRV indices is an area of great interest as they provide a low-cost and non-invasive window into ANS regulation of the heart.

Data Acquisition and Processing

HRV analysis was performed on 3-min running windows taken every 30 s. In each window, outlier RR intervals were identified by imposing a limit on the derivative of the instantaneous HR, which cannot exceed a time-varying threshold based on the median of its previous values (Mateo and Laguna, 2003). Only those windows with less than 10 outliers (always below 5% in this study) were considered for further analysis. Two different HRV representations were used for time and frequency domain HRV indices estimation.

For time domain indices, the RR series was used, after correction of identified outlier RR values using the interpolation proposed in Mateo and Laguna (2003). The following indices were computed: MHR, RMSSD, pNN50, SDSD, and SDNN (Malik et al., 1996).

For frequency domain indices, the HRV representation used is the modulating signal, based on the heart timing signal, since it was shown to outperform other HRV representations for frequency domain indices estimation (Mateo and Laguna, 2000). The modulating signal, assumed to carry information from the ANS, was estimated from the beat occurrence time series, derived from the recorded RR intervals, based on the time-varying integral pulse frequency modulation model (Bailón et al., 2011). First, the instantaneous HR signal was estimated, sampled at a sampling frequency Fs = 4 Hz, and denoted by $d_{HR}(n)$. Subsequently, the time-varying MHR signal, $d_{MHR}(n)$, was estimated by low-pass filtering $d_{HR}(n)$ with a cutoff frequency of 0.03 Hz. The modulating signal, $m(n)$, was estimated by normalizing the HRV signal, $d_{HRV}(n) = d_{HR}(n) - d_{MHR}(n)$, by the time-varying MHR, i.e., $m(n) = d_{HRV}(n)/d_{MHR}(n)$. Note that the modulating signal $m(n)$ is adimensional. The purpose of this normalization is to alleviate the effect that changes in MHR have on HRV (Bailón et al., 2011). Then, the power spectral density (PSD) of the modulating signal $m(n)$ was estimated using Welch periodogram with internal window of 2 min and 50% overlap. The power in the following bands was estimated: (i) LF, from 0.04 to 0.15 Hz; (ii) HF, from 0.15 to 0.40 Hz; (iii) extended HF (HFext), from 0.15 to half the MHR, to avoid misestimation of the HF component when respiratory rate is above 0.4 Hz (24 breaths per minute; Bailón et al., 2007). The LFn power was computed by dividing LF power by the sum of LF and HF powers (LFn), and the extended LFn was determined by dividing LF by the sum of LF and HFext (LFn_ext). Finally, the ratio between the LF and HF powers (LF/HF) and the ratio between LF and HFext powers (LF/HFext) were calculated.

As HRV analysis was performed on running 3-min windows taken every 30 s, the mean of each HRV variable in all running windows with less than 10 outlier RR values was computed to characterize each subject.

Statistical Analysis

Descriptive values are presented as mean ± SD and HRV values are reported as median and (1st quartile–3rd quartile). The normality of data was checked with the Shapiro-Wilk test. Since the data distribution violated the assumption of normality required by parametric tests and could not be corrected by common transformations, a non-parametric analysis was used. To assess differences between the three age groups, Kruskal-Wallis test (non-parametric equivalent of one-way ANOVA) with Bonferroni correction was performed.

The Dunn-Bonferroni *post hoc* method was used for pairwise comparisons. A multiple linear regression was performed to control for potential confounding effects like the body mass index (BMI). To evaluate the magnitude of the difference, effect size (ES) was calculated as: ES = $chi^2/(k-1)$, where k = total number of subjects. The difference was considered as small when ES < 0.2, small to medium when ES = 0.2–0.5, medium to large when ES = 0.5–0.8, and large when ES > 0.8 (Cohen, 1992).

A sub-analysis was carried out by following centenarians until their death and calculating Spearman's correlation coefficient (ρ) between HRV variables and "time to death." For the HRV variables showing significant correlation to "time to death," centenarians were divided into two groups by setting a cut point that defined a high risk group containing one third of the centenarian population and a low risk group containing the remaining two thirds. The Mann-Whitney U test was used to compare differences between the statistical distributions of the two groups. Kaplan-Meier survival analysis was performed and the log-rank test was used to test survival differences between the two groups. Additionally, the value of HRV variables in predicting survival was determined by Cox proportional hazards analyses. Statistical analyses were performed using IBM SPSS (version 25; Chicago, IL, United States). The significance level was set at $p < 0.05$.

RESULTS

Table 1 shows the descriptive characteristics of the groups.

An illustrative example of the RR interval series for each group is shown in **Figure 1**. Results obtained for the HRV variables in each age group are shown in **Table 2**. Differences between groups were only observed when analyzing the parasympathetic variables: RMSSD, pNN50, HF, HFext, and SDSD as well as LF and SDNN. All these variables decreased significantly with age (main effect: $p < 0.05$) but no statistically significant differences were found between octogenarians and centenarians.

We were able to follow up 14 centenarians until death. The three subjects with incomplete data were females but no significant differences with the 14 subjects included in the sub-analysis were found either in the descriptive variables or in the HRV variables. The only HRV variable that presented significant correlation with survival prognosis in centenarians (**Table 3**) was SDNN (ρ = 0.536, p = 0.048).

The 14 centenarians were divided according to the SDNN variable into two groups based on a cut point of 19 ms: Group 1 presenting low SDNN values (N = 4; 15 ± 4 ms, range: 10–18) and Group 2 presenting high SDNN values (N = 10; 49 ± 25 ms, range: 27–110). The difference between the statistical distributions of the two groups was statistically significant. Group 2 was associated with greater survival (1.6 ± 0.9 years, range: 0.3–3.5) than Group 1 (0.6 ± 0.3 years, range: 0.3–1.0) in Kaplan-Meier analysis (*Log-rank test* = 0.010, **Figure 2**). Mortality risk in Group 1 was five times higher (p = 0.028; Hazard Ratio = 5.72) than in Group 2.

TABLE 1 | Descriptive characteristics of the groups.

Variable	Young adults (N = 20)	Octogenarians (N = 18)	Centenarians (N = 17)
Age (years)	20.6 ± 2.3 (18–26)	84.1 ± 2.6 (80–88)	101.9 ± 1.9 (100–105)
Women (%)	100	100	76.5
BMI (kg/m^2)	20.7 ± 1.9 (17–24)	27.0 ± 2.8 (22–31)	23.1 ± 3.4 (17–28)
Chronic diseases (%)*	Osteoarthritis	50	59
	CVD	44	53
	Dementia	11	47
	Diabetes	33	18
	AHT	61	53
	COPD	6	6
	Others	61	65
	Total number	3.3 ± 1.7 (0–5)	3.3 ± 1.0 (2–6)

All young adults were healthy.
Scale values are mean ± standard deviation (SD) and min-max; BMI, body mass index; CVD, cardiovascular disease; AHT, arterial hypertension; COPD, chronic obstructive pulmonary disease.

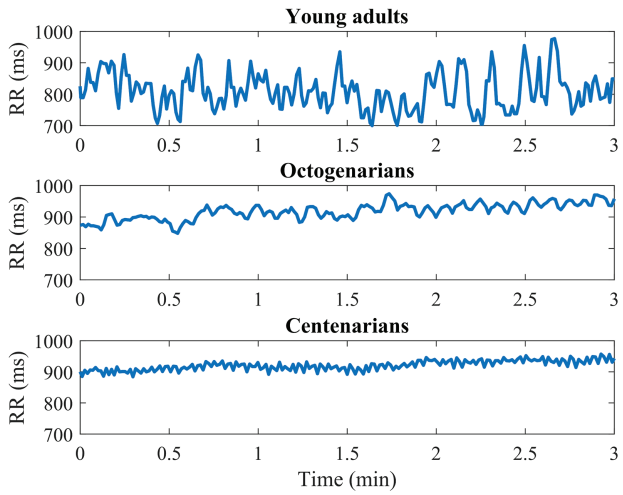

FIGURE 1 | Example of the RR interval series for one subject from each age group.

No relation was found between subjects' age at RR recording time and time to death (p = 0.477).

DISCUSSION

The present study shows that parasympathetic time-domain HRV measures as well as SDNN and LF all decrease with age; moreover, other variables such as LFn or LF/HF ratio do not indicate differences between age groups. In relation to survival prognosis, SDNN was the only HRV measure showing moderate correlation (ρ = 0.5–0.7) with time to death in centenarians, with SDNN values below 19 ms being associated with early mortality (≤1 year) in centenarians (Hazard Ratio = 5.72).

TABLE 2 | Differences between groups using Kruskal-Wallis non parametric test.

Variable	Young adults (*N* = 20)	Octogenarians (*N* = 18)	Centenarians (*N* = 17)	Main effect	
	Median (Q_1–Q_3)	Median (Q_1–Q_3)	Median (Q_1–Q_3)	*p*	ES
LF	0.0015 (0.0012–0.0031)†‡	0.0004 (0.0001–0.0017)	0.0002 (0.0001–0.0004)	<0.001*	0.441
LFn	0.5708 (0.5204–0.6266)	0.6131 (0.4608–0.7031)	0.5366 (0.3867–0.6456)	0.504	0.025
LFn_ext	0.5517 (0.4969–0.5997)	0.5701 (0.4286–0.6202)	0.4426 (0.2840–0.5887)	0.168	0.066
HF	0.0016 (0.0007–0.0032)†‡	0.0002 (0.0000–0.0009)	0.0001 (0.0001–0.0003)	<0.001*	0.414
HFext	0.0018 (0.0008–0.0035)†‡	0.0002 (0.0001–0.0010)	0.0002 (0.0001–0.0005)	<0.001*	0.367
RMSSD	57.97 (41.83–109.35)†‡	24.37 (10.14–67.29)	36.37 (18.71–50.55)	0.005*	0.194
pNN50	24.31 (12.61–41.24)†‡	2.62 (0.26–22.00)	6.88 (0.85–9.29)	<0.001*	0.283
SDSD	58.02 (41.91–109.59)†‡	24.41 (10.16–67.44)	36.44 (18.74–50.57)	0.005*	0.194
SDNN	68.13 (52.55–130.75)†‡	41.38 (17.41–82.03)	36.77 (21.25–45.90)	0.001*	0.276
LF/HF	1.44 (1.12–1.91)	1.78 (0.91–2.39)	1.36 (0.68–2.19)	0.650	0.016
LF/HFext	1.32 (1.03–1.61)	1.41 (0.80–1.75)	0.85 (0.43–1.60)	0.303	0.044
MHR	72.49 (65.87–79.05)	70.50 (64.44–76.62)	72.61 (62.23–85.50)	0.687	0.014

*$p < 0.05$.
†Different to octogenarian.
‡Different to centenarian.
Values are expressed as median and (1st quartile–3rd quartile). LF, low frequency; LFn, normalized LF; LFn_ext, extended LFn; HF, high frequency; HFext, extended HF; RMSSD, root mean square of successive differences; pNN50, percentage of RR intervals which exceed 50 ms from the previous one; SDSD, SD of successive differences; SDNN, SD of the RR series; LF/HF, ratio between LF and HF; LF/HFext, ratio between LF and HFext; MHR, mean heart rate; p, p-value for Kruskal-Wallis test; ES, effect size.

TABLE 3 | Survival prognosis in centenarians (*N* = 14).

	LF	LFn	LFn_ext	HF	HFext	RMSSD	pNN50	SDSD	SDNN	LF/HF	LF/HFext	MHR
ρ	0.423	0.172	0.295	0.190	0.232	0.304	0.247	0.304	0.536	0.214	0.251	–0.082
p	0.131	0.557	0.305	0.516	0.426	0.290	0.395	0.290	0.048*	0.463	0.386	0.782

*$p < 0.05$. *LF, low frequency; LFn, normalized LF; LFn_ext, extended LFn; HF, high frequency; HFext, extended HF; RMSSD, root mean square of successive differences; pNN50, percentage of RR intervals which exceed 50 ms from the previous one; SDSD, SD of successive differences; SDNN, SD of the RR series; LF/HF, ratio between LF and HF; LF/HFext, ratio between LF and HFext; MHR, mean heart rate; ρ, Spearman's correlation coefficient with "time to death"; p, p-value for Pearson's correlation coefficient.*

HRV Measures

When analyzing the main parasympathetic HRV variables (RMSSD, pNN50, HF, and SDSD; Malik et al., 1996; Laborde et al., 2017), a clear decrease with age is observed in all of them, with very remarkable differences between young adults and older adults but without significant differences between octogenarians and centenarians. This age-related decrease can be appreciated in the illustrative examples of **Figure 1** and has already been reported by other authors (Umetani et al., 1998; Bonnemeier et al., 2003; Abhishekh et al., 2013). Also, to the best of our knowledge, this is the first time that it has been described in centenarians. It should be emphasized that our results differ from those of Paolisso et al., Zulfiqar et al., and Almeida-Santos et al. since they establish a parasympathetic nadir at 75–80, 70–79, and 60–69 years, respectively, and our study indicates that parasympathetic HRV variables continue to decrease in centenarians (Piccirillo et al., 1998; Zulfiqar et al., 2010; Almeida-Santos et al., 2016). There are several possible explanations for our sample of centenarians not showing a reversal of the decrease in parasympathetic function. First, erratic rhythms may have a confounding effect on age-related changes in parasympathetic HRV indices (Nicolini et al., 2012), which is why our study only analyzes RR segments free of erratic patterns, allowing the presence of no more than 10 outlier values in each 3-min window of analysis. Second, although the BMI of the octogenarian sample was significantly higher, BMI had no effect as a confounding variable ($p > 0.05$ in the multiple linear regression). Some studies have found reduced HRV in underweight and overweight adult women (Triggiani et al., 2017; Gerardo et al., 2019), but studies in the literature investigating the oldest old are scarce. The lower BMI in the centenarian sample could be an indicator of healthy body composition but also a simple consequence of age-associated sarcopenia or osteoporosis. On the other hand, previous studies in centenarians have been very restrictive in the selection of subjects, including only very healthy and independent subjects (Piccirillo et al., 1998; Paolisso et al., 1999; Zulfiqar et al., 2010), which may involve a selection bias (Tan et al., 2019). The parasympathetic decrease in centenarians found in our study could, thus, be more representative of a natural exhaustion of allostatic systems related to age.

As already mentioned, the interpretation of the standard HF band (0.15–0.4 Hz) is compromised when respiratory rate does not fall within this band (9–24 bpm). Since breathing was not monitored, power in the extended HF band (0.15-half MHR) was computed to account for respiratory rates that

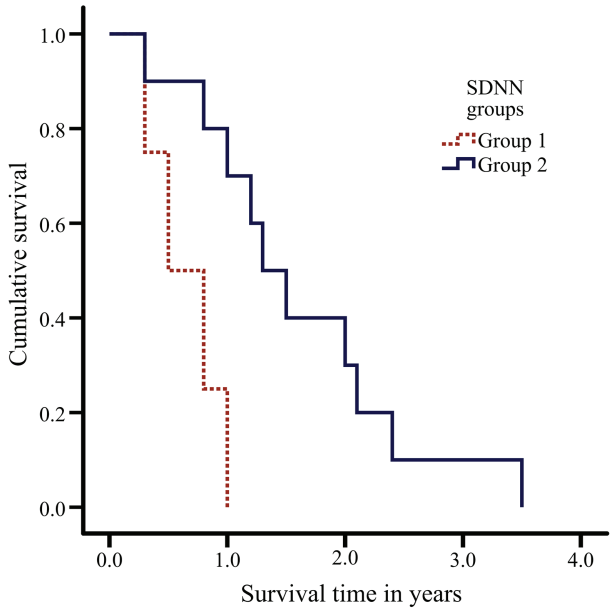

FIGURE 2 | Kaplan-Meier curves depicting association between survival in centenarians (N = 14) and SDNN groups based on a cut point of 19 ms.

might exceed 24 bpm, as suggested in Bailón et al., 2007. As it can be seen in **Table 2**, results of the standard HF band were parallel to those of the extended HF band, suggesting that in this database, respiratory rates were in the standard HF band (0.15–0.40 Hz). Therefore, HF could be considered as a measure of the vagal tone (Laborde et al., 2017).

