TESIS DE LA UNIVERSIDAD DE ZARAGOZA
2021
49

Jorge Marín Puyalto

Relación de la actividad y condición físicas con la composición, estructura y metabolismo óseos durante el crecimiento, estrategias para la prevención temprana de la osteoporosis

Departamento
Fisiatría y Enfermería

Director/es
Casajús Mallén, José Antonio
Vicente Rodríguez, Germán
Gómez Cabello, Alba María

ISSN 2254-7606

Prensas de la Universidad
Universidad Zaragoza

ISSN 2254-7606

Universidad Zaragoza
1542

Tesis Doctoral

RELACIÓN DE LA ACTIVIDAD Y CONDICIÓN FÍSICAS CON LA COMPOSICIÓN, ESTRUCTURA Y METABOLISMO ÓSEOS DURANTE EL CRECIMIENTO, ESTRATEGIAS PARA LA PREVENCIÓN TEMPRANA DE LA OSTEOPOROSIS

Autor

Jorge Marín Puyalto

Director/es

Casajús Mallén, José Antonio
Vicente Rodríguez, Germán
Gómez Cabello, Alba María

UNIVERSIDAD DE ZARAGOZA

Fisiatría y Enfermería

2019

Tesis Doctoral Internacional *[International Doctoral Thesis]*

Curso Académico 2019-2020

RELACIÓN DE LA ACTIVIDAD Y CONDICIÓN FÍSICAS CON LA COMPOSICIÓN, ESTRUCTURA Y METABOLISMO ÓSEOS DURANTE EL CRECIMIENTO. ESTRATEGIAS PARA LA PREVENCIÓN TEMPRANA DE LA OSTEOPOROSIS.

ASSOCIATIONS BETWEEN PHYSICAL ACTIVITY AND FITNESS WITH BONE COMPOSITION, STRUCTURE AND METABOLISM DURING GROWTH. STRATEGIES FOR THE EARLY PREVENTION OF OSTEOPOROSIS.

JORGE MARÍN PUYALTO

Departamento de Fisiatría y Enfermería

Facultad de Ciencias de la Salud y del Deporte

Universidad de Zaragoza

Relación de la actividad y condición físicas con la composición, estructura y metabolismo óseos durante el crecimiento. Estrategias para la prevención temprana de la osteoporosis.

Associations of physical activity and fitness with bone composition, structure and metabolism during growth. Strategies for the early prevention of osteoporosis.

JORGE MARÍN PUYALTO

A Germán, José Antonio y Alba, mis directores y a mis compañeros del grupo GENUD

A mi familia y amigos

A Elena

Gracias por guiarme y acompañarme

Ser capaces de perseguir nuestros propios sueños

es lo que nos hace fuertes

Chester Bennington

Relación de la actividad y condición físicas con la composición, estructura y metabolismo óseos durante el crecimiento. Estrategias para la prevención temprana de la osteoporosis.

Associations of physical activity and fitness with bone composition, structure and metabolism during growth. Strategies for the early prevention of osteoporosis.

DIRECTORES DE TESIS:

Dr. Germán Vicente Rodríguez	**Prof. José A. Casajús Mallén**	**Dra. Alba María Gómez Cabello**
Facultad de Ciencias de la Salud y del Deporte	*Facultad de Ciencias de la Salud*	
Universidad de Zaragoza	Universidad de Zaragoza	Centro Universitario de la Defensa de Zaragoza

MIEMBROS DEL TRIBUNAL:

Presidente	***Secretario***	***Vocal***
Prof. Luis Alberto Moreno Aznar	**Dr. Hugo Olmedillas Fernández**	**Dr. Armando Manuel de Mendoça Raimundo**
Facultad de Ciencias de la Salud y del Deporte	*Facultad de Biología*	*Departamento de Desporto e Saúde*
Universidad de Zaragoza	Universidad de Oviedo	Universidade de Évora

Suplente 1º	***Suplente 2º***
Dr. Jorge Pérez Gómez	**Dra. Inge Huybrechts**
Facultad de Ciencias del Deporte	*Section of Nutrition and Metabolism*
Universidad de Extremadura	International Agency for Research on Cancer

Dr. GERMÁN VICENTE RODRÍGUEZ
Profesor Titular de Universidad

Departamento de Fisiatría y Enfermería
Facultad de Ciencias de la Salud y del Deporte
Universidad de Zaragoza