SDNN can be considered as an indicator of global autonomic regulation, although it has been claimed that in short-term recordings, the primary source of its variations is parasympathetically-mediated RSA (Shaffer and Ginsberg, 2017). In agreement with previous studies (Zulfiqar et al., 2010; Almeida-Santos et al., 2016; Sammito and Böckelmann, 2016), SDNN values decline with age, further reflecting an age-dependent decline in ANS activity. LF results were in the same line as the parasympathetic HRV variables and SDNN, probably because the recording was made while sitting upright during resting and under these conditions the primary contributors to HRV have been suggested to be related to parasympathetic and baroreflex activity rather than to sympathetic activity (Shaffer and Ginsberg, 2017).

Other HRV variables, LFn and LF/HF, whose physiological interpretation is usually controversial, have been additionally investigated in our study, neither of them showing statistically significant differences between groups.

HRV and Survival Prognosis in Centenarians

In recent decades, HRV has been confirmed as a strong, independent predictor of morbidity and all-cause mortality (Billman, 2011; Kemp et al., 2017). To investigate HRV variables that may be associated with survival prognosis in centenarians, we followed up subjects until death. Only SDNN showed significant correlation (ρ = 0.536, p = 0.048) with survival prognosis in centenarians. The group of centenarians with low SDNN values presented five times greater mortality risk than centenarians with high SDNN values. In the framework of the research topic "Horizon 2030: Innovative Applications of Heart Rate Variability," we discuss about HRV and exceptional longevity. However these results should be read with perspective, as the sample of centenarians followed until death is heterogeneous in gender, including 4 men and 10 women.

Since Kleiger et al. set the basis for the use of HRV in post-acute myocardial infarction risk stratification in 1987, SDNN is considered as a "gold standard" when recorded over a 24-h period. SDNN values below 50 ms are classified as unhealthy, 50–100 ms as compromised health, and above 100 ms as healthy (Kleiger et al., 1987). According to Bilchick et al. (2002), each 10-ms increase in SDNN confers a 20% decrease in risk of mortality. SDNN is the only variable presenting significant correlation with time to death in our cohort of centenarians. In particular, SDNN <19 ms turns out to be indicative of early mortality (≤1 year). Of note, one subject presented a value of SDNN of 110 ms and was the one who lived the longest time (3.5 years) calculated from the time point when RR was recorded.

It should be noted that there are other RR-derived variables that have been related to increased mortality risk in the literature. The fact that they have not been found to be associated with time to death in our study could be due to the small sample of our cohort or to the particular characteristics of the studied centenarians. A classic example is high resting HR (Zhang et al., 2015). Additionally, a recent meta-analysis has established LF/HF ratio and SDNN as two of the variables with greater potential as predictors of mortality (Sen and McGill, 2018). Shimizu et al. (2002) have also observed the relevance of LF/HF ratio in a cohort of 27 centenarians. Finally, LF is one of the most controversial HRV indices in the literature. Some studies, such as the Framingham Heart Study, have associated a 1-SD decrement in LF with 1.70 times greater hazard for all-cause mortality (Tsuji et al., 1994). On the other hand, cross-sectional studies in healthy centenarians have reported that high LF values are associated with increased mortality risk (Piccirillo et al., 1998; Paolisso et al., 1999).

Centenarians and the "Neurovisceral Integration Across a Continuum of Time" Framework

Centenarians are considered to be a model of healthy and successful aging. It is well known that exceptional longevity is a partially inheritable phenotype that could be explained in 20–35% by the genetic load (Rea et al., 2016). Consequently, it could be that the ANS of the centenarians had a greater and innate adaptation level, and therefore they will take 20 years more than the general population to reach a level of depletion of the allostatic systems related to mortality. On the other hand, another feasible explanation would be that centenarians

have healthy behaviors that allow them to experience a less marked decrease in the function of the ANS. Non-genetic factors, including diet, physical activity, health habits, and psychosocial factors contribute approximately 50% of the variability in human lifespan (Rea et al., 2016).

Recently, Kemp et al. (2017) published a theoretical framework called "Neurovisceral Integration Across a Continuum of Time (NIACT)" where they propose that the function of the vagus nerve, indexed by resting-state HRV, plays a regulatory role on a variety of allostatic systems, therefore contributing to an increase or decrease in the risk of future morbidity and mortality. NIACT proposes that while age decreases vagal function, there are many interventions that may be applied to contend such decreases including health behavior, meditation, and positive psychological interventions (Kemp et al., 2017). Health behaviors related to improvements in HRV are similar to those that characterize the lifestyle of centenarians in different populations: regular physical activity, dietary habits, no drinking, and no smoking (Ozaki et al., 2007; Kim et al., 2012; Wu et al., 2017). But psychological moments are also a key element in the NIACT framework, and in the same way, active engagement in community activities, high levels of self-perceived well-being, and satisfaction with life are defining elements of the centenarian population (Ozaki et al., 2007; Kim et al., 2012; Wu et al., 2017; Hitchcott et al., 2018; Yorgason et al., 2018). Therefore, the characteristic lifestyle of centenarians would imply a greater resilience, indexed by greater variability of the HR and, as described above, higher SDNN values would mean better survival prognosis in centenarians.

Strengths and Limitations

The main strength of the present study is the exceptionality of the sample, considering than being centenarian is a rare phenotype (17.3 centenarians per 100.000 inhabitants; Teixeira et al., 2017). Secondly, centenarians were followed up to death and our study proposes SDNN <19 ms as a cutoff point to define a marker of early mortality (≤1 year), which is obtained from short-term measurements, thus more suitable for ambulatory care and patient monitoring. Given its ease of recording, short-term variability allow measurements under homogeneous conditions, enabling the control of confounding factors and the reproducibility of the study (Li et al., 2019). Moreover, RR measurements were acquired using a validated device and processed with methods that allow better identification of the erratic patterns.

On the other hand, one of the main limitations was gender heterogeneity in the centenarian group, with 76.5% of our centenarians being women. A meta-analysis has highlighted that women show greater vagal activity compared to men, noting the following possible etiological factors: estrogen, oxytocin, and neural control (Koenig and Thayer, 2016). Our sample, however, is very similar to the overall centenarian population in Europe (83.5% women; Teixeira et al., 2017), and sex differences have been reported to disappear in the last age decades, especially in short-term HRV, presumably by the hormonal restructuring especially caused by the menopause in women (Bonnemeier et al., 2003; Voss et al., 2015; Koenig and Thayer, 2016). Indeed, when the statistical analysis of our study was performed by excluding men (N = 4) from the sample of centenarians, results were similar to those reported in **Table 2**. Finally, recording conditions should be taken into account before generalizing the results. For example, paced breathing was not considered in our work. Under resting conditions, the respiratory rate (9–24 bpm) is expected to be in the 0.15–0.40 Hz band (Shaffer and Ginsberg, 2017), but processing should account for the possibility that the respiratory rate goes outside this frequency band, as performed in the present work. A within-subject repeated measure design would have contributed to assess the reproducibility of our evaluations. In future research, more representative samples of centenarians would allow to confirm the results obtained by this study.

In conclusion, HRV indices reflecting parasympathetic outflow (RMSSD, pNN50, HF, and SDSD) as well as SDNN and LF all present an age-related reduction, which could be representative of a natural exhaustion of allostatic systems related to age. Moreover, low SDNN values (<19 ms) are indicative of early mortality (≤1 year) in centenarians. HRV seems to play a role in exceptional longevity, which could be accounted for by centenarians' exposome.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Clinical Research Ethics Committee of the University Hospital of Alcorcón (ID of the approval: 16/50). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

NG designed the overall study. AS-L, GR-R, and AH-V collected the data. All authors contributed equally in the interpretation and analysis of the data, revision of manuscript for important intellectual content and have read and approved the final version.

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4.6 Artículo VI

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MDPI

Article

ECG Ventricular Repolarization Dynamics during Exercise: Temporal Profile, Relation to Heart Rate Variability and Effects of Age and Physical Health

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Abstract: Periodic repolarization dynamics (PRD) is a novel electrocardiographic marker of cardiac repolarization instability with powerful risk stratification capacity for total mortality and sudden cardiac death. Here, we use a time-frequency analysis approach to continuously quantify PRD at rest and during exercise, assess its dependence on heart rate variability (HRV) and characterize the effects of age (young adults/middle-aged adults/older adults), body mass index (non-overweight/overweight) and cardiorespiratory fitness level (fit/unfit). Sixty-six male volunteers performed an exercise test. RR and dT variabilities (RRV, dTV), as well as the fraction of dT variability unrelated to RR variability, were computed based on time-frequency representations. The instantaneous LF power of dT (P_{dTV}), representing the same concept as PRD, and of its RRV-unrelated component ($P_{dTVuRRV}$) were quantified. dT angle was found to mostly oscillate in the LF band. Overall, 50–70% of P_{dTV} was linearly unrelated to RRV. The onset of exercise caused a sudden increase in P_{dTV} and $P_{dTVuRRV}$, which returned to pre-exercise levels during recovery. Clustering analysis identified a group of overweight and unfit individuals with significantly higher P_{dTV} and $P_{dTVuRRV}$ values at rest than the rest of the population. Our findings shed new light on the temporal profile of PRD during exercise, its relationship to HRV and the differences in PRD between subjects according to phenotypic characteristics.

Keywords: electrocardiography; ventricular repolarization; time-frequency analysis; sympathetic nervous system; periodic repolarization dynamics; heart rate variability; exercise test; cluster analysis

1. Introduction

Sudden cardiac death is responsible for 15–20% of all deaths in Western societies [1]. It is strongly associated with, and can be caused by, ventricular arrhythmias. Although rare, when an athlete's life is claimed by sudden cardiac death, the impact on society is very high. Nevertheless, the absolute number of cases in athletes is not higher than in the general population, but intense exercise appears to increase the risk of sudden cardiac death in individuals harboring certain cardiac conditions [2]. Considering that less than 5%

of people with an out-of-hospital cardiac arrest survive, the search for reliable markers able to identify athletes and non-athletes at high arrhythmic risk is urgently needed. This would help in the election of a cost-effective treatment, such as antiarrhythmic drugs, prophylactic implantation of a cardioverter defibrillator or catheter ablation [3]. Among the variety of non-invasive methods proposed in the literature to assess arrhythmic risk, methods can be found that quantify heart rate variability (HRV), baroreflex sensitivity or ventricular repolarization characteristics, such as the QT interval duration and hysteresis, T-wave alternans, T-peak-to-end/RR interval curvature or T-wave morphology restitution [4–8].

Sympathetic nervous system (SNS) hyperactivity has been shown to increase triggered activity and enhance dispersion of ventricular repolarization under different clinical conditions, thus contributing to accentuate the vulnerability to fatal ventricular arrhythmias and sudden cardiac death [9]. Recent studies have proposed an electrocardiogram (ECG)-based risk predictor, which has been suggested to reflect sympathetic effects on ventricular myocardium [10]. This marker, called periodic repolarization dynamics (PRD), quantifies the magnitude of low-frequency (LF) oscillations ($\leq$0.1 Hz) in the angle dT between T-wave vectors of consecutive heart beats [10]. Elevated PRD measured at rest has been shown to be a strong predictor of all-cause mortality, cardiac mortality or sudden cardiac death in patients with acute and chronic myocardial infarction and in patients with chronic heart failure [10–14]. The stratification capacity of PRD has been shown to be independent of that of other clinical and ECG variables including left ventricular ejection fraction, HRV, diabetes mellitus and Global Registry of Acute Coronary Events score in myocardial infarction populations and New York Heart Association class in chronic heart failure populations [13,14]. In addition, PRD has been shown to predict mortality reduction associated with prophylactic implantation of defibrillators in cardiomyopathy patients and could thus help guide treatment decisions [15].

The physiological mechanisms underlying the genesis of PRD are yet to be fully described, particularly regarding the direct involvement of sympathetic oscillatory activity on regulation of the ventricular myocardium [9]. The importance of LF oscillations in providing information potentially related to sympathetic neural activity has been described through different markers obtained from the T-wave vector, sympathetic nerve activity recordings, HRV, action potentials or systolic arterial blood pressure [9]. Specifically regarding PRD, clinical and experimental studies have so far provided evidence that it is enhanced by sympathetic activation, induced by either tilt table test or exercise, and suppressed by pharmacological β-adrenergic blockade [10,16]. PRD has been verified to occur independently of respiratory activity in volume-controlled ventilated swine [10] and in humans when comparing respiratory rates of 10 and 20/min with constant minute ventilation [17]. Moreover, PRD has been confirmed not to be an epiphenomenon of HR and HRV, as substantiated by the fact that fixed atrial pacing exerts modest effects on PRD despite fully abolishing HRV and despite varying HR at fixed values in a large range [10,18]. This has been later supported by studies using an incremental exercise test, which have described low correlation between PRD and HR or HRV [16]. Nevertheless, there is yet limited information on how PRD varies continuously with time so that a full characterization of its temporal profile following physiological sympathoexcitatory interventions can be established. In addition, no study has so far provided quantifications of the PRD fractions related and unrelated to HRV and on the variation of these two fractions with time in response to sympathetic provocations. This is of interest, both in a general population but also in subpopulations stratified by certain phenotypic characteristics.

The aim of this study is to continuously quantify PRD at rest and during exercise, assess its dependence on heart rate variability (HRV) and characterize the effects of age, body mass index (BMI) and cardiorespiratory fitness level. For this purpose, we use a time-varying nonparametric methodology to evaluate the instantaneous power of LF oscillations in the dT angle, representing the same concept as PRD, and we ascertain the part of it that is unrelated to HRV and could thus reflect direct sympathetic effects on the ventricular myocardium. Next, we characterize how the LF power of dT and its HRV-

unrelated portion change in response to incremental exercise in a population of subjects with highly varied age, BMI and cardiorespiratory fitness level. By clustering analysis, we identify groups of individuals with similar phenotypic characteristics and we assess differences in the magnitude of their LF oscillatory ventricular activities that could offer hints on the relationship between elevated PRD and cardiovascular risk.

2. Materials and Methods

2.1. Participants

Recruitment posters were distributed in public establishments, such as sports centers, hospitals and universities. Sixty-six males agreed to participate in the study. The sample consisted of three age groups: young adults from 20 to 30 years (N = 24), middle-aged adults from 40 to 50 years (N = 21) and older adults from 60 to 70 years (N = 21). Exclusion criteria included the following: subjects going through an acute disease, being on cardiac medication, suffering from heart diseases (e.g., atrial fibrillation or heart failure) or presenting any clinical contraindication for the practice of physical exercise. The descriptive characteristics of the three age groups are shown in Table 1. The study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the ethical committee for clinical research of Aragón (ID of the approval: PI17/0409). After detailed explanation of potential risks, informed consent was obtained from all subjects involved in the study.

Table 1. Descriptive characteristics of the three age groups.

Outcome	Young Adults (n = 24)	Middle-Aged Adults (n = 21)	Older Adults (n = 21)
Age (years)	25.41 ± 2.74	42.86 ± 3.06	63.82 ± 2.97
Height (m)	1.75 ± 0.06	1.77 ± 0.06	1.71 ± 0.05
Weight (kg)	71.81 ± 11.45	78.44 ± 10.49	76.53 ± 7.96
BMI ($kg \cdot m^{-2}$)	23.30 ± 2.86	25.09 ± 2.88	26.17 ± 2.82
"Overweight" (%)	20.8 (5)	47.6 (10)	61.9 (13)
$PWC_{80\%}$ ($W \cdot kg^{-1}$)	2.01 ± 0.61	2.02 ± 0.59	1.74 ± 0.59
"Unfit" (%)	50.0 (12)	52.4 (11)	52.4 (11)

Continuous variables are expressed as mean ± standard deviation. Dichotomous variables are expressed as percentage (number of subjects). BMI = Body mass index; $PWC_{80\%}$ = Physical Work Capacity at 80% of estimated HR_{max} (208-0.7*age in years) in watts per kg bodyweight.

2.2. Procedure

All subjects performed an exercise test during a session at the laboratory between 16:00–20:00 h. Before the session, volunteers were asked to follow some guidelines: (1) refrain from doing heavy exercise the day before the test; (2) get enough sleep (6–8 h) the night before the test; (3) avoid substances such as alcohol, tobacco or stimulants (theine, taurine, caffeine, etc.) in the 8 h preceding the test; (4) do not eat for 3 h prior to the test; (5) ensure being well hydrated; and (6) wear comfortable clothing. Volunteers were prepared by using a razor to shave any hair from the electrode sites and by cleaning their skin with alcohol and gauze. To place the ECG electrodes, the manufacturer's instructions were followed (H12 +, Mortara Instrument; Milwaukee, WI, USA).

The test was conducted in an environmentally controlled room (22–23 °C, 40–60% humidity) and was divided into 3 consecutive segments: resting (S_{REST}), cycling (S_{CY}) and recovery (S_{REC}). During S_{REST}, participants were monitored while seated at rest for 5 min, without moving or talking. A 3-min period was set to change from being seated in the chair during S_{REST} to being seated in the cycle ergometer during S_{CY}. During this 3-min period, the volunteer rode the cycle-ergometer (Ergoselect 200 K, Ergoline; Bitz, Germany) at 50 W workload and chose a cadence that was maintained during the whole test. S_{CY} was a submaximal cycle-ergometer test divided into three stages lasting 5 min each. The workload was adjusted during each stage to 60, 70 and 80% of estimated maximum heart

rate (HR_{max}), with these stages denoted as S_{CY60}, S_{CY70} and S_{CY80}, respectively. HR_{max} was estimated for each subject by using HR_{max} = 208-0.7*age (years) to avoid a maximal exercise test [19]. Finally, during S_{REC}, participants remained seated in the chair again for 5 min without moving or talking.

2.3. Data Recording

Volunteers self-reported their birth date, current medication and pathologies. Height was measured with a stadiometer (SECA 225; Hamburg, Germany) to the nearest 0.001 m, with participants standing and their heels, buttocks and scapula resting against a wall with the heels touching and forming a 45° angle and the head in the Frankfort's plane. An electronic scale (SECA 861; Hamburg, Germany) was used to weight the subject to the nearest 0.1 kg, in underwear and after urination. BMI was calculated by dividing weight in kilograms by the square of height in meters. Based on World Health Organization standards, weight status was split into 2 groups: "non-overweight" (BMI < 25 $kg \cdot m^{-2}$) and "overweight" (BMI $\geq$ 25 $kg \cdot m^{-2}$) [20].

Submaximal exercise test is a safe and feasible method to estimate VO_{2max}, showing good validity against maximal exercise tests (correlation coefficients from 0.69 to 0.98) [21]. Rather than commonly used tests with stages of short or variable duration, an ad hoc test with 5-min stages was defined to allow reliable estimation of the LF power of HRV and repolarization variability. This enabled assessment of cardiac response to increased sympathetic activity with each cycling stage [22]. Consequently, cardiorespiratory fitness was assessed using the approach of "Physical Work Capacity" (PWC) [23,24]. PWC was measured in watts during S_{CY80} of the submaximal cycle-ergometer test and was subsequently divided by the participant's body weight ($PWC_{80\%}$ in $W \cdot kg^{-1}$). Alternatively to the use of fixed HR thresholds, this method incorporates the age-dependent decline of HR_{max} [23,24] and has previously been used as an objective assessment of cardiorespiratory fitness [25,26]. In each age group, cardiorespiratory fitness status was dichotomized: subjects above the $W \cdot kg^{-1}$ age group median were classified as "fit" and subjects below the age group median as "unfit".

A twelve-lead high-resolution (1000 Hz) Holter recorder (H12+, Mortara Instrument; Milwaukee, WI, USA) was used to record the ECG.

2.4. Data Analysis and Processing

QRS detection and ECG wave delineation were performed by using a wavelet-based single-lead automatic system [27]. The detection and delineation annotations from each lead were combined by using rules to obtain multi-lead ECG delineation marks [27] and additional updates were applied to account for the high levels of noise during stress testing [28]. From these annotations, the RR time series (measured from one QRS complex to the next one) was extracted. In addition, the onset and end of the T-waves (T_{on} and T_{end}, respectively) were obtained.

The time series of angles between consecutive T-wave vectors, denoted as dT series, was obtained as described in [29], which uses a method updated from the original one proposed in [10]. First, the orthogonal leads X, Y, Z were obtained from the 12-lead ECG using the inverse Dower matrix [30]. Each T-wave was delimited based on the T_{on} and T_{end} time points identified as described previously and an average T-wave vector was calculated for each wave. The angle dT between two consecutive T-waves was calculated by the dot product of each pair of consecutive average T-wave vectors.

Outlier values in both RR and dT time series were detected and corrected as described next. First, a 30-th order median filter was applied over the times series of absolute differences between successive intervals. Outliers were identified if their absolute difference was above 5 times the corresponding value in the median filtered series. These outlier values were replaced with the mean of their adjacent values.

RR variability (RRV), dT variability (dTV) and dT variability unrelated to RR variability (dTVuRRV) were computed based on time-frequency representations following

previously developed approaches [31], as described next. First, a highpass filter with a cut-off frequency of 0.03 Hz was applied to both RR and dT series. To obtain the time-frequency (TF) representations, Cohen's class distributions were used with temporal and spectral resolutions of 11.7 s and 0.039 Hz, respectively. TF representations of the dTV and RRV series, as well as the TF coherence between dTV and RRV series, were obtained and denoted as $S_{dTV}(t,f)$, $S_{RRV}(t,f)$ and $\gamma_{dTV,RRV}(t,f)$, respectively. The TF spectrum of dTV was decomposed into two separate spectra, which allowed characterizing the part of dTV linearly related to RRV (dTVrRRV) and the part of dTV unrelated to RRV (dTVuRRV). The TF spectrum of dTVuRRV was calculated as:

$$S_{dTVuRRV}(t,f) = \left(1 - \left|\gamma_{dTV,RRV}(t,f)\right|^2\right) S_{dTV}(t,f) \quad (1)$$

The bias from the TF coherence estimators was estimated and corrected [31].

The instantaneous powers of LF oscillations for dTV, RRV, dTVuRRV and dTVrRRV series were calculated by integrating their TF distributions, $S_{dTV}(t,f)$, $S_{RRV}(t,f)$, $S_{dTVuRRV}(t,f)$ and $S_{dTVrRRV}(t,f)$ respectively, in the 0.03–0.15 Hz band, and denoted as $P_{dTV}(t)$, $P_{RRV}(t)$, $P_{dTVuRRV}(t)$ and $P_{dTVrRRV}(t)$. The normalized LF power of dTVuRRV was estimated as:

$$P_{dTVuRRVn}(t) = \frac{P_{dTVuRRV}(t)}{P_{dTV}(t)} \quad (2)$$

From the instantaneous power series $P_{RRV}(t)$, $P_{dTV}(t)$, $P_{dTVuRRV}(t)$, $P_{dTVrRRV}(t)$, and $P_{dTVuRRVn}(t)$, the indices used in the statistical analysis were obtained as the mean of the corresponding segment (S_{REST}, S_{CY60}, S_{CY70}, S_{CY80} and S_{REC}) after removing the first 30 s of each of them.

2.5. Statistical Analysis

The normality of data was checked using the Kolmogorov–Smirnov test. Since the data distribution violated the assumption of normality necessary for the parametric tests and could not be corrected by commonly employed transformations, non-parametric analysis was conducted. Descriptive variables are presented as mean ± standard deviation and markers related to cardiac variability series are reported as median and interquartile range. Statistical analyses were performed using IBM SPSS (version 25; Chicago, IL, USA). The significance level was set at $p \leq 0.05$.

Friedman's two-way ANOVA, the non-parametric equivalent of one-way related analysis of variance ANOVA, was used to test for differences in variables between test segments, i.e., S_{REST}, S_{CY60}, S_{CY70}, S_{CY80}, and S_{REC}. The Dunn–Bonferroni post hoc method was used for pairwise comparisons.

Cluster analysis was performed to identify groups of subjects with similar characteristics in terms of the following three variables of interest: age, BMI and cardiorespiratory fitness level ($PWC_{80\%}$). Following the methodology of previous studies [32,33], two types of cluster analyses were combined: hierarchical clustering (Ward's method) and k-means clustering. First, individual and multivariate outliers (according to Mahalanobis distance) were detected to reduce the sensitivity of the Ward's method to outliers. Second, hierarchical cluster analysis was used, as the number of clusters in the data was unknown beforehand. Examination of dendrograms showed that a two-cluster solution produced good differentiation between groups. Finally, k-means cluster was performed with two possible solutions. Compared to hierarchical methods, k-means cluster analysis is considered less sensitive to outliers and has been found to result in greater within-cluster homogeneity and between-cluster heterogeneity [32].

A Kruskal–Wallis test (non-parametric equivalent of one-way independent ANOVA) with Bonferroni correction was performed to assess differences in variables between the three age groups, i.e., young adults, middle-aged adults and older adults. The Dunn–Bonferroni post hoc method was used for pairwise comparisons. To evaluate the magnitude

of the differences, ES was calculated as: $ES = H/((n^2-1)/(n+1))$, where H stands for the Kruskal–Wallis test statistic and n is the total number of observations [34].

The Mann–Whitney U-test, the non-parametric equivalent of the unpaired samples t-test, was used to determine differences in variables between dichotomous groups i.e., BMI (non-overweight/overweight), $PWC_{80\%}$ (fit/unfit) and clusters (CLUSTER A/B). The magnitude of the difference was calculated by determining the effect size (ES): $ES = Z/\sqrt{n}$ where Z represents the Z-score for the Mann–Whitney U-test and n is the total number of observations [34]. The difference was considered small when ES < 0.2, small to medium when ES = 0.2–0.5, medium to large when ES = 0.5–0.8 and large when ES > 0.8 [35].

3. Results

3.1. LF Oscillations of dT in Response to Exercise and Relation to HRV

Figure 1 shows the concept of dT, with representation of the orthogonal leads X, Y, and Z derived from the twelve standard leads (Figure 1a) and pairs of T-wave vectors corresponding to consecutive beats (Figure 1b). The time series of dT shows the time course of the angles between pairs of T-wave vectors (Figure 1d, zoomed version in Figure 1c).

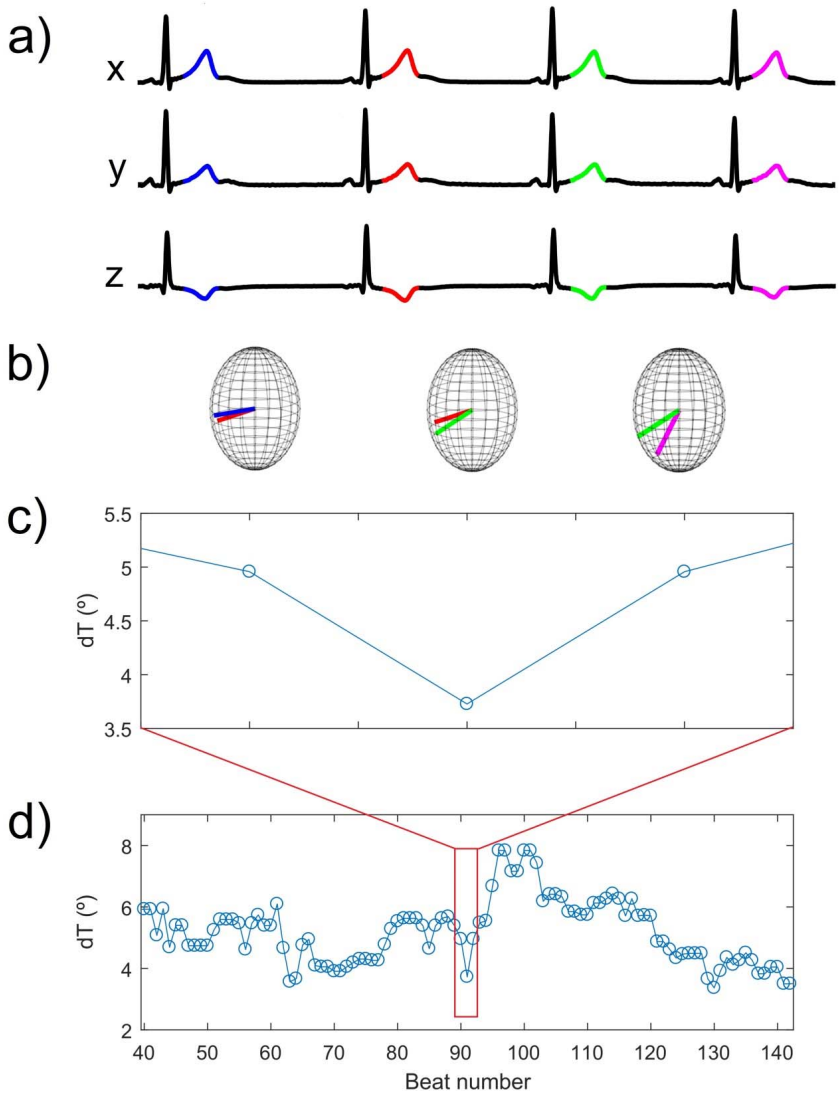

Figure 1. Computation of dT from an ECG in the Frank lead configuration. (**a**) T-waves from four consecutive heart beats. (**b**) Pairs of T-wave vectors from consecutive beats are illustrated in three-dimensional spheres. (**c**) Angle dT between the consecutive T-wave vectors shown in panel b. (**d**) Time series of dT for a piece of an ECG recording.

Figures 2–4 illustrate the temporal evolution of the RR and dT indices and their variabilities throughout the test, including rest, cycling and recovery. Figure 2 shows an example of dT and RR time series from a subject of the study population, from which it can be observed that there are decreases in both RR and its variability during exercise, concomitant to increases in dT and its variability. The corresponding temporal evolution of the instantaneous power of LF oscillations for RRV (P_{RRV}, i.e., LF component of RRV), dTV (P_{dTV}, representing the PRD concept), dTV unrelated to RRV ($P_{dTVuRRV}$, i.e., the fraction of PRD unrelated to the LF component of RRV) and dTV related to RRV ($P_{dTVrRRV}$) is displayed in Figure 3. The relevant contribution to dTV of both its RRV-related and RRV-unrelated components can be clearly appreciated, with the two of them showing remarkable increases during exercise. The time-frequency distributions of RRV, dTV, dTVuRRV and dTVrRRV are depicted in Figure 4. Oscillations in dT are very notable in magnitude during exercise and are mostly concentrated in the LF band. Although a portion of dTV is related to RRV, there is an important fraction of it that provides information additional to RRV.

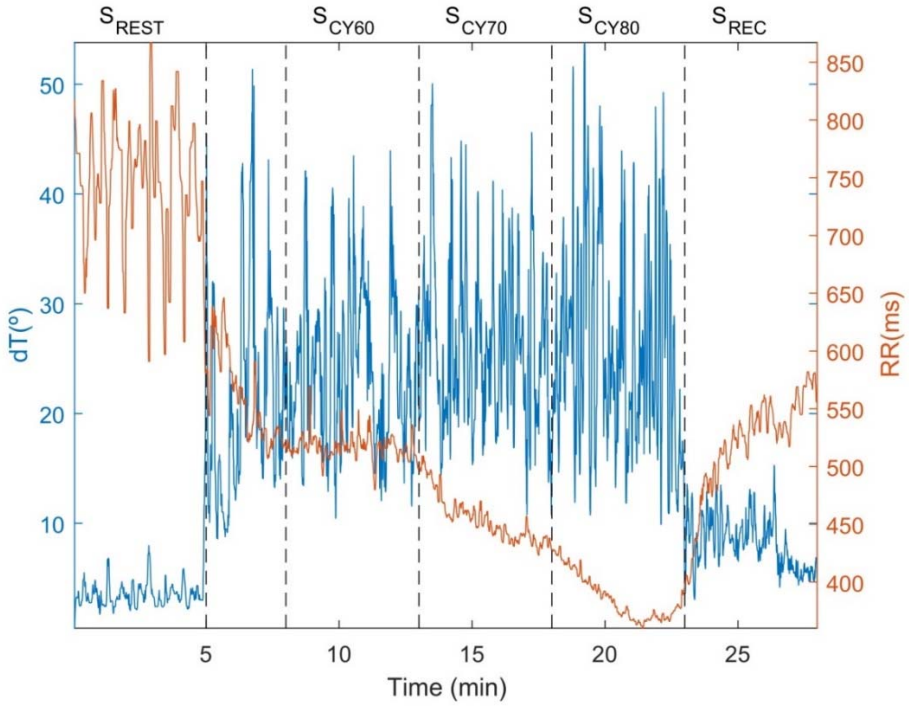

Figure 2. Example of the dT angle and RR interval time series for one subject throughout the entire test. Dotted lines separate the different test segments: resting (S_{REST}), cycling (S_{CY}) and recovery (S_{REC}). S_{CY} was divided into three stages corresponding to 60, 70 and 80% of estimated HR_{max}, denoted as S_{CY60}, S_{CY70} and S_{CY80}, respectively.