GERMÁN VICENTE RODRÍGUEZ, PROFESOR TITULAR DE LA UNIVERSIDAD DE ZARAGOZA, CERTIFICA:

Que la Tesis Doctoral titulada *"Relación de la actividad y condición físicas con la composición, estructura y metabolismo óseos durante el crecimiento. Estrategias para la prevención temprana de la osteoporosis."* que presenta D. **JORGE MARÍN PUYALTO** al superior juicio del Tribunal que designe la Universidad de Zaragoza, ha sido realizada bajo mi dirección durante los años 2014-2019, siendo expresión de la capacidad técnica e interpretativa de su autor en condiciones tan aventajadas que le hacen merecedor del Título de Doctor, siempre y cuando así lo considere el citado Tribunal.

Fdo. Germán Vicente Rodríguez

En Zaragoza, a 23 de septiembre de 2019

Prof. JOSÉ ANTONIO CASAJÚS MALLÉN
Catedrático de Universidad

Departamento de Fisiatría y Enfermería
Facultad de Ciencias de la Salud
Universidad de Zaragoza

JOSÉ ANTONIO CASAJÚS MALLÉN, CATEDRÁTICO DE LA UNIVERSIDAD DE ZARAGOZA, CERTIFICA:

Que la Tesis Doctoral titulada *"Relación de la actividad y condición físicas con la composición, estructura y metabolismo óseos durante el crecimiento. Estrategias para la prevención temprana de la osteoporosis."* que presenta D. **JORGE MARÍN PUYALTO** al superior juicio del Tribunal que designe la Universidad de Zaragoza, ha sido realizada bajo mi dirección durante los años 2014-2019, siendo expresión de la capacidad técnica e interpretativa de su autor en condiciones tan aventajadas que le hacen merecedor del Título de Doctor, siempre y cuando así lo considere el citado Tribunal.

Fdo. José A. Casajús Mallén

En Zaragoza, a 23 de septiembre de 2019

Dra. ALBA MARÍA GÓMEZ CABELLO

Profesor Contratado Doctor

Centro Universitario de la Defensa de Zaragoza

ALBA MARÍA GÓMEZ CABELLO, PROFESOR CONTRATADO DOCTOR DEL CENTRO UNIVERSITARIO DE LA DEFENSA DE ZARAGOZA, CERTIFICA:

Que la Tesis Doctoral titulada *"Relación de la actividad y condición físicas con la composición, estructura y metabolismo óseos durante el crecimiento. Estrategias para la prevención temprana de la osteoporosis."* que presenta D. **JORGE MARÍN PUYALTO** al superior juicio del Tribunal que designe la Universidad de Zaragoza, ha sido realizada bajo mi dirección durante los años 2014-2019, siendo expresión de la capacidad técnica e interpretativa de su autor en condiciones tan aventajadas que le hacen merecedor del Título de Doctor, siempre y cuando así lo considere el citado Tribunal.