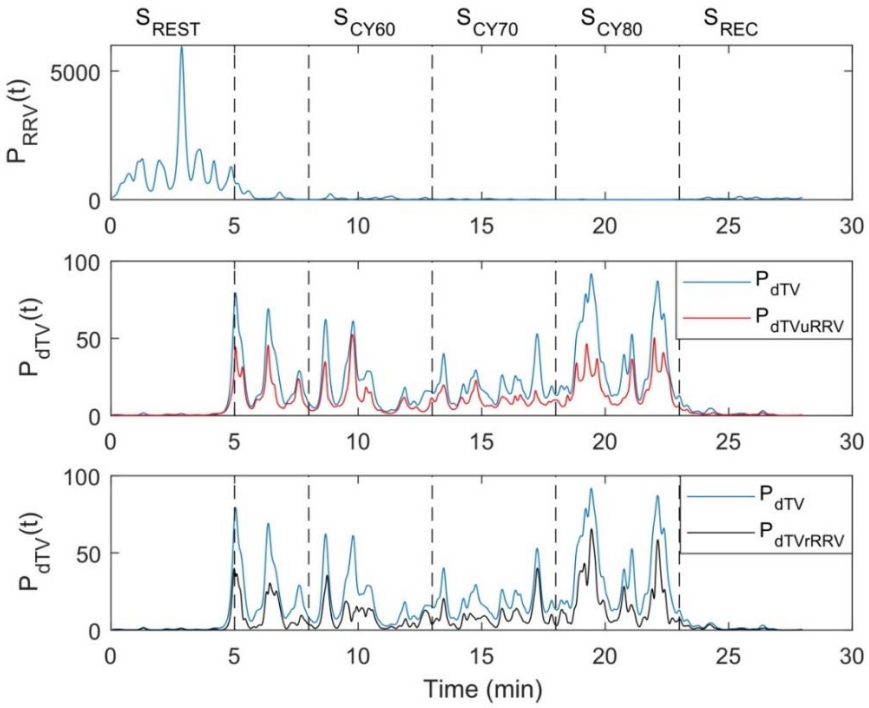

Figure 3. Example of the instantaneous power of LF oscillations for: RR variability (P_{RRV}), dT variability (P_{dTV}), dTV unrelated to RRV ($P_{dTVuRRV}$) and dTV related to RRV ($P_{dTVrRRV}$) obtained for the same subject as in Figure 2. Dotted lines separate the different test segments: resting (S_{REST}), cycling (S_{CY}) and recovery (S_{REC}). S_{CY} was divided into three stages corresponding to 60, 70 and 80% of estimated HR_{max}, denoted as S_{CY60}, S_{CY70} and S_{CY80}, respectively.

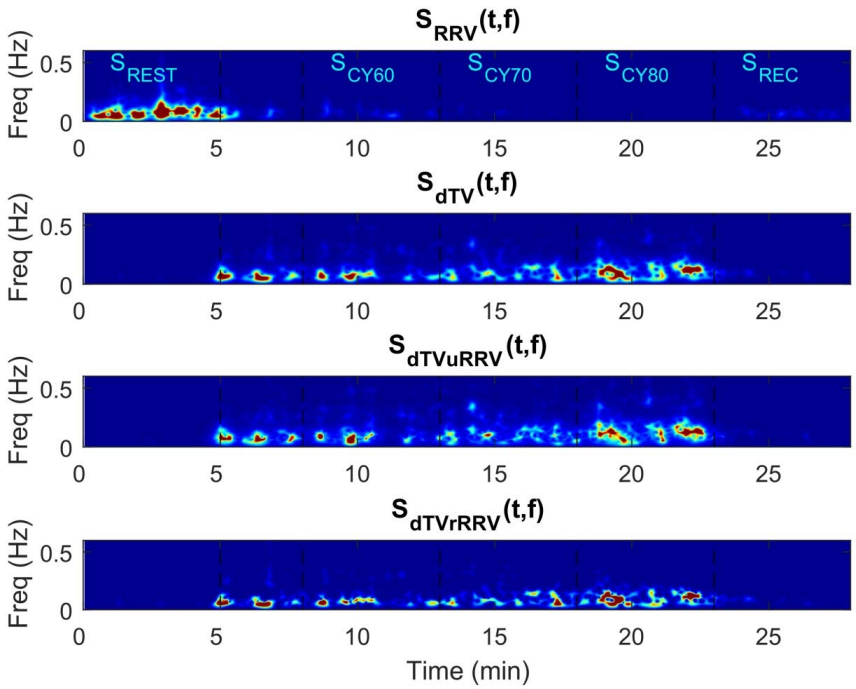

Figure 4. Example of the time-frequency distribution for RR variability (RRV), dT variability (dTV), dTV unrelated to RRV ($P_{dTVuRRV}$) and dTV related to RRV ($P_{dTVrRRV}$) obtained for the same subject as in Figure 2. Dotted lines separate the different test segments: resting (S_{REST}), cycling (S_{CY}) and recovery (S_{REC}). S_{CY} was divided into three stages corresponding to 60, 70 and 80% of estimated HR_{max}, denoted as S_{CY60}, S_{CY70} and S_{CY80}, respectively.

Figure 5 shows box plots representing the distributions of P_{RRV}, P_{dTV}, $P_{dTVuRRV}$ and $P_{dTVuRRVn}$ over the study population (N = 66) for the different test segments. Regarding P_{RRV} shown in Panel 5.a, the beginning of exercise elicits a drop in the LF oscillations of RRV ($p \leq 0.001$), followed by a progressive decrease with each increment in exercise intensity (all $p \leq 0.05$), upon which P_{RRV} returns ($p \leq 0.001$) to pre-exercise levels in the recovery segment. The P_{dTV} profile in Panel 5.b shows that exercise onset causes a sudden increase well above the resting level ($p \leq 0.001$), followed by a variable behavior among subjects during exercise (note the wide boxes and whiskers in S_{CY60}, S_{CY70} and S_{CY80}), with a subsequent decrease in P_{dTV} corresponding to the recovery segment ($p \leq 0.001$), at the end of which values similar to rest are attained. From Panel 5.c, it can be seen that $P_{dTVuRRV}$ has a similar pattern to P_{dTV}, in this case with a more remarkable tendency to increase in median with exercise intensity, although this increase is not statistically significant due to the wide distribution of values at S_{CY60}, S_{CY70} and S_{CY80}. $P_{dTVrRRV}$ also has a similar pattern to P_{dTV}, but without any observable tendency to increase in median with exercise intensity as seen in $P_{dTVuRRV}$. Finally, Panel 5.d shows the normalized $P_{dTVuRRV}$, i.e., $P_{dTVuRRVn}$, with values at S_{CY60} and S_{CY70} being significantly lower than at S_{CY80} ($p \leq 0.001$).

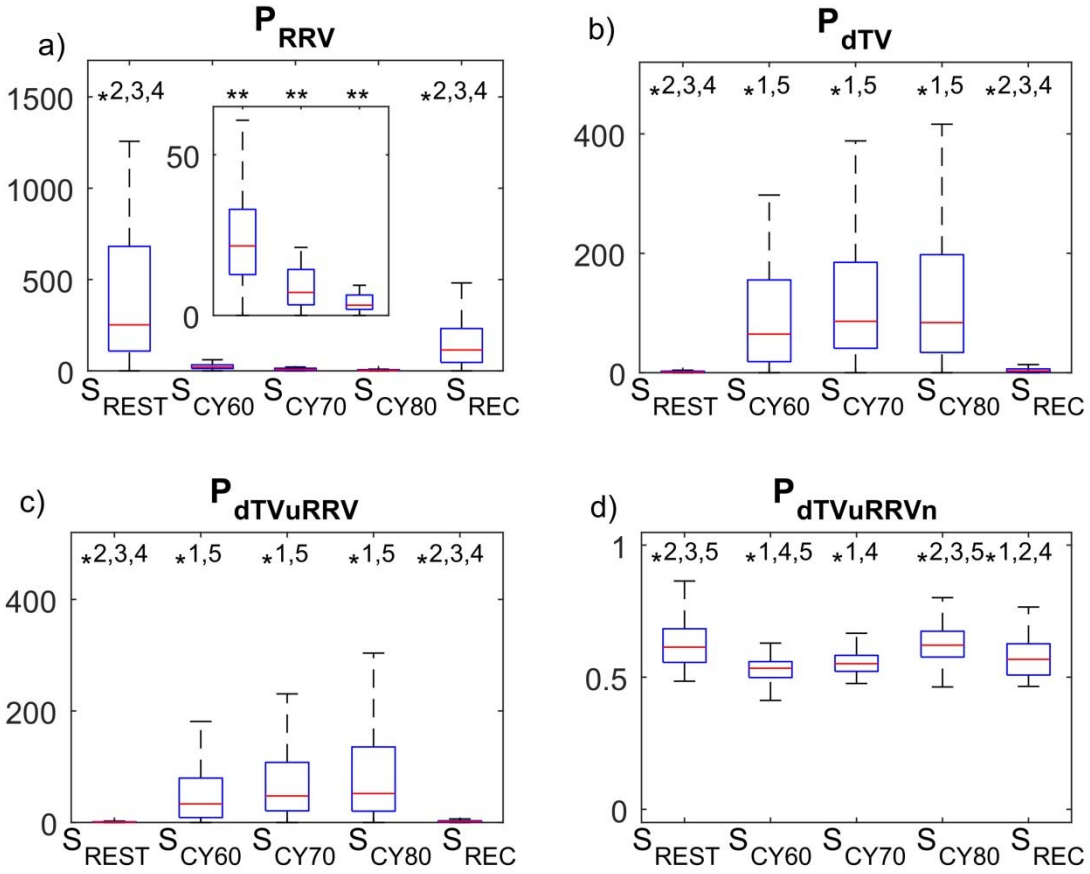

Figure 5. Box plots representing the distributions of P_{RRV}, P_{dTV}, $P_{dTVuRRV}$ and $P_{dTVuRRVn}$ (N = 66) over the study population (N = 66) for the different test segments: resting (S_{REST}), cycling (S_{CY}) and recovery (S_{REC}). S_{CY} was divided into three stages corresponding to 60, 70 and 80% of estimated HR_{max}, denoted as S_{CY60}, S_{CY70} and S_{CY80}. (**a**) LF oscillations of RR variability (P_{RRV}), with the inset showing the three S_{CY} stages. (**b**) LF oscillations of dT variability (P_{dTV}). (**c**) P_{dTV} unrelated to P_{RRV} ($P_{dTVuRRV}$). (**d**) Normalized P_{dTV} unrelated to P_{RRV} ($P_{dTVuRRVn}$). * = Significant differences between test segments ($p \leq 0.05$, Friedman's ANOVA): * 1 = Different to S_{REST}; * 2 = Different to S_{CY60}; * 3 = Different to S_{CY70}; * 4 = Different to $S_{CY80;}$ * 5 = Different to $S_{REC;}$ ** = Different to all.

3.2. Effects of Age, BMI and Cardiorespiratory Fitness Level on LF Oscillations of dT

Table A1 shows averaged values of P_{RRV}, P_{dTV}, $P_{dTVuRRV}$, $P_{dTVrRRV}$ and $P_{dTVuRRVn}$

for young, middle-aged and older adults across the different test segments. Significant reductions with age were found in P_{RRV} at S_{REST}, S_{CY60} and S_{REC}. Table A2 presents the comparison of the same indices according to BMI groups. P_{dTV} and $P_{dTVrRRV}$ at S_{REST} were significantly higher in the overweight group, while $P_{dTVuRRVn}$ at S_{CY60} and P_{RRV} at S_{REC} were significantly higher in the non-overweight group. The corresponding comparison according to cardiorespiratory fitness levels is shown in Table A3. Only $P_{dTVuRRVn}$ at the highest exercise intensity, i.e., S_{CY80}, was significantly higher in the more fit individuals.

3.3. Identification of Individuals with Elevated LF Oscillations of dT

Table 2 shows the descriptive characteristics of the two cluster groups, which were described as CLUSTER A "non-overweight and fit" (normal BMI and high $PWC_{80\%}$), and CLUSTER B "overweight and unfit" (high BMI and low $PWC_{80\%}$).

Table 2. Descriptive characteristics of the two cluster groups.

Outcome	CLUSTER A (n = 31)	CLUSTER B (n = 35)	p	Effect Size
Age (years)	32.99 ± 11.48	52.22 ± 14.38	**<0.001** *	0.580
Height (m)	175.37 ± 6.07	173.51 ± 6.20	0.203	0.157
Weight (kg)	69.36 ± 8.88	80.79 ± 8.57	**<0.001** *	0.506
BMI ($kg \cdot m^{-2}$)	22.48 ± 1.87	26.82 ± 2.38	**<0.001** *	0.757
% of "overweight"	*6.5 (2)*	*74.3 (26)*		
$PWC_{80\%}$ ($W \cdot kg^{-1}$)	2.33 ± 0.59	1.57 ± 0.33	**<0.001** *	0.628
% of "unfit"	*32.3 (10)*	*68.6 (24)*		

Continuous variables are expressed as mean ± standard deviation. *Dichotomous variables* are expressed as *percentage (number of subjects)*. BMI = Body mass index; $PWC_{80\%}$ = Physical Work Capacity at 80% of estimated HR_{max} (208-0.7*age in years) in watts per kg bodyweight. Clusters were based on: age, BMI and cardiorespiratory fitness level ($PWC_{80\%}$). * = Significant differences between clusters ($p \leq 0.05$, Mann–Whitney U-test).

Table 3 shows averaged values of P_{RRV}, P_{dTV}, $P_{dTVuRRV}$, $P_{dTVrRRV}$ and $P_{dTVuRRVn}$ for the two cluster groups. At S_{REST}, P_{dTV}, $P_{dTVuRRV}$ and $P_{dTVrRRV}$ were significantly higher for CLUSTER B, while P_{RRV} was significantly higher for CLUSTER A. During the other test segments, results were not significantly different between groups, except for P_{RRV} at S_{REC}.

Table 3. Comparison of P_{RRV}, P_{dTV}, $P_{dTVuRRV}$, $P_{dTVrRRV}$ and $P_{dTVuRRVn}$ between cluster groups.