Fdo. Alba María Gómez Cabello

En Zaragoza, a 23 de septiembre de 2019

Tabla de contenidos

Listado de publicaciones ..... ***- 21 -***

Características de las revistas ..... ***- 23 -***

Contribución del doctorando ..... ***- 25 -***

Proyectos de investigación ..... ***- 26 -***

Becas ..... ***- 29 -***

Listado de abreviaturas ..... ***- 30 -***

Listado de tablas y figuras ..... ***- 32 -***

Resumen general ..... ***- 34 -***

Capítulo 1. Introducción y justificación ..... ***- 39 -***

1.1 Estructura y función del tejido óseo ..... ***- 41 -***

1.2 Evolución de los parámetros óseos a lo largo de la vida ..... ***- 44 -***

1.2.1 Remodelado óseo ..... *- 44 -*

1.2.2 Evolución del contenido mineral óseo ..... *- 45 -*

1.2.3 Evolución de la estructura ósea ..... *- 48 -*

1.2.4 Evolución de los marcadores óseos ..... *- 49 -*

1.3 Osteoporosis ..... ***- 51 -***

1.4 Métodos de medición de la masa ósea ..... ***- 52 -***

1.4.1 Absorciometría de rayos X de energía dual (DXA) ..... *- 52 -*

1.4.2 Tomografía axial computerizada periférica (pQCT) ..... *- 54 -*

1.4.3 Resonancia magnética ..... *- 54 -*

1.4.4 Ultrasonidos ..... *- 54 -*

1.5 Actividad física y masa ósea ..... ***- 55 -***

1.5.1 Mecanismos de adaptación ósea ..... *- 56 -*

1.5.2 Relación entre actividad física y hueso ..... *- 57 -*

1.5.3 Natación y tejido óseo ..... *- 58 -*

1.5.4 Entrenamiento con plataformas vibratorias ..... *- 59 -*

Capítulo 2. Hipótesis y objetivos ..... ***- 61 -***

Hipótesis ..... ***- 63 -***

Objetivos ..... ***- 64 -***

Capítulo 3. Material y métodos ..... ***- 69 -***

3.1 Consideraciones éticas y legales ..... ***- 71 -***

3.2 Proyecto PREFIT ..... ***- 72 -***

3.2.1 Diseño y participantes ..... *- 72 -*

3.2.2 Mediciones antropométricas y evaluación de la masa ósea ..... - 73 -

3.2.3 Pruebas de condición física ..... - 74 -

3.3 Proyecto internacional..... - 76 -

3.3.1 Diseño y participantes..... - 76 -

3.3.2 Mediciones antropométricas y evaluación de la masa ósea ..... - 77 -

3.3.3 Registro de la práctica de actividad física..... - 78 -

3.4 Proyecto RENACIMIENTO..... - 78 -

3.4.1 Diseño y participantes..... - 79 -

3.4.2 Protocolo de entrenamiento vibratorio..... - 80 -

3.4.3 Mediciones antropométricas y evaluación de la masa ósea ..... - 81 -

3.4.4 Biomarcadores de metabolismo óseo..... - 81 -

3.4.5 Pruebas de condición física ..... - 82 -

3.4.6 Otras variables..... - 83 -

3.4.7 Revisión de revisiones sistemáticas..... - 83 -

3.5 Análisis estadísticos ..... - 85 -

Capítulo 4. Resultados y discusión [en inglés]..... - 87 -

4.1 Asociación entre la condición física y la fortaleza y estructura óseas en niños de 3 a 5 años ..... - 91 -

4.2 La frecuencia y la duración de las tandas de actividad física vigorosa están asociadas con el estado mineral óseo de los adolescentes varones. Un estudio transversal..... - 113 -

4.3 Los patrones de actividad física vigorosa afectan al crecimiento óseo durante la pubertad temprana en chicos..... - 131 -

4.4 ¿Es el entrenamiento vibratorio bueno para tus huesos? Una revisión de revisiones sistemáticas ..... - 151 -

4.5 Efectos del entrenamiento vibratorio de cuerpo completo sobre los marcadores de remodelamiento óseo en nadadores adolescentes..... - 177 -

4.6 Desarollo de un modelo informático para la identificación de nadadores adolescentes con baja densidad mineral ósea ..... - 195 -