	Outcome	CLUSTER A (n = 31)	CLUSTER B (n = 35)	p	ES
S_{REST}	P_{RRV} (e^{-4})	18.14 (6.31 to 38.94)	9.79 (3.65 to 17.54)	**0.020** *	0.287
	P_{dTV}	1.00 (0.41 to 1.87)	1.50 (0.82 to 2.96)	**0.021** *	0.284
	$P_{dTVuRRV}$	0.51 (0.22 to 1.23)	0.75 (0.49 to 1.56)	**0.039** *	0.254
	$P_{dTVrRRV}$	0.39 (0.23 to 0.79)	0.75 (0.37 to 1.43)	**0.018** *	0.290
	$P_{dTVuRRVn}$	0.62 (0.60 to 0.70)	0.62 (0.56 to 0.73)	0.743	0.040
S_{CY60}	P_{RRV} (e^{-4})	1.40 (0.98 to 3.41)	1.64 (0.89 to 2.39)	0.400	0.104
	P_{dTV}	63.42 (24.28 to 162.91)	99.78 (33.81 to 186.33)	0.289	0.130
	$P_{dTVuRRV}$	32.34 (11.83 to 72.16)	53.95 (15.66 to 93.05)	0.295	0.129
	$P_{dTVrRRV}$	30.84 (12.45 to 74.75)	48.50 (11.08 to 93.27)	0.415	0.100
	$P_{dTVuRRVn}$	0.55 (0.52 to 0.58)	0.53 (0.50 to 0.56)	0.141	0.181
S_{CY70}	P_{RRV} (e^{-4})	0.66 (0.32 to 1.21)	0.60 (0.33 to 1.15)	0.974	0.004
	P_{dTV}	98.08 (54.45 to 186.19)	88.19 (69.84 to 243.71)	0.724	0.043
	$P_{dTVuRRV}$	56.59 (29.10 to 106.70)	49.70 (30.67 to 126.59)	0.733	0.042
	$P_{dTVrRRV}$	42.96 (21.45 to 79.49)	47.58 (22.86 to 83.74)	0.832	0.026
	$P_{dTVuRRVn}$	0.56 (0.54 to 0.60)	0.55 (0.52 to 0.60)	0.272	0.135
S_{CY80}	P_{RRV} (e^{-4})	0.24 (0.12 to 0.40)	0.22 (0.14 to 0.43)	0.729	0.043
	P_{dTV}	136.21 (53.69 to 223.58)	79.23 (51.54 to 150.67)	0.352	0.115
	$P_{dTVuRRV}$	83.28 (33.82 to 152.10)	48.80 (23.36 to 117.25)	0.372	0.110
	$P_{dTVrRRV}$	47.41 (19.87 to 91.11)	30.82 (17.09 to 59.68)	0.256	0.140
	$P_{dTVuRRVn}$	0.64 (0.61 to 0.70)	0.60 (0.57 to 0.67)	0.052	0.240

Table 3. *Cont.*

	Outcome	CLUSTER A (*n* = 31)	CLUSTER B (*n* = 35)	*p*	ES
S_{REC}	P_{RRV} (e^{-4})	9.03 (5.18 to 17.32)	5.29 (2.49 to 13.55)	**0.036** *	0.259
	P_{dTV}	2.36 (1.40 to 5.70)	3.82 (1.91 to 7.94)	0.079	0.216
	$P_{dTVuRRV}$	1.21 (0.83 to 3.07)	2.35 (1.26 to 4.06)	0.060	0.232
	$P_{dTVrRRV}$	1.14 (0.58 to 2.55)	1.90 (0.66 to 3.88)	0.250	0.142
	$P_{dTVuRRVn}$	0.59 (0.53 to 0.64)	0.57 (0.53 to 0.63)	0.559	0.072

Values are expressed as median and interquartile range. Segments are based on the test phases: resting (S_{REST}), cycling (S_{CY}) and recovery (S_{REC}). S_{CY} was divided in three stages at 60, 70 and 80% of estimated HR_{max}, denoted as S_{CY60}, S_{CY70} and S_{CY80}, respectively. P_{RRV} = LF oscillations for RR variability; P_{dTV} = LF oscillations for dT variability; $P_{dTVuRRV}$ = P_{dTV} unrelated to P_{RRV}; $P_{dTVrRRV}$ = P_{dTV} related to $P_{RRV;}$ $P_{dTVuRRVn}$ = normalized $P_{dTVuRRV}$. Clusters were based on: age, BMI and cardiorespiratory fitness level ($PWC_{80\%}$). ES = Effect size. * = Significant differences between groups ($p \leq 0.05$, Mann–Whitney *U*-test).

Figure 6 shows examples of dT time series at rest for two subjects that are representative of each of the two clusters. The more pronounced LF oscillations in dT for the subject belonging to CLUSTER B can be clearly appreciated (top panels). This is manifested in higher instantaneous P_{dTV}, with the RRV-unrelated fraction of it, $P_{dTVuRRV}$, remaining higher too (bottom panels).

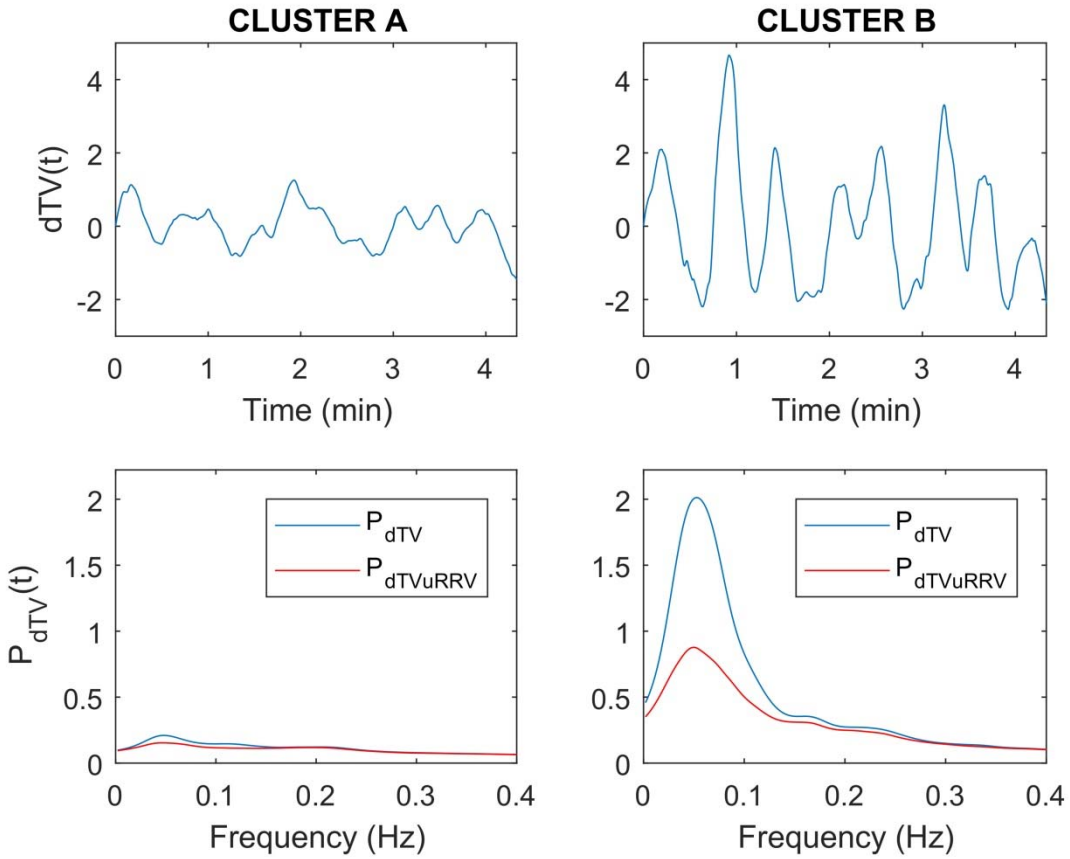

Figure 6. Examples of dTV, P_{dTV} and $P_{dTVuRRV}$ at rest obtained for two subjects that are representative of each of the two clusters. A and B, described in the text.

4. Discussion

By using time-frequency methods, we confirmed that the dT angle between consecutive T-wave vectors mainly oscillates in the LF band and, for the first time, we showed that its variability can be decomposed into two components with relevant contributions, one related to RRV and the other one unrelated to it. As an advantage of our methods over other methods quantifying LF oscillations of dT, we could characterize the temporal

profile of dTV and of its RRV-related and unrelated components during an exercise test. In line with previous findings, we observed a significant exercise-induced increase in the instantaneous LF power of dTV, P_{dTV}, with respect to rest and recovery, which we proved to be accompanied by gradual increases in its RRV-unrelated component, $P_{dTVuRRV}$, but not in its RRV-related one, $P_{dTVrRRV}$, in response to incremental exercise. The temporal profile of P_{dTV} and $P_{dTVuRRV}$ as a function of exercise intensity was highly inter-individual. Importantly, our study provides first evidence on the behavior of P_{dTV} as a function of age, BMI and cardiorespiratory fitness level, both when these variables are analyzed individually and in combination. We showed that, at rest but not along incremental exercise, P_{dTV}, $P_{dTVuRRV}$ and $P_{dTVrRRV}$ were significantly elevated in a group of overweight and unfit individuals, while no clear relationship with age could be established.

4.1. dT Mainly Oscillates in the LF Band, Being Not Completely Unrelated to HRV

To the best of our knowledge, this study is the first one using time-frequency methods to evaluate the frequency components of dTV and RRV during rest, exercise and recovery. The results of our ECG analysis corroborate that dT oscillations are mainly contained in the LF band and their magnitude is enhanced in response to exercise-induced sympathetic stimulation [10,16,18]. These results agree well with previous reports showing enhancement of PRD, which represents the same concept as P_{dTV}, subsequent to tilt table test and to mild exercise as well as decrease following β-adrenergic blockade [10]. On top of clinical and experimental studies assessing LF oscillations in ventricular repolarization from the surface ECG [13,14], these oscillations have additionally been demonstrated by in vivo studies at the level of ventricular electrograms and action potentials, which have characterized the LF oscillatory pattern [36–39], its magnification by sympathetic provocations [37] and its reduction following β-adrenergic blockade [38]. In silico studies have suggested that synergistic β-adrenergic stimulation and mechanical stretch could contribute to explain the LF oscillatory pattern of ventricular repolarization [40,41]. Although further research is needed to mechanistically link LF oscillations in ventricular repolarization to LF rhythmic discharge of sympathetic neurons [9], our study, together with all cited evidences from cell to whole-body levels, provide indirect support to the involvement of the sympathetic nervous system in the generation of the observed oscillatory behavior.

An important aspect that could render repolarization risk markers, such as PRD, inaccurate in representing the sympathetic effect on ventricular repolarization is their dependence on HR. Here, we show that P_{dTV} has an RRV-unrelated fraction accounting for 50–70% of it and an RRV-related one accounting for the remaining 30–50%. For PRD to be more meaningful from a clinical point of view, its RRV-unrelated fraction could be analyzed, as it could more closely reflect ventricular repolarization instabilities occurring under excessive sympathetic activity that may increase susceptibility to ventricular arrhythmias and sudden cardiac death. Prior studies investigating PRD have assessed its modulation by HR by evaluating the response to physiological interventions, such as hyperventilation or incremental exercise, and have established the independence of dT and PRD with respect to HR and HRV by reporting a non-significant correlation [16,17]. Other studies have determined that PRD is not an epiphenomenon of HRV by proving that it presents small (25% in mean) changes following fixed atrial pacing to abolish HRV [10]. Here, we provide instantaneous quantification of the percentages of P_{dTV} that are related and unrelated to RRV at any time instant during rest, exercise and recovery. This quantification could prove useful to assess whether increases in RRV-unrelated oscillations of dT could be more sensitive in predicting impeding ventricular arrhythmias than the combined RRV-related and unrelated oscillations measured through PRD. Previous studies in the literature have confirmed the value of specifically measuring RRV-unrelated oscillations of other ventricular repolarization markers such as the QT interval. In a recent investigation, the LF power of QT variability (QTV) unrelated to RRV, but not of the full QTV, was able to identify patients with coronary artery disease (CAD) from the first phases of a stress test [42]. In [43], QTV was quantified at given HRV levels and it was reported to be greater in heart failure

patients with spontaneous ventricular tachycardia than in normal heart subjects, with inter-group QTV differences being further amplified in response to atrial pacing (i.e., in the absence of HRV). These evidences on the existence and value of mechanisms additional to RRV-dependent effects on LF oscillations of ventricular repolarization provide new avenues for the development of arrhythmic risk markers with improved stratification capacity by refinement of PRD, as suggested in this study.

4.2. Incremental Exercise Enhances LF Oscillations of dT, with the Temporal Oscillatory Profile Being Highly Inter-Individual

While the pattern of change in RRV along a full exercise test has been extensively described in the literature, the pattern of dTV remains less well characterized. In line with previous reports [44], we describe a sudden drop in P_{RRV} with the beginning of exercise, followed by a more gradual decay as exercise intensity increases and a return to resting P_{RRV} levels during the recovery segment. Regarding dT and P_{dTV}, only two studies have provided an in-depth description of the pattern of change during the exercise test [16,18]. In agreement with these two studies, we show that, with the start of the exercise and the elevation in the sympathetic activity, there is an increase in dT and P_{dTV}, with such an increase being sustained along the different exercise intensities in mean over the analyzed population. In some individuals, P_{dTV} is magnified by a factor above 200 at maximum exercise intensity. In accordance to previous works, we show a decrease in P_{dTV} towards pre-exercise values during recovery [16,18].

The specific characteristics of the dT and P_{dTV} profiles along an exercise test vary across studies depending on the design of the exercise protocol. Hamm et al. used a step-wise incremental protocol and described that dT increased concordantly to HR until reaching the lactate anaerobic threshold and then started to decline discordantly to HR [18]. Milagro et al. considered a more exigent ramp protocol and did not observe such a transient drop in dT but reported a three-phase profile of dT and P_{dTV} during exercise. This profile consisted of an initial rapid rise and plateau-like behavior at light-intensity exercise, followed by a slight increase around the point when P_{RRV} reached its minimum and a final sudden increase after reaching the second ventilatory threshold [16]. Here, we find a tendency for P_{dTV} to increase with exercise intensity, even if not reaching statistical significance possibly due to the fact that, at our analyzed intensities, not all subjects reached the second ventilatory threshold (around 80–90% of HR_{max}) after which dT and P_{dTV} would be expected to grow remarkably [16,45].

On top of characterizing the P_{dTV} profile, we provide the profiles of its RRV-related and unrelated fractions, not investigated so far in previous studies. While the RRV-unrelated fraction, $P_{dTVuRRV}$, presents a similar pattern to P_{dTV}, with an even more marked increment in relation to exercise intensity, this was not the case of the RRV-related fraction, $P_{dTVrRRV}$, which did not show an increasing tendency with exercise intensity in mean over subjects. These results support the observation that the RRV-unrelated part of PRD could better reflect sympathetic effects on ventricular repolarization, with increased repolarization lability levels accompanying increased sympathetic activity [46]. Other studies in the literature have investigated the profile of repolarization variability and its RRV-unrelated component during exercise by analysis of QTV. In [42], the RRV-unrelated fraction of QTV is shown to be increased with exercise and to represent nearly 80% of all QTV at maximum exercise intensity. While this is true for both non-CAD and CAD patients, significant differences between these two groups are appreciated only at the first phases of the stress test and only for the RRV-unrelated fraction of QTV, which highlights the relevance of using methods able to separate the two repolarization variability components and to monitor them over the course of time, as proposed in this study. In addition, the time course of the LF power of the two QTV components has been investigated in response to maneuvers that shift the sympathovagal balance towards more sympathetic predominance, such as the tilt table test [31,47]. The unrelated component, but not the related one, increases significantly along the tilt test, again confirming the importance of the time-varying methodologies used in our study for characterization of LF oscillations of repolarization unrelated to RRV.

4.3. LF Oscillations of dT Are Significantly Elevated in a Group of Overweight and Unfit Individuals

The measurements of cardiac variability quantified in our study have been compared between groups stratified by age, BMI and cardiorespiratory fitness level. In accordance to the literature [48], we show that age is associated with a reduction in P_{RRV} at rest, light-intensity exercise and recovery. Additionally, we analyze, for the first time, the relationship between age and P_{dTV}, $P_{dTVuRRV}$ and $P_{dTVrRRV}$ and we describe no significant differences between age groups. Similarly, when comparing according to BMI or cardiorespiratory fitness level individually, most variables did not show differences between groups either, with only P_{dTV} and $P_{dTVrRRV}$ at rest being significantly higher in the overweight group.

Next, we performed cluster analysis to identify subjects with common phenotypic characteristics. CLUSTER A, composed of "non-overweight and fit" individuals, presents higher P_{RRV} at rest than CLUSTER B comprising "overweight and unfit" individuals, which is congruent with studies associating higher HRV with better health and lower HRV with poorer prognosis in different clinical conditions [49]. In addition, CLUSTER B shows higher P_{dTV}, $P_{dTVuRRV}$ and $P_{dTVrRRV}$ at rest, which agrees with investigations linking elevated resting P_{dTV} with higher cardiovascular risk (Rizas et al. 2014). Indeed, PRD has been shown to be a strong predictor of all-cause mortality, cardiac mortality and sudden cardiac death in different patient populations [10–14]. It should be noted that the way to calculate P_{dTV} in the present study is not the same as in some of the aforementioned clinical studies and, thus, our reported P_{dTV} values should not be compared with the PRD threshold set in those studies for mortality prediction. Future work in larger study populations should confirm whether P_{dTV} (equivalent to PRD) and its RRV-unrelated fraction, $P_{dTVuRRV}$, can be used as tools to measure the chronic effects of age, BMI or fitness on sympathetically-modulated ventricular repolarization and how this could be related to increased cardiac and arrhythmic risk.