Capítulo 5. Conclusiones y aportaciones principales..... - 213 -

Conclusiones..... - 215 -

Aportaciones principales de la Tesis Doctoral..... - 219 -

Referencias..... - 223 -

Agradecimientos ..... - 259 -

Material suplementario ..... - 267 -

Anexos..... - 277 -

Table of contents

List of publications ..... ***- 21 -***

Journal characteristics ..... ***- 23 -***

Contribution of the PhD candidate ..... ***- 25 -***

Research project ..... ***- 27 -***

Grants ..... ***- 29 -***

Abbreviation list ..... ***- 30 -***

List of tables and figures ..... ***- 33 -***

General abstract ..... ***- 36 -***

Chapter 1. Introduction and justification [Spanish] ..... ***- 39 -***

1.1 Bone tissue function and structure ..... ***- 41 -***

1.2 Evolution of bone parameters across the lifespan ..... ***- 44 -***

1.2.1 Bone remodelling ..... *- 44 -*

1.2.2 Evolution of bone mineral content ..... *- 45 -*

1.2.3 Evolution of bone structure ..... *- 48 -*

1.2.4 Evolution of bone turnover markers ..... *- 49 -*

1.3 Osteoporosis ..... ***- 51 -***

1.4 Bone measurement methods ..... ***- 52 -***

1.4.1 Dual-energy X-ray absorptiometry (DXA) ..... *- 52 -*

1.4.2 Peripheral quantitative computed tomography ..... *- 54 -*

1.4.3 Magnetic resonance imaging ..... *- 54 -*

1.4.4 Quantitative ultrasound ..... *- 54 -*

1.5 Physical activity and bone mass ..... ***- 55 -***

1.5.1 Bone adaption mechanisms ..... *- 56 -*

1.5.2 Relationships between physical activity and bone ..... *- 57 -*

1.5.3 Swimming and bone tissue ..... *- 58 -*

1.5.4 Whole body vibration training ..... *- 59 -*

Chapter 2. Hypothesis and aims ..... ***- 61 -***

Hipothesis ..... ***- 63 -***

Aims ..... ***- 66 -***

Chapter 3. Material and methods [Spanish] ..... ***- 69 -***

3.1 Ethical and legal considerations ..... ***- 71 -***

3.2 PREFIT project ..... ***- 72 -***

3.2.1 Study design and participants ..... *- 72 -*

3.2.2 Anthropometric and bone measurements ..... *- 73 -*

3.2.3 Physical fitness tests ..... *- 74 -*

3.3 International project ..... ***- 76 -***

3.3.1 Study design and participants ..... *- 76 -*

3.3.2 Anthropometric and bone measurements ..... *- 77 -*

3.3.3 Physical activity register ..... *- 78 -*

3.3 RENACIMIENTO project ..... ***- 78 -***

3.4.1 Study design and participants ..... *- 79 -*

3.4.2 Vibration training protocol ..... *- 80 -*

3.4.3 Anthropometric and bone measurements ..... *- 81 -*

3.4.4 Biomarkers of bone metabolism ..... *- 81 -*

3.4.5 Physical fitness tests ..... *- 82 -*

3.4.6 Other variables ..... *- 83 -*

3.4.7 Overview of systematic reviews ..... *- 83 -*

3.5 Statistical analyses ..... ***- 85 -***

Chapter 4. Results and discussion ..... ***- 87 -***

4.1 Associations between physical fitness and bone strength and structure in 3 to 5-year-old children ..... ***- 91 -***

4.2 Frequency and duration of vigorous physical activity bouts are associated with adolescent boys' bone mineral status: A cross-sectional study ..... ***- 113 -***

4.3 Vigorous physical activity patterns affect bone growth during early puberty in boys ***- 131 -***

4.4 Is vibration training good for your bones? An overview of systematic reviews ..... ***- 151 -***

4.5 Effects of whole-body vibration training on bone turnover markers in adolescent swimmers ..... ***- 177 -***

4.6 Design of a computer model for the identification of adolescent swimmers with low BMD ..... ***- 195 -***

Chapter 5. Conclusions and main contributions ..... ***- 213 -***

Conclusions ..... ***- 217 -***

Main contributions of the Thesis ..... ***- 221 -***

References ..... ***- 223 -***

Acknowledgements [Spanish] ..... ***- 266 -***

Supplementary material ..... ***- 267 -***

Annexes ..... ***- 277 -***

Listado de publicaciones [List of publications]

A pesar de que para la confección de la presente Tesis Doctoral se ha optado por seguir la modalidad tradicional, parte del contenido desarrollado también se encuentra disponible como trabajos científicos previamente publicados, aceptados para publicación o sometidos a revisión, cuyas referencias se presentan a continuación:

I. Gomez-Bruton A, **Marin-Puyalto J**, Muniz-Pardos B, Lozano-Berges G, Cadenas-Sanchez C, Matute-Llorente A, Gomez-Cabello A, Moreno LA, Gonzalez-Aguero A, Casajus JA, Vicente-Rodriguez G. Associations between physical fitness and bone strength in 3 to 6-year-old children. **Sports Health**. Sometido *[Submitted]*.

II. **Marin-Puyalto J**, Mäestu J, Gomez-Cabello A, Lätt E, Remmel L, Purge P, Vicente-Rodríguez G, Jürimäe J. Frequency and duration of vigorous physical activity bouts are associated with adolescent boys' bone mineral status. **Bone**. 2019 Mar; 120: 141-147. https://doi.org/10.1016/j.bone.2018.10.019

III. **Marin-Puyalto J**, Mäestu J, Gomez-Cabello A, Lätt E, Remmel L, Purge P, Casajús JA, Vicente-Rodríguez G, Jürimäe J. Vigorous physical activity patterns affect bone growth during early puberty in boys. **Osteoporos Int**. 2018 Dec; 29(12): 2693-2701. https://doi.org/10.1007/s00198-018-4731-2

IV. **Marin-Puyalto J,** Gomez-Cabello A, Gonzalez-Agüero A, Gomez-Bruton A, Matute-Llorente A, Casajús JA, Vicente-Rodríguez G. Is vibration training good for your bones? An overview of systematic reviews. **Biomed Res Int**. 2018 Nov; 4. https://doi.org/10.1155/2018/5178284

V. **Marin-Puyalto J,** Gomez-Cabello A, Gonzalez-Agüero A, Matute-Llorente A, Gomez-Bruton A, Jürimäe J, Casajus JA, Vicente-Rodriguez G. Effects of whole-body vibration training on bone turnover markers. **J Pediatr Endocrinol Metab**. Sometido *[Submitted]*.

VI. **Marin-Puyalto J**, Gomez-Cabello A, Gomez-Bruton A, Matute-Llorente A, Gonzalez-Agüero A, Casajus JA, Vicente-Rodriguez G. Design of a computer model for the identification of adolescent swimmers with low BMD. **Bone**. Sometido *[Submitted]*.

Características de las revistas [Journal characteristics]

Factor de impacto y ránking 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.]*

Artículos publicados o aceptados [Published or accepted manuscripts]

Artículo *[Manuscript]*	Revista *[Journal]*	Factor de impacto *[Impact factor]*
II	Bone JCR 2018 (Endocrinology & Metabolism): 32/145 – Q1	**4,360**
III	Osteoporosis International JCR 2018 (Endocrinology & Metabolism): 43/145 – Q2	**3,819**
IV	Biomed Research International JCR 2018 (Medicine, Research & Experimental): 83/136 – Q3 JCR 2018 (Biotechnology & Applied Microbiology): 94/162 – Q3	**2,197**

Artículos sometidos [Submitted manuscripts]

Artículo *[Manuscript]*	Revista *[Journal]*	Factor de impacto *[Impact factor]*
I	Sports Health-A Multidisciplinary Approach JCR 2018 (Sport Sciences): 24/83 – Q2	**2,649**
V	Journal of Pediatric Endocrinology and Metabolism JCR 2018 (Pediatrics): 86/124 – Q3 JCR 2018 (Endocrinology & Metabolism): 132/145 – Q4	**1,239**
VI	Bone JCR 2018 (Endocrinology & Metabolism): 32/145 – Q1	**4,360**

Contribución del doctorando

En el artículo I, el doctorando participó en la toma de datos y analizó las imágenes radiográficas. Además, colaboró en el análisis estadístico y la redacción del artículo.

En los artículos II y III, recopiló y analizó los datos de acelerometría, creó la base de datos con la que realizó los análisis estadísticos y escribió los artículos.

En el artículo IV que se trata de una revisión de revisiones sistemáticas, el doctorando realizó la búsqueda sistemática de los artículos en las bases de datos, evaluó y seleccionó los artículos que cumplían los criterios de inclusión, y escribió el documento.

En los artículos V y VI, el doctorando colaboró en las tomas de datos y en las distintas evaluaciones del proyecto, creó la base de datos con la que realizó los análisis estadísticos y escribió los artículos.

Contribution of the PhD candidate

In manuscript I, the student took part in the data acquisition and analyzed the radiographic images. He also collaborated in the statistical analysis and manuscript writing.

In manuscripts II and III, the PhD candidate analyzed the accelometry data, created the database with which he performed the statistical analyses and redacted the documents.

In manuscript number IV, which is an overview of systematic reviews, the PhD candidate performed the literature search in different electronic databases, evaluated and selected the articles that met the inclusion criteria, and wrote the document.

In manuscripts V and VI, the PhD student was involved in the data aqcuisition and participant evaluation, created the database, performed the statistical analyses and wrote the manuscripts.

Proyectos de investigación

El trabajo desarrollado para dar lugar a los artículos y, por lo tanto, a la presente Tesis Doctoral se ha llevado dentro del marco de tres proyectos de investigación diferentes.