As an observation from our research, we could not find significant differences between clusters A and B in terms of LF oscillations of cardiac activity during exercise. Our initial hypothesis was that exercise would accentuate potential resting differences between individuals with distinct phenotypes. However, the temporal profile of P_{dTV} presents high inter-individual variability even among subjects of the same cluster, which results in a large standard deviation of the P_{dTV} measures. In terms of the median of P_{dTV} and its RRV-related and unrelated components (see Table 3), CLUSTER A shows an increasing trend with exercise intensity, whereas the opposite behavior is observed in CLUSTER B. Particularly for CLUSTER A, we show a marked increase in P_{dTV} from S_{CY70} to S_{CY80}, which matches the findings by Milagro et al., who reported a sudden increase around the second ventilatory threshold in trained subjects [16]. Our observed differences between the two clusters could potentially be a reflection of differences in the sympathetic modulation of ventricular activity with exercise, with the profile reported for subjects of CLUSTER A being representative of a better health status.

4.4. Strengths, Limitations and Future Research

A key strength of the present study is the in-depth analysis of PRD (quantified through P_{dTV}) using a time-frequency approach to track the frequency components of the dT time series and of their portions related and unrelated to RRV. Second, previous studies describing PRD patterns during exercise have been carried out in groups of 20 young lean volunteers and, in some cases, all of them being physically fit [16,18]. In contrast, our study population is larger, comprises volunteers of ages spanning from 20 to 70 years old and is much more heterogeneous in terms of weight and physical fitness, thus being more representative of the general population. Third, cluster analysis is used to evaluate the extent to which LF oscillations of HR and ventricular repolarization are modulated by the concurrence of phenotype characteristics, such as age, BMI and cardiorespiratory fitness levels. This perspective is particularly relevant considering that, by 2050, 1 out of 6 people in the world will be an older adult [50] and advanced age has been associated

with changes in body composition and reduced cardiorespiratory fitness [51,52]. Last but not least, all the measurements and signal recordings of this study are performed in the laboratory, under homogeneous conditions, thus enabling control of confounding factors and guaranteeing the reproducibility of the study.

On the other hand, some limitations of the study are to be acknowledged. Although larger than in previous similar studies, the sample size is still relatively small. In future research, larger, more representative samples would allow confirming the findings of the present study regarding the temporal profile of PRD during incremental exercise, with dissection of the portion attributable to HR-dependent effects and the portion related to intrinsic autonomic modulation of the ventricular myocardium. Furthermore, it should be born in mind that a mesomorph subject may be overweight according to its BMI, so the results on ECG ventricular repolarization dynamics in these subjects should be critically interpreted taking this into account. As another limitation of our study, all the participants were Spanish white men. Further work should aim at applying the methodologies here reported onto other populations including women and other racial or ethnic groups.

In this paper, PRD is quantified during exercise and values are found to be two orders of magnitude higher than at rest. Previous studies have established thresholds for cardiac and arrhythmic risk stratification based on resting PRD measurements. Future studies could take the present work as a basis and measure PRD in clinical populations to assess the value of exercise-induced PRD increments for risk prediction. This, together with other more mechanistic investigations, could help elucidate the grounds underlying the predictive capacity of elevated PRD. Those grounds could involve not only a higher vulnerability of the myocardium to arrhythmogenic LF repolarization oscillations but a higher release of norepinephrine and arrhythmogenic co-transmitters due to larger neuronal synchronization, as proposed in [9]. Additionally, further research should confirm whether PRD and its RRV-unrelated component, both measured at rest and in response to exercise, could be useful to assess chronic effects of age, BMI and cardiorespiratory fitness level on ventricular activity and its relationship to cardiac risk, in general, and arrhythmic risk, in particular.

5. Conclusions

This study characterizes the frequency content along time of the dT angle between consecutive ECG T-wave vectors as a measure of repolarization instability. Oscillations in dT mostly occur in the low-frequency band and as much as 50–70% of them are unrelated to heart rate variability. The instantaneous LF power of dT, P_{dTV}, increases by two orders of magnitude during an incremental exercise protocol as compared to values at rest and during recovery from exercise, although high inter-individual variability is observed in the temporal profiles of P_{dTV}. By clustering analysis, we show that a group of overweight and unfit individuals presents significantly larger P_{dTV} values at rest, whereas no clear relationship with age is observed. Notwithstanding the limitations of the study, concerning sample size, BMI and sample characteristics, these findings extend our knowledge of periodic repolarization dynamics (PRD), a promising ECG risk marker, and set the stage for future studies to investigate exercise-induced heart rate-unrelated changes in PRD as a strategy to improve its prognostic cardiac and arrhythmic risk stratification capacity.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the ethical committee for clinical research of Aragón (ID of the approval: PI17/0409; date: 17 January 2018).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The datasets analyzed during the current study are available from the corresponding author on reasonable request.

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Appendix A

Table A1. Comparison of P_{RRV}, P_{dTV}, $P_{dTVuRRV}$, $P_{dTVrRRV}$ and $P_{dTVuRRVn}$ between age groups at the different evaluated test segments.

	Outcome	Young Adults (n = 24)	Middle-Aged Adults (n = 21)	Older Adults (n = 21)	Main Effect	
					p	Effect Size
S_{REST}	P_{RRV} (e^{-4})	23.17 (15.06 to 43.17) ^{O}	11.96 (7.90 to 23.84) ^{O}	3.65 (1.86 to 10.82) ^{Y,M}	**<0.001** *	0.333
	P_{dTV}	1.31 (0.64 to 2.55)	1.33 (0.71 to 4.95)	0.92 (0.48 to 2.76)	0.666	0.012
	$P_{dTVuRRV}$	0.80 (0.39 to 1.24)	0.64 (0.41 to 2.75)	0.61 (0.26 to 1.41)	0.712	0.010
	$P_{dTVrRRV}$	0.54 (0.24 to 1.15)	0.69 (0.31 to 2.20)	0.43 (0.21 to 1.09)	0.480	0.023
	$P_{dTVuRRVn}$	0.61 (0.59 to 0.66)	0.67 (0.59 to 0.77)	0.62 (0.56 to 0.76)	0.393	0.029
S_{CY60}	P_{RRV} (e^{-4})	2.43 (1.15 to 3.52) ^{O}	1.24 (0.89 to 2.12)	1.13 (0.71 to 1.95) ^{Y}	**0.013** *	0.133
	P_{dTV}	63.30 (25.45 to 97.67)	141.55 (40.01 to 192.05)	99.11 (26.17 to 125.91)	0.307	0.036
	$P_{dTVuRRV}$	32.34 (12.18 to 47.36)	72.16 (16.47 to 97.98)	48.60 (12.91 to 71.78)	0.340	0.033
	$P_{dTVrRRV}$	30.55 (13.27 to 55.29)	67.71 (19.33 to 104.23)	42.66 (8.72 to 56.85)	0.301	0.037
	$P_{dTVuRRVn}$	0.54 (0.50 to 0.57)	0.53 (0.50 to 0.55)	0.55 (0.51 to 0.58)	0.471	0.023
S_{CY70}	P_{RRV} (e^{-4})	0.94 (0.45 to 1.67)	0.75 (0.33 to 1.03)	0.44 (0.24 to 0.91)	0.164	0.056
	P_{dTV}	103.00 (57.80 to 222.93)	87.38 (66.51 to 172.59)	88.19 (49.36 to 213.62)	0.929	0.002
	$P_{dTVuRRV}$	60.75 (29.50 to 122.93)	56.59 (33.15 to 95.72)	48.87 (26.85 to 117.75)	0.953	0.001
	$P_{dTVrRRV}$	43.61 (27.82 to 105.93)	48.79 (24.36 to 76.87)	44.75 (15.30 to 93.96)	0.886	0.004
	$P_{dTVuRRVn}$	0.56 (0.54 to 0.60)	0.55 (0.54 to 0.57)	0.55 (0.52 to 0.60)	0.555	0.018

Table A1. *Cont.*

	Outcome	Young Adults (*n* = 24)	Middle-Aged Adults (*n* = 21)	Older Adults (*n* = 21)	Main Effect	
					p	Effect Size
S_{CY80}	P_{RRV} (e^{-4})	0.32 (0.15 to 0.48)	0.19 (0.12 to 0.36)	0.20 (0.14 to 0.38)	0.345	0.033
	P_{dTV}	151.85 (41.39 to 221.44)	96.18 (46.42 to 143.97)	79.23 (53.30 to 169.55)	0.789	0.007
	$P_{dTVuRRV}$	85.69 (21.61 to 150.95)	58.99 (28.59 to 117.91)	53.67 (31.37 to 116.53)	0.898	0.003
	$P_{dTVrRRV}$	53.54 (20.21 to 89.76)	31.17 (16.32 to 49.83)	31.73 (21.17 to 62.73)	0.506	0.021
	$P_{dTVuRRVn}$	0.62 (0.58 to 0.69)	0.64 (0.61 to 0.70)	0.60 (0.56 to 0.68)	0.255	0.042
S_{REC}	P_{RRV} (e^{-4})	7.09 (3.45 to 16.39)	10.71 (5.34 to 17.35) ^{O}	5.16 (2.28 to 9.47) ^{M}	**0.014** *	0.130
	P_{dTV}	2.46 (1.42 to 6.59)	2.83 (1.36 to 4.20)	4.92 (2.86 to 7.52)	0.086	0.075
	$P_{dTVuRRV}$	1.25 (0.75 to 4.09)	1.30 (0.86 to 2.32)	2.66 (1.40 to 3.98)	0.067	0.083
	$P_{dTVrRRV}$	1.27 (0.60 to 2.75)	1.43 (0.49 to 2.21)	2.23 (0.93 to 3.67)	0.257	0.042
	$P_{dTVuRRVn}$	0.57 (0.54 to 0.64)	0.58 (0.50 to 0.62)	0.61 (0.54 to 0.64)	0.613	0.015

Values are expressed as median and interquartile range. Segments are based on the test phases: resting (S_{REST}), cycling (S_{CY}) and recovery (S_{REC}). S_{CY} was divided in three stages at 60, 70 and 80% of estimated HR_{max}, denoted as S_{CY60}, S_{CY70} and S_{CY80}, respectively. P_{RRV} = LF oscillations for RR variability; P_{dTV} = LF oscillations for dT variability; $P_{dTVuRRV}$ = P_{dTV} unrelated to P_{RRV}; $P_{dTVrRRV}$ = P_{dTV} related to P_{RRV}; $P_{dTVuRRVn}$ = normalized $P_{dTVuRRV}$. * = Significant differences between groups ($p \leq 0.05$, Kruskal–Wallis test). ^{Y} = Different to Young adults; ^{M} = Different to Middle-aged adults; ^{O} = Different to Older adults.

Table A2. Comparison of P_{RRV}, P_{dTV}, $P_{dTVuRRV}$, $P_{dTVrRRV}$ and $P_{dTVuRRVn}$ between BMI groups.

	Outcome	Non-Overweight (*n* = 38)	Overweight (*n* = 28)	*p*	ES
S_{REST}	P_{RRV} (e^{-4})	16.93 (6.18 to 30.57)	10.09 (3.91 to 19.57)	0.143	0.180
	P_{dTV}	1.10 (0.44 to 2.07)	1.60 (0.84 to 3.37)	**0.030** *	0.267
	$P_{dTVuRRV}$	0.57 (0.27 to 1.24)	0.75 (0.51 to 2.05)	0.078	0.217
	$P_{dTVrRRV}$	0.46 (0.21 to 0.80)	0.75 (0.38 to 1.47)	**0.019** *	0.289
	$P_{dTVuRRVn}$	0.63 (0.60 to 0.71)	0.60 (0.56 to 0.73)	0.364	0.112
S_{CY60}	P_{RRV} (e^{-4})	1.74 (1.01 to 2.95)	1.44 (0.73 to 2.33)	0.173	0.168
	P_{dTV}	69.61 (27.79 to 165.01)	98.71 (23.81 to 176.69)	0.716	0.045
	$P_{dTVuRRV}$	33.32 (12.88 to 74.32)	47.73 (12.13 to 90.98)	0.736	0.042
	$P_{dTVrRRV}$	37.01 (14.92 to 78.70)	43.54 (8.67 to 89.04)	0.815	0.029
	$P_{dTVuRRVn}$	0.55 (0.53 to 0.58)	0.51 (0.50 to 0.55)	**0.002** *	0.388
S_{CY70}	P_{RRV} (e^{-4})	0.72 (0.33 to 1.13)	0.54 (0.30 to 1.26)	0.645	0.057
	P_{dTV}	99.71 (56.84 to 218.33)	87.78 (68.89 to 177.39)	0.887	0.018
	$P_{dTVuRRV}$	53.14 (30.30 to 116.59)	56.33 (30.53 to 102.87)	0.979	0.003
	$P_{dTVrRRV}$	43.61 (26.17 to 94.15)	46.17 (15.76 to 74.53)	0.640	0.057
	$P_{dTVuRRVn}$	0.56 (0.54 to 0.60)	0.55 (0.51 to 0.59)	0.208	0.155
S_{CY80}	P_{RRV} (e^{-4})	0.29 (0.14 to 0.41)	0.19 (0.13 to 0.46)	0.559	0.072
	P_{dTV}	116.95 (51.73 to 235.29)	83.25 (51.81 to 150.11)	0.392	0.105
	$P_{dTVuRRV}$	74.30 (31.29 to 148.63)	51.23 (25.08 to 114.92)	0.429	0.097
	$P_{dTVrRRV}$	43.68 (19.03 to 93.20)	31.00 (19.81 to 55.13)	0.270	0.136
	$P_{dTVuRRVn}$	0.64 (0.60 to 0.69)	0.61 (0.57 to 0.68)	0.254	0.141

Table A2. *Cont.*

	Outcome	Non-Overweight (n = 38)	Overweight (n = 28)	*p*	ES
S_{REC}	P_{RRV} (e^{-4})	9.47 (6.05 to 18.15)	4.06 (2.17 to 8.44)	**<0.001** *	0.436
	P_{dTV}	2.76 (1.47 to 5.99)	3.62 (1.68 to 6.60)	0.517	0.080
	$P_{dTVuRRV}$	1.27 (0.85 to 3.22)	2.16 (1.28 to 3.82)	0.259	0.139
	$P_{dTVrRRV}$	1.49 (0.64 to 2.82)	1.59 (0.45 to 3.43)	0.825	0.027
	$P_{dTVuRRVn}$	0.58 (0.53 to 0.64)	0.58 (0.53 to 0.63)	0.959	0.006

Values are expressed as median and interquartile range. Segments are based on the test phases: resting (S_{REST}), cycling (S_{CY}) and recovery (S_{REC}). S_{CY} was divided in three stages at 60, 70 and 80% of estimated HRmax, denoted as S_{CY60}, S_{CY70} and S_{CY80}, respectively. P_{RRV} = LF oscillations for RR variability; P_{dTV} = LF oscillations for dT variability; $P_{dTVuRRV}$ = P_{dTV} unrelated to P_{RRV}; $P_{dTVrRRV}$ = P_{dTV} related to P_{RRV}; $P_{dTVuRRVn}$ = normalized $P_{dTVuRRV}$. ES = Effect size. * = Significant differences between groups ($p \leq 0.05$, Mann–Whitney *U*-test).

Table A3. Comparison of P_{RRV}, P_{dTV}, $P_{dTVuRRV}$, $P_{dTVrRRV}$ and $P_{dTVuRRVn}$ between cardiorespiratory fitness level ($PWC_{80\%}$) groups.