El artículo I se engloba dentro del proyecto "Assessing FITness in PREschoolers (PREFIT)", un proyecto multicéntrico en el que se evaluó la condición física de preescolares en 10 ciudades españolas. Dentro de la cohorte de Zaragoza, se llevó a cabo un proyecto adicional, titulado "Evaluación de la condición física en preescolares y su utilidad como marcador de riesgo de enfermedades actuales y futuras: obesidad y osteoporosis". Este proyecto, liderado por el Doctor ***Germán Vicente Rodríguez***, fue financiado por la Universidad de Zaragoza (referencia JIUZ-2014-BIO-08).

Durante sus periodos de estancia de investigación en el extranjero, el doctorando tuvo la ocasión de colaborar en el proyecto titulado "*Physical performance and health: adaptational and age-related aspects.*", elaborando los artículos II y III de la presente Tesis Doctoral. Este proyecto fue financiado por el *Ministerio de Educación y Ciencia* del Gobierno de Estonia (referencia IUT 20-58) y su investigador principal fue el Doctor ***Jaak Jürimae***.

Finalmente, el proyecto que incluye los artículos IV, V y VI del presente documento se denomina "***Re****percusión del entrenamiento y la práctica de la* ***na****tación sobre el desarrollo metabólico y estructural del hueso en cre****cimiento****. Beneficios de la incorporación de entrenamiento pliométrico o vibratorio."*, formando el acrónimo RENACIMIENTO. Se trata de un proyecto nacional de 3 años de duración, el cual fue financiado por el *Ministerio de Ciencia e Innovación* (referencia DEP2011-29093). Su investigador principal fue asimismo el Doctor ***Germán Vicente Rodríguez.***

Research projects

The studies leading to the elaboration of the manuscripts and the present Thesis have been carried out within the framework of three different research projects.

Article I is part of the project "Assessing FITness in PREschoolers (PREFIT)", a multi-centric project that evaluated the physical fitness of preschoolers in 10 different Spanish cities. Within the Zaragoza cohort, an additional project was carried out: "Evaluación de la condición física en preescolares y su utilidad como marcador de riesgo de enfermedades actuales y futuras: obesidad y osteoporosis". This project was also led by ***Germán Vicente Rodríguez*** and was supported by the Universidad de Zaragoza (reference JIUZ-2014-BIO-08).

During the course of his international research stays, the student had the opportunity to collaborate in the project entitled "*Physical performance and health: adaptational and age-related aspects.*" elaborating manuscripts II and III of the present Thesis. The project was funded by the *Estonian Minstry of Education and Science* (reference IUT 20-58) and its principal investigator was ***Jaak Jürimäe***.

Lastly, the project including manuscripts IV, V and VI of this document is named "***Re****percusión del entrenamiento y la práctica de la* ***na****tación sobre el desarrollo metabólico y estructural del hueso en cre****cimiento****. Beneficios de la incorporación de entrenamiento pliométrico o vibratorio."*, forming the acronym RENACIMIENTO. This three-year national project was funded by the *Ministerio de Ciencia e Innovación* (reference DEP2011-29093). The principal investigator principal was ***Germán Vicente Rodríguez.***

Becas

Jorge Marín Puyalto recibió una beca destinada a la Formación de Profesorado Universitario del *Ministerio de Educación, Cultura y Deporte del Gobierno de España* (FPU014/04302).

Grants

Jorge Marín Puyalto received a grant from the *Ministerio de Educación, Cultura y Deporte del Gobierno de España* (FPU14/04302).

Listado de abreviaturas [Abbreviation list]

ANCOVA	Análisis de covarianza *[Analysis of covariance]*
ANOVA	Análisis de varianza *[Analysis of variance]*
AUC	Área bajo la curva *[Area under the curve]*
BMAD	Densidad mineral ósea aparente *[Bone mineral apparent density]*
BMC	Contenido mineral óseo *[Bone mineral content]*
BMD	Densidad mineral ósea *[Bone mineral density]*
BMI	Índice de masa corporal *[Body mass index]*
BUA	Atenuación del ultrasonido de banda ancha *[Broadband ultrasound attenuation]*
CON	Grupo control *[Control group]*
CRF	Condición cardiorrespiratoria *[Cardiorespiratory fitness]*
CTX	Telopéptido carboxi-terminal del colágeno tipo I *[Collagen type I carboxi-terminal telopeptide]*
DXA	Absorciometría de rayos X de energía dual *[Dual-energy X-ray absorptiometry]*
FN	Cuello femoral *[Femoral neck]*
Frac_X	Resistencia a la fractura en el eje X *[Resistance to fracture in the X axis]*
LS	Columna lumbar *[Lumbar spine]*