	Outcome	Fit (*n* = 32)	Unfit (*n* = 34)	*p*	ES
S_{REST}	P_{RRV} (e^{-4})	12.45 (5.61 to 24.60)	14.25 (6.12 to 28.66)	0.581	0.068
	P_{dTV}	1.10 (0.43 to 2.22)	1.43 (0.80 to 2.70)	0.166	0.171
	$P_{dTVuRRV}$	0.63 (0.27 to 1.38)	0.71 (0.43 to 1.24)	0.419	0.099
	$P_{dTVrRRV}$	0.45 (0.23 to 0.80)	0.71 (0.33 to 1.44)	0.061	0.231
	$P_{dTVuRRVn}$	0.65 (0.60 to 0.73)	0.61 (0.55 to 0.67)	0.059	0.232
S_{CY60}	P_{RRV} (e^{-4})	1.23 (0.96 to 2.51)	1.82 (0.96 to 2.91)	0.441	0.095
	P_{dTV}	74.15 (19.97 to 175.41)	92.39 (36.08 to 165.01)	0.635	0.058
	$P_{dTVuRRV}$	35.28 (10.57 to 91.96)	44.43 (15.99 to 81.78)	0.663	0.054
	$P_{dTVrRRV}$	36.43 (10.35 to 90.40)	48.12 (14.57 to 73.71)	0.599	0.065
	$P_{dTVuRRVn}$	0.55 (0.52 to 0.57)	0.53 (0.50 to 0.55)	0.121	0.191
S_{CY70}	P_{RRV} (e^{-4})	0.54 (0.32 to 1.03)	0.76 (0.35 to 1.32)	0.223	0.150
	P_{dTV}	85.90 (42.46 to 208.57)	101.57 (70.22 to 175.55)	0.496	0.084
	$P_{dTVuRRV}$	49.52 (21.85 to 121.80)	59.95 (34.25 to 91.91)	0.663	0.054
	$P_{dTVrRRV}$	38.97 (16.83 to 86.07)	46.17 (31.58 to 78.83)	0.488	0.085
	$P_{dTVuRRVn}$	0.56 (0.54 to 0.60)	0.55 (0.52 to 0.57)	0.166	0.171
S_{CY80}	P_{RRV} (e^{-4})	0.21 (0.13 to 0.35)	0.26 (0.14 to 0.53)	0.133	0.185
	P_{dTV}	130.30 (59.72 to 291.39)	83.25 (50.12 to 152.08)	0.281	0.133
	$P_{dTVuRRV}$	82.98 (37.23 to 156.10)	49.39 (23.61 to 92.27)	0.178	0.166
	$P_{dTVrRRV}$	42.06 (16.91 to 108.72)	31.45 (21.28 to 57.86)	0.635	0.058
	$P_{dTVuRRVn}$	0.67 (0.61 to 0.70)	0.61 (0.58 to 0.64)	**0.016** *	0.295
S_{REC}	P_{RRV} (e^{-4})	8.39 (5.23 to 17.51)	5.22 (2.89 to 13.56)	0.063	0.229
	P_{dTV}	3.55 (1.93 to 6.02)	2.86 (1.47 to 6.83)	0.710	0.046
	$P_{dTVuRRV}$	2.03 (0.94 to 3.08)	1.36 (0.89 to 3.85)	0.672	0.052
	$P_{dTVrRRV}$	1.81 (0.86 to 2.81)	1.44 (0.53 to 3.78)	0.691	0.049
	$P_{dTVuRRVn}$	0.60 (0.54 to 0.65)	0.57 (0.53 to 0.62)	0.178	0.166

Values are expressed as median and interquartile range. Segments are based on the test phases: resting (S_{REST}), cycling (S_{CY}) and recovery (S_{REC}). S_{CY} was divided in three stages at 60, 70 and 80% of estimated HR_{max}, denoted as S_{CY60}, S_{CY70} and S_{CY80}, respectively. P_{RRV} = LF oscillations for RR variability; P_{dTV} = LF oscillations for dT variability; $P_{dTVuRRV}$ = P_{dTV} unrelated to P_{RRV}; $P_{dTVrRRV}$ = P_{dTV} related to P_{RRV}; $P_{dTVuRRVn}$ = normalized $P_{dTVuRRV}$. ES = Effect size. * = Significant differences between groups ($p \leq 0.05$, Mann–Whitney *U*-test).

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5. Discusión global

Uno de los principales objetivos de la tesis fue analizar las limitaciones de los métodos de medición de los niveles de actividad física y la electrofisiología cardíaca al utilizarse en población mayor. El artículo II de la presente tesis evaluó las métricas basadas en puntos de corte *versus* métricas libres de puntos de corte. Las limitaciones de los puntos de corte son bien conocidas y conducen a algunos de los problemas observados en el artículo II (132). En primer lugar, los puntos de corte son protocolo-específicos y población-específicos, por lo tanto: i) los resultados no son comparables entre estudios; ii) hemos observado que el tiempo que un centenario pasa en actividad física de intensidad-baja e intensidad moderada-vigorosa varía ampliamente en función del punto de corte utilizado para el cálculo; iii) los científicos tienen que seleccionar uno de los muchos puntos de corte disponibles para un segmento poblacional Ej. Personas mayores, pero no hay puntos para sub-segmentos específicos y extremos como los centenarios (56,133,134). La segunda limitación de los puntos de corte es que dos participantes puntúan muy diferente si uno realiza actividad justo por encima del punto de corte y el otro realiza actividad justo por debajo (132), evidentemente estas fronteras súbitas no existen en la fisiología humana. En tercer y último lugar, muchos participantes no alcanzan ninguna actividad por encima de los puntos de corte (particularmente en el rango vigoroso) (132). En concreto, nuestros centenarios mostraron un potencial efecto suelo para la actividad física moderada-vigorosa, que se intensificaba al usar los puntos de corte más exigentes, dicho efecto suelo se evitó al usar las métricas libres de puntos de corte. Las métricas libres de puntos de corte emergen como una sólida alternativa, puesto que son población-independientes, aunque son protocolo-específicas y podrían diferir entre algunas marcas y modelos de acelerómetros (59). Además con el enfoque Mx, los resultados no están colapsados en categorías, sino que se mantiene la naturaleza continua

de los datos y se pueden realizar interpretaciones post-hoc en relación a cualquier punto de corte (59). También merece ser destacado que en las métricas Mx, los minutos de actividad física pueden ser acumulados en cualquier momento del día, sin necesidad de ser acumulados en «*bouts*», siendo coherentes con la perspectiva *«cada movimiento cuenta»* de la Organización Mundial de la Salud (35). Nuestros resultados sugieren que los nuevos enfoques analíticos podrían superar las limitaciones de los puntos de corte al estudiar a los muy mayores.

Por su parte, las limitaciones de los métodos de medición de la electrofisiología cardíaca fueron analizadas en el artículo IV de esta tesis. Particularmente el artículo evaluó la validez del análisis de variabilidad de la frecuencia cardíaca registrado por el sensor de frecuencia cardíaca *PolarH7*. De acuerdo a estudios previos, el *PolarH7* mostró un excelente acuerdo con el electrocardiograma en reposo (83,85). Durante el ejercicio de intensidad moderada-alta se observó un desacuerdo entre los dispositivos en las oscilaciones de alta frecuencia de la frecuencia cardíaca, hallazgos similares fueron descritos al evaluar el modelo anterior *Polar RS800* (88), tanto las oscilaciones de alta frecuencia como los índices de dominio temporal relacionados, tales como RMSSD (135), son comúnmente usados para monitorizar el sistema nervioso autónomo antes, durante y después del ejercicio (114), por lo que estos datos deberían interpretarse con cuidado cuando hayan sido registrados por el *PolarH7* a más del 70% de la frecuencia cardíaca máxima. Es la primera vez que ha sido estudiada la validez del *PolarH7* durante la recuperación, las mediciones de frecuencia cardíaca fueron precisas, al contrario que las de variabilidad de la frecuencia cardíaca, por lo cual de acuerdo a Schneider et al. es recomendable el uso de la frecuencia cardíaca durante la recuperación post-ejercicio (98). Los resultados del análisis de conglomerados confirman la hipótesis de que la edad y características fenotípicas de los sujetos son una de las causas, no la única, de desacuerdo

entre dispositivos (88), factor a considerar cuando se use el *PolarH7* y en general en la validación de cualquier sensor.

Otro de los principales objetivos de la tesis fue aportar resultados descriptivos de niveles de actividad física y electrofisiología cardíaca en personas muy mayores así como estudiar su relación con diferentes marcadores de salud. Este objetivo se llevó a cabo en los artículos I, II, V y VI. Los artículos I y II evaluaron objetivamente la actividad física y el sedentarismo en centenarios, confirmando la hipótesis de que se observaría un descenso asociado a la edad en los niveles de actividad física. El artículo I utilizó métricas basadas en puntos de corte, mostrando que los centenarios son menos activos y más sedentarios que los nonagenarios. Este artículo también analizó el número de pasos, gracias a lo que fueron observados dos hechos interesantes: i) al comparar nuestros resultados con los de Davis et al. se vio que el número de pasos podría presentar un descenso acelerado durante las últimas décadas de vida (67); ii) de acuerdo con anteriores estudios en ancianos de 71-91 años (136,137), el periodo más activo del día para los nonagenarios y los centenarios fue la mañana, la identificación de estos patrones comportamentales podrían orientar futuras intervenciones con esta población. Por su parte, el artículo II utilizó además métricas libres de puntos de corte, las cuales ratificaron una reducción relacionada con la edad en el volumen y la intensidad de la actividad física, presentando nuestros centenarios los valores más bajos del espectro de edades en todas las variables Ej. Aceleración media, gradiente de intensidad y Mx (138–140). Hay que tener en cuenta que la muestra del artículo II está compuesta por centenarios institucionalizados y que puntualmente algunos centenarios podrían presentar resultados similares o incluso mayores a los reportados por personas mayores, de hecho existen ejemplos inspiradores de atletas centenarios que continúan compitiendo (68). Se conoce que la actividad física presenta una relación dosis respuesta con la salud (38), incluso en

centenarios, el artículo II mostró que tanto las medidas basadas en puntos de corte como las libres de puntos de corte encontraron diferencias entre grupos con un estado positivo *versus* negativo en diferentes marcadores de salud tales como: independencia funcional, capacidad cognitiva y capacidad física. Sin embargo, más del 7.2% de las muertes por cualquier causa y un 8% de las enfermedades no transmisibles son atribuidas a la inactividad física (141), por lo que resulta realmente preocupante que el sub-segmento que más rápido crece de la población (los muy mayores) sea el más inactivo. La inactividad física de los muy mayores debería ser vista por los políticos como un problema prioritario, dadas sus implicaciones para la calidad de vida relacionada con la salud y los costes sanitarios asociados (4).

Los resultados descriptivos de electrofisiología cardíaca en personas muy mayores y su relación con diferentes marcadores de salud fueron estudiados en los artículos V y VI. En concreto, el artículo V investigó las posibles diferencias en la variabilidad de la frecuencia cardíaca de las mujeres adultas, octogenarias y centenarias. Encontramos una clara disminución con la edad en los principales índices de variabilidad de frecuencia cardíaca que reflejan la actividad parasimpática, este descenso ha sido reportado por otros autores (99,101,142), aunque es la primera vez que se describe en centenarios y contradice el nadir parasimpático en los 60-80 años que ha sido descrito por varios autores (108,110,143), esta discrepancia podría explicarse, entre otros, por que los estudios previos no hubieran filtrado patrones erráticos o por que hubieran seleccionado muestras incluyendo únicamente centenarios sanos e independientes (108–110,144). Los resultados de SDNN y las oscilaciones de baja frecuencia siguieron la misma línea que las variables parasimpáticas, probablemente debido a que el SNP es una de las principales fuentes de variabilidad en estos índices durante mediciones de corta duración realizadas en sedestación (145). El artículo V también evaluó si los índices de variabilidad de la

frecuencia cardíaca pueden predecir la mortalidad por todas las causas en centenarios. Únicamente SDNN se asoció con mortalidad temprana (≤1 año), el grupo de centenarios con SDNN <19ms mostró un riesgo de mortalidad temprana cinco veces superior que aquellos con SDNN >19ms, aunque los resultados deben leerse con perspectiva ya que se trata de una muestra pequeña y heterogénea. En las últimas décadas la variabilidad de la frecuencia cardíaca ha sido confirmada como un predictor independiente de morbilidad y mortalidad (96,146). Específicamente SDNN ya fue planteado en 1987 para la estratificación del riesgo tras infarto de miocardio y de acuerdo con Bilchick et al. cada incremento de 10ms en SDNN conferiría un descenso del 20% en el riesgo de mortalidad (94,95).

El artículo VI también aporta resultados descriptivos de electrofisiología cardíaca, si el artículo V se centraba en el intervalo RR, el artículo VI pone el foco sobre la onda T, es decir en las DRP. Uno de los objetivos específicos de este artículo fue cuantificar de forma continua las DRP en reposo, durante el ejercicio y la recuperación, evaluando su dependencia de la variabilidad de la frecuencia cardíaca. Según nuestro conocimiento, este estudio es el primero que evalúa los componentes frecuenciales de dT y de la variabilidad de la frecuencia cardíaca; revelando dos aspectos importantes: i) las oscilaciones de dT ocurren principalmente a baja frecuencia y su magnitud aumenta en respuesta al estímulo simpático que supone el ejercicio, lo que unido a evidencias previas, apoya de forma indirecta la idea de que el SNS estaría involucrado en la generación de dicho comportamiento oscilatorio (120,129,130,147–152); ii) Un 50-70% de las oscilaciones dT de baja frecuencia (es decir, DRP) no están relacionadas con la variabilidad de la frecuencia cardíaca, esta parte no relacionada podría ser más significativa desde el punto de vista clínico, anteriores estudios han confirmado en otros marcadores de repolarización ventricular, como el intervalo QT, el valor de medir de

forma específica la parte no relacionada con la variabilidad de la frecuencia cardíaca (153,154). En lo concerniente al patrón de DRP durante un test de ejercicio incremental, en la misma línea que los estudios previos DRP mostró un aumento durante el ejercicio respecto a los valores de reposo y recuperación (124,126), observándose una alta variabilidad inter-sujeto durante el ejercicio, que podría deberse a que se utilizó un protocolo sub-máximo (124,126). Cabe destacar que, mientras que la componente no relacionada presentó un patrón durante el ejercicio similar a DRP, la componente relacionada no lo hizo, lo que refuerza que la componente no relacionada podría reflejar mejor el efecto simpático en la repolarización ventricular. El segundo objetivo específico de este artículo fue caracterizar los efectos de la edad, el índice de masa corporal y el nivel de aptitud cardiorrespiratoria sobre las DRP. No se observaron diferencias en DRP al analizar grupos divididos según una única variable, por ejemplo la edad, sin embargo el análisis de conglomerados identificó un grupo de sujetos con sobrepeso y baja condición física con valores de DRP en reposo significativamente más altos que el resto de la muestra, de hecho un DRP elevado ha mostrado ser predictor de mortalidad y muerte súbita en diferentes grupos de pacientes (120,127–130).

Esta tesis culmina con el estudio III, un estudio único que por primera vez en centenarios pasa del plano descriptivo a la intervención. Concretamente, se examinaron los efectos de 12 semanas de entrenamiento supervisado de fuerza sobre la condición física, independencia funcional, fragilidad y calidad de vida relacionada con la salud en centenarios frágiles institucionalizados. La evidencia científica respalda los efectos beneficiosos del ejercicio físico, incluso en personas nonagenarias, frágiles, institucionalizadas u hospitalizadas (18,73–75), sin embargo en la literatura científica únicamente encontramos dos estudios de caso que reportan el entrenamiento mediante ejercicio físico en dos atletas centenarios que competían a nivel mundial, la intervención

duró más de un año y estaba pobremente detallada (29,78), nuestro estudio es por tanto el primero que incluye un grupo control y evalúa los efectos del ejercicio físico en centenarios que no son atletas. El envejecimiento lleva a una progresiva disminución en la función de todos los sistemas (9), consecuentemente era esperable la tendencia al deterioro de todas las variables que se observó en el grupo control tras el periodo de 12 semanas. Hay que subrayar que el ANOVA de medidas repetidas reveló una interacción grupo-tiempo significativa en todas las variables evaluadas, extendiendo los conocidos beneficios del ejercicio físico en los muy mayores hasta el límite de la esperanza de vida humana (18). Además, debemos destacar que el grupo intervención no se limitó a eludir el descenso asociado a la edad, sino que mejoró significativamente su condición física, independencia funcional, fragilidad y calidad de vida relacionada con la salud; en otras palabras, nuestros resultados sugieren que nadie es demasiado mayor para beneficiarse del entrenamiento de fuerza. Por último pero no menos importante, la *«National Strength and Conditioning Association»* asegura que las lesiones que sufren las personas mayores durante el entrenamiento de fuerza están principalmente relacionadas con una técnica incorrecta o con una selección inadecuada de los ejercicios (72). A pesar de la fragilidad de nuestra muestra de centenarios, no se observó ningún efecto adverso importante durante el periodo de intervención, en la misma línea que estudios previos en personas mayores (155).