OC	Osteocalcina *[Osteocalcin]*
P1NP	Propéptido amino-terminal del procolágeno tipo I *[Procollagen type I amino-terminal propeptide]*
PA	Actividad física *[Physical activity]*
pQCT	Tomografía axial computerizada periférica *[Peripheral quantitative computed tomography]*
Rel_handgrip	Fuerza de prensión manual relativa *[Relative handgrip strength]*
ROC	Características receptor-operador *[Receiver-operator characteristics]*
SLJ	Salto de longitud a pies juntos *[Standing long jump]*
SOS	Velocidad del sonido *[Speed of sound]*
SPSS	Paquete estadístico para las Ciencias Sociales *[Statistical Package for the Social Sciences]*
SSIPOL	Índice de tensión-deformación polar *[Polar stress-strain index]*
SWI	Grupo de nadadores *[Swimming group]*
vBMD	Densidad mineral ósea volumétrica *[Volumetric bone mineral density]*
VIB	Grupo de entrenamiento vibratorio *[Vibration group]*
VPA	Actividad física vigorosa *[Vigorous physical activity]*
WB	Cuerpo completo *[Whole body]*
WBV	Vibración de cuerpo completo *[Whole-body vibration]*

Listado de tablas y figuras

Tabla 1. Parámetros del protocolo de vibración

Figura 1. Estructura y propiedades mecánicas del tejido óseo compacto y esponjoso

Figura 2. Sección transversal de un hueso largo

Figura 3. Etapas del proceso de remodelado óseo

Figura 4. Primeras etapas del desarrollo óseo

Figura 5. Pico de ganancia de masa ósea en chicos y chicas europeos

Figura 6. Evolución de la masa ósea durante el ciclo vital en hombres y mujeres

Figura 7. Evolución de la estructura ósea durante el ciclo vital en hombres y mujeres

Figura 8. Valores normativos de osteocalcina en chicos (círculos sombreados) y chicas (círculos claros) de entre 0 y 18 años

Figura 9. Umbrales de estimulación ósea propuestos por Frost

Figura 10. Modalidades de transmisión de la vibración en plataformas vibratorias

Figura 11. Ejercicios realizados durante la sesión de entrenamiento vibratorio

List of tables and figures

Table 1. Vibration protocol parameters

Figure 1. Structure and mechanical properties of compact and spongy bone tissue

Figure 2. Transversal section of a long bone

Figure 3. Bone remodelling stages

Figure 4. First stages of bone development

Figure 5. Peak of bone mass attainment in European boys and girls

Figure 6. Bone mass evolution across the life cycle in men and women

Figure 7. Bone structure evolution across the life cycle in men and women

Figure 8. Normative values of osteocalcin in boys (filled circles) and girls (white circles) between 0 and 18 years.

Figure 9. Bone stimulation thresholds proposed by Frost

Figure 10. Modes of vibration transmission in vibrating platforms

Figure 11. Exercises performed during whole-body vibration training

Resumen general

La prevención temprana de la osteoporosis es uno de los grandes retos a los que se enfrentan los sistemas sanitarios de los países desarrollados. Una de las maneras de lograr este objetivo consiste en alcanzar un pico de masa ósea lo más elevado posible en el inicio de la edad adulta. Para ello, un correcto desarrollo óseo durante la pubertad resulta decisivo, puesto que es en este periodo cuando se forma la mayor parte de la masa ósea que tendrá la persona.

La práctica de actividad física de impacto es uno de los factores controlables que más influencia tienen sobre la obtención de masa ósea durante la adolescencia, por lo que es vital comprender el mecanismo mediante el que se relacionan. Asimismo, siguiendo el enfoque de la prevención temprana de la osteoporosis, se debería proponer un ejercicio complementario a aquellos adolescentes que practiquen deportes que no generen impacto como es la natación.

Por lo tanto, el principal objetivo de la presente Tesis Doctoral es analizar el papel de la actividad física en la mineralización ósea durante las primeras etapas de la vida, poniendo particular atención en los adolescentes practicantes de natación y en el entrenamiento vibratorio de cuerpo completo.