6. Conclusiones [Conclusions]

Artículo I.

- Nuestros resultados sugieren que la disminución en los niveles de actividad física continúa empeorando hasta el límite de la esperanza de vida humana, siendo los centenarios menos activos y más sedentarios que los nonagenarios.
- Al observar la distribución horaria, el período del día más activo para los nonagenarios y centenarios fue la mañana, con un pico entre las 10:00 y las 11:59.

Artículo II.

- Las métricas de acelerometría libres de puntos de corte también muestran una reducción relacionada con la edad en el volumen y la intensidad de la actividad física, presentando nuestros centenarios los valores más bajos del espectro de edades en todas las variables Ej. Aceleración media, gradiente de intensidad y Mx.
- Tanto las medidas basadas en puntos de corte como las libres de puntos de corte estuvieron relacionadas con los estados de salud, pero la variable M30, libre de puntos de corte, fue la única relacionada con mortalidad temprana
- El tiempo diario que un centenario pasa en actividad física de intensidad-baja e intensidad moderada-vigorosa varía ampliamente en función del punto de corte utilizado para el cálculo, además se encontró un potencial efecto suelo para la actividad física moderada-vigorosa. El uso en futuros estudios de medidas libres de puntos de corte está justificado para proporcionar información más completa y comparable entre muestras de centenarios.

Artículo III.

- Es la primera vez que se presenta un ensayo controlado aleatorizado investigando los efectos de 12 semanas de entrenamiento de fuerza en centenarios. Los resultados sugieren que nadie es demasiado mayor para beneficiarse del entrenamiento de fuerza, retrasar la pérdida de funcionalidad relacionada con la edad y mejorar su calidad de vida.
- No se observaron efectos adversos importantes durante el periodo de intervención a pesar de la fragilidad de los centenarios.

Artículo IV.

- En una muestra relativamente amplia y heterogénea, el *PolarH7* y el electrocardiograma fueron intercambiables en reposo. Durante el ejercicio, la frecuencia cardíaca media y las oscilaciones de baja frecuencia de la frecuencia cardíaca (hasta 0,15 Hz) también mostraron un excelente acuerdo entre los dispositivos. Sin embargo, existió desacuerdo entre los dispositivos al evaluar las oscilaciones de alta frecuencia de la frecuencia cardíaca durante el ejercicio de intensidad moderada-alta.
- La validez de las mediciones del *PolarH7* durante la recuperación en sedestación ha sido estudiada por primera vez. Los resultados de la presente investigación respaldan que el *PolarH7* es apropiado para evaluar la recuperación post-ejercicio de la frecuencia cardíaca, pero no para evaluar la recuperación post-ejercicio de la variabilidad de la frecuencia cardíaca.
- La edad, composición corporal y nivel de condición física pueden representar una de las causas de desacuerdo entre los sensores de frecuencia cardíaca y los dispositivos de electrocardiograma, lo que debe considerarse específicamente

al usar el *PolarH7* y, en general, en la validación de cualquier sensor de frecuencia cardíaca para el análisis de variabilidad de la frecuencia cardíaca.

Artículo V.

- Encontramos una clara disminución con la edad en los principales índices de variabilidad de la frecuencia cardíaca que reflejan la actividad parasimpática, así como en la desviación estándar de la serie RR (SDNN) y en las oscilaciones de baja frecuencia de la frecuencia cardíaca, lo que podría ser representativo de un agotamiento natural de los sistemas alostáticos relacionado con la edad.
- SDNN <19 ms se asoció con mortalidad temprana (≤1 año) en personas centenarias (Cociente de riesgos instantáneos = 5,72).

Artículo VI.

- Las oscilaciones en dT ocurren principalmente a baja frecuencia y un 50-70% no están relacionadas con la variabilidad de la frecuencia cardíaca.
- La potencia instantánea de baja frecuencia de dT (DRP) aumenta en dos órdenes de magnitud durante un protocolo de ejercicio incremental en comparación con los valores en reposo y en recuperación del ejercicio, aunque se observa una alta variabilidad inter-sujeto en los perfiles temporales de DRP.
- El análisis de conglomerados identificó un grupo de sujetos con sobrepeso y baja condición física con valores de DRP en reposo significativamente más altos que el resto de la muestra, pero no se observó una clara relación con la edad.

- Estos hallazgos amplían nuestro conocimiento sobre las dinámicas de repolarización periódica, un prometedor marcador de riesgo basado en el electrocardiograma, y sientan las bases para futuros estudios que investiguen los cambios inducidos por el ejercicio en la componente de DRP no relacionada con la variabilidad de la frecuencia cardíaca, como una estrategia para mejorar su capacidad de estratificación del riesgo cardiaco y arrítmico.

6. Conclusions

Manuscript I.

- Our results suggest that the decline in physical activity levels continues to worsen until the end of the human lifespan, with centenarians being less active and more sedentary than their nonagenarian peers.
- When observing time distribution, the most active day period for nonagenarians and centenarians was the morning, with a peak between 10:00 and 11:59.

Manuscript II.

- Cut-point-free metrics also shows an age-related reduction in physical activity volume and intensity, with our centenarians presenting the lowest values of the age spectrum in all the variables, E.g. Average acceleration, intensity gradient and Mx.
- Both cut-point-based and cut-point-free measures were related to health states, but the cut-point-free M30 was the only one related to early mortality.
- The daily time spent by a centenarian in light-intensity physical activity and moderate-to-vigorous physical activity varies greatly depending on the cut-point used for the calculation, moreover a potential floor effect was found for moderate-to-vigorous physical activity. Future studies using the new cut-point-free physical activity metrics are warranted to provide more complete and comparable information across samples of centenarians.

Manuscript III.

- This is the first randomized control trial study investigating the effects of 12-week resistance training in centenarians. The results suggest that no one is too old to benefit from resistance training, delay the age-related loss of functionality and improve their quality of life.
- No major adverse effects were noted over the intervention period despite the frailty of the centenarians.

Manuscript IV.

- In a relative large and heterogeneous sample, *PolarH7* and electrocardiogram were interchangeable at rest. During exercise, mean heart rate and low-frequency oscillations of heart rate (up to 0.15 Hz) also showed an excellent agreement between devices. However, there was disagreement between devices when evaluating high-frequency heart rate oscillations during moderate-to-high intensity exercise.
- The validity of *PolarH7* measurements during sitting recovery has been studied for the first time. The results of the present research support the notion that *PolarH7* is appropriate to study heart rate recovery rather than post-exercise heart rate variability.
- Age, body composition and fitness level can represent one of the causes for disagreement between heart rate sensors and electrocardiogram devices, which should be considered specifically when using *PolarH7* and, generally, in the validation of any heart rate sensor for heart rate variability analysis.

Manuscript V.

- We founded a clear decrease with age in the main heart rate variability indices reflecting parasympathetic outflow as well as in the standard deviation of the RR series (SDNN) and in low-frequency heart rate oscillations, which could be representative of a natural exhaustion of allostatic systems related to age.
- SDNN <19 ms was associated with early mortality (≤1 year) in centenarians (Hazard Ratio = 5.72).

Manuscript VI.

- Oscillations in dT mostly occur in the low-frequency band and as much as 50–70% of them are unrelated to heart rate variability.
- The instantaneous low-frequency power of dT (PRD) increases by two orders of magnitude during an incremental exercise protocol as compared to values at rest and during recovery from exercise, although high inter-individual variability is observed in the temporal profiles of PRD.
- Clustering analysis identified a group of overweight and unfit individuals with significantly higher PRD values at rest than the rest of the sample, whereas no clear relationship with age was observed.
- These findings extend our knowledge of periodic repolarization dynamics, a promising electrocardiogram risk marker, and set the stage for future studies to investigate exercise-induced heart rate-unrelated changes in PRD as a strategy to improve its prognostic cardiac and arrhythmic risk stratification capacity.

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Apéndice

Características de las revistas *[Journal characteristics]*

Factor de impacto y clasificación de cada revista en el "*ISI Web of Knowledge – Journal Citation Reports"* dentro de sus áreas correspondientes.

[Impact factor and ranking of each journal in "ISI Web of Knowledge – Journal Citation Reports" within their subject categories.]

Artículo *[Manuscriptt]*	Revista *[Journal]*	Factor de impacto *[Impact factor]*
I	Journal of aging and physical activity JCR 2019 (Gerontology - Social Sciences Citation Index): 18/36– Q2 JCR 2019 (Geriatrics & gerontology - Science Citation Index Expanded): 37/51– Q3 JCR 2019 (Sport sciences - Science Citation Index Expanded): 52/85– Q3	**1,763**
II	Under review at Medicine and Science in Sports and Exercise. JCR 2020 (Sport sciences - SCIE): 8/88– Q1	**5,411**
III	Draft with preliminary results	**-**
IV	Sensors JCR 2020 (Instruments & instrumentation - Social Sciences Citation Index): 14/64 – Q1 JCR 2020 (Chemistry, analytical - Social Sciences Citation Index): 26/87 – Q2 JCR 2020 (Engineering, electrical & electronic - Social Sciences Citation Index): 82/273 – Q2	**3,576**
V	Frontiers in physiology JCR 2020 (Physiology - Social Sciences Citation Index): 14/81 – Q1	**4,566**
VI	International Journal of Environmental Research and Public Health JCR 2020 (Public, environmental & occupational health - Social Sciences Citation Index): 41/176 – Q1 JCR 2020 (Public, environmental & occupational health - Science Citation Index Expanded): 68/203 – Q2 JCR 2020 (Environmental sciences - Science Citation Index Expanded): 118/274 – Q2	**3,390**

Contribución del doctorado *[Contribution of the PhD candidate]*

En el artículo I, el doctorado solicitó el comité ético, analizó los datos de acelerometría, creó la base de datos con la que realizó los análisis estadísticos y escribió el artículo.

En los artículos II y III, solicitó el comité ético, reclutó a 19 centenarios, participó en las mediciones, entrenó al grupo intervención, realizó el análisis estadístico y escribió los artículos.

En los artículos IV y VI, solicitó el comité ético, reclutó a los participantes, participó en las mediciones y en el procesamiento de datos, realizó el análisis estadístico y escribió los artículos.

En el artículo V, el doctorado participó en las mediciones, colaboró en el procesamiento de datos, realizó el análisis estadístico y escribió el artículo.

[In manuscript I, the student requested the ethics committee, analyzed the accelerometry data, created the database with which he performed the statistical analyses and redacted the document.

In manuscripts II and III, the PhD candidate requested the ethics committee, recruited 19 centenarians, took part in the measurements, was the physical trainer of the intervention group, performed the statistical analyses and wrote the documents.

In manuscripts IV and VI, the student applied for the ethics committee, recruited the participants, was involved in the measurements and data processing, performed the statistical analysis, and wrote the manuscripts.

In manuscript number V, the PhD student participated in the measurements, collaborated in the data processing, performed the statistical analysis and wrote the document.]

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Germán, gracias por tu confianza y apoyo. Aunque diriges muchas más cosas aparte de esta tesis, siempre has sumado, siempre has sido facilitador. Por grande que sea el problema, siempre tienes una solución y transmites el optimismo necesario para superarlo. Además, cualquier reunión es más amena si comienza hablando sobre montañas.

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Me considero muy afortunado de haber podido desarrollarme como investigador en Aragón, además he tenido el privilegio de poder realizar la tesis en un grupo como **GENUD**, cuya calidad científica es superlativa. Pero la mayor de las suertes ha sido encontrar personas maravillosas con las que he compartido momentos más allá del trabajo, compañeros que a los que llamo amigos. Paso a continuación a darles las gracias por su ayuda, afecto y soporte.

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experimentales de esta tesis. Por último, Gracias **José Luis**, *¿Quién es José Luis?*, tía, ese que hace la tesis con una FPU en GENUD, *¿?*, si hombre, el que lleva una chaqueta North Face, así delgadillo con los ojos azules, *¿?*, que sí mujer, este que hace esquí de montaña, vaya y también carreras de montaña, escalada y así…, *¿?*, que es compañero de piso de Noel, *¿?*, ya no sé cómo explicarte, José Luis, que su novia es muy maja, con acento andaluz..., *¿Pero ese no es Adrián Hernández?*. Pues eso, que gracias a Joseadrián, por haber completado esta ruta como buenos montañeros; con compañerismo, decisión, perseverancia y cerveza.

"Cuando todos piensan igual, es que ninguno está pensando", esta es una de mis citas favoritas, y adquirió nuevos matices al colaborar con los investigadores de **BSICoS**, cuyos cerebros están sintonizados en otra frecuencia. Me gustaría comenzar dando las gracias a **Raquel Bailón**, quien ha sido prácticamente la 4ª directora de esta tesis. **David** gracias por tu jovialidad y tu infinita paciencia para enseñar Matlab a un niño de 5 años. **Milagro**, escribir sobre umbrales ventilatorios con un ingeniero fue una de las más gratas experiencias multidisciplinares que he vivido. Formar parte de un ambiente de trabajo tan internacional ha sido genial: **Kostas, Jorge Mario, Carmen, Spiros, Saúl, Pablo**… pero los cafés con discusiones lingüísticas o desarrollando algoritmos de clasificación de vegetales, eso es otro nivel, gracias por todo BSICoS.

I would like to thank those who strive to remove all barriers in the way of science. Particularly, I will be forever grateful to ***Fabián Sanchís*** *and* ***Thijs Eijsvogels****, who enabled international collaboration in the midst of a global pandemic. The* ***Radboud UMC*** *team made me feel at home, both inside and outside the Department of Physiology, I really appreciate it. The stay has been a finishing touch for the PhD, and I would repeat it with without a shadow of a doubt.*

Y aunque los científicos somos parte importante, las imprescindibles son las **personas anónimas** que han participado en esta tesis. Gracias a todas las personas que

voluntariamente colaboraron con la ciencia. En particular, agradezco enormemente a los trabajadores de las residencias, familiares y centenarios que creyeron en el ejercicio físico.

Antes de cerrar la parte más académica-profesional, no quiero olvidarme de todos los maestros que dejan huella durante la formación de una persona, entre otros: **Candi, Chusé, Iranzo, Joaquín Ríos, Generelo, Eva, Whyte**… Mi entrenador, **Lázaro**, merece una mención especial dentro de este apartado, puesto que jugó un papel fundamental para que yo terminara estudiando el grado en CCAFD. En el otro lado de la moneda, también quiero agradecer **a mis estudiantes** de la Facultad de CCSyD; me conformo si han aprendido la mitad que yo de ellos. Para terminar, gracias **a mis deportistas**, quienes me mantienen con un pie en el mundo real y me recuerdan lo bonito que es el deporte, sirva de ejemplo cuando **Ángel**, en un año, pasó de una grave lesión de rodilla a ganar el Campeonato de Aragón de BTT.

Para continuar con los agradecimientos, quiero hablaros de albóndigas. Pero no de una albóndiga cualquiera, sino de las albóndigas de mi madre, perfectas bolas de amor y energía cocinadas a partir de las mejores carnes. Mis amigos y familiares son similares, pero en lugar de llenar el estómago y ser pequeños ladrillos con los que construir un amasijo de huesos, tendones, músculos…, llenan el corazón de ganas de vivir y me constituyen como ser humano.

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Este documento está dedicado a todas las personas que he ido mencionando y a alguna que se me habrá olvidado mencionar. Me siento muy afortunado y agradecido de que forméis parte de mi vida.