Esta Tesis Doctoral incluye estudios pertenecientes a tres proyectos diferentes. En el proyecto PREFIT se analizó la relación existente entre la condición física y parámetros estructurales del hueso en 139 niños y niñas de preescolar. El segundo proyecto se centró en estudiar detalladamente y de manera objetiva los distintos patrones de práctica de actividad física en un grupo de 180 adolescentes sanos en etapas tempranas de la pubertad y cómo estos patrones afectan su salud ósea. Por último, en el proyecto RENACIMIENTO se evaluaron los efectos de 6 meses de entrenamiento vibratorio de cuerpo completo en los

marcadores de metabolismo óseo mediante un ensayo aleatorizado en el que participaron casi una centena de nadadores adolescentes.

Los principales resultados son los siguientes:

1) La condición física ya determina la estructura ósea en una edad tan temprana como la preescolar.
2) La actividad física realizada durante el inicio de la pubertad debe ser de carácter vigoroso y con una duración de al menos cinco minutos consecutivos para optimizar su potencial osteogénico.
3) El protocolo de vibración utilizado no consiguió incrementar el metabolismo óseo en nadadores adolescentes.
4) Se ha propuesto una metodología para el cribado e identificación de nadadores susceptibles de presentar baja densidad mineral ósea.

General abstract

Early prevention of osteoporosis is one of the main challenges that healthcare systems of developed countries face. One of the pathways to reach this goal relies on obtaining the highest peak bone mass during early adulthood. A correct bone development during puberty is critical for that purpose, given that most of the adult bone is formed during this period.

Weight-bearing physical activity is one of the main controllable factors that affect bone accretion during adolescence and therefore it is crucial to comprehend its mechanism. Also, considering the approach of the early prevention of osteoporosis, additional training alternatives should be offered to those adolescents engaged in non-weight-bearing sports such as swimming.

Therefore, the main aim of the present Doctoral Thesis is to analyze the role of physical activity and fitness on bone mineralization during the first stages of life, with a particular focus on adolescent swimmers and whole-body vibration training.

This Doctoral Thesis features studies from three different research projects. On the PREFIT project, the relationship between the physical fitness and bone structural parameters of 139 preschool children was analyzed. A second project was aimed at the objective assessment of physical activity participation patterns and their relationship with bone health in 180 healthy early pubertal adolescents. Finally, within the RENACIMIENTO project, the effects of a 6-month vibration training protocol on bone turnover markers were tested in a randomized controlled trial with almost one hundred adolescent swimmers.

The main results found are the following:

1) Physical fitness already affects bone structure at the prescholar stage
2) Vigorous physical activity should be performed in bouts of at least 5 minutes during early puberty to elicit an optimized osteogenic response.
3) Regarding the adolescent swimmers, the vibration protocol did not increase the levels of bone turnover markers.
4) A tool for the screening of swimmers at risk of having low bone mineral density was devised.

Capítulo 1

Introducción y justificación

La introducción se basará en distintos aspectos relativos a la masa ósea, su medición, evolución a lo largo de la vida y respuesta a distintos estímulos, dado que el objetivo central de este trabajo gravita en torno a estos aspectos. Asimismo, el análisis de la salud ósea es el nexo común de los proyectos y manuscritos que se incluyen en la presente Tesis Doctoral.

1.1 Estructura y función del tejido óseo

El tejido óseo es un sistema complejo y en constante evolución. El hueso está formado en un 75% por contenido mineral (principalmente en forma de pequeños cristales de fosfato de calcio impuro) que proporciona rigidez, mientras que el resto es contenido orgánico, siendo su principal componente el colágeno pero contando también con otras proteínas, lípidos, agua y células (1). Estos tejidos se disponen de manera que presentan una resistencia óptima frente a las cargas impuestas por la actividad habitual, como fue descrito por Wolff ya en el siglo XIX (2).

Existen dos tipos de tejido óseo claramente definidos histológicamente y radiológicamente: el tejido denso o compacto y el tejido esponjoso o trabecular (3). Estos tipos de hueso se diferencian en su disposición estructural, con el tejido compacto dispuesto en lamelas concéntricas formando unidades denominadas osteonas, mientras que el tejido esponjoso se organiza en una red de finas espículas interconectadas.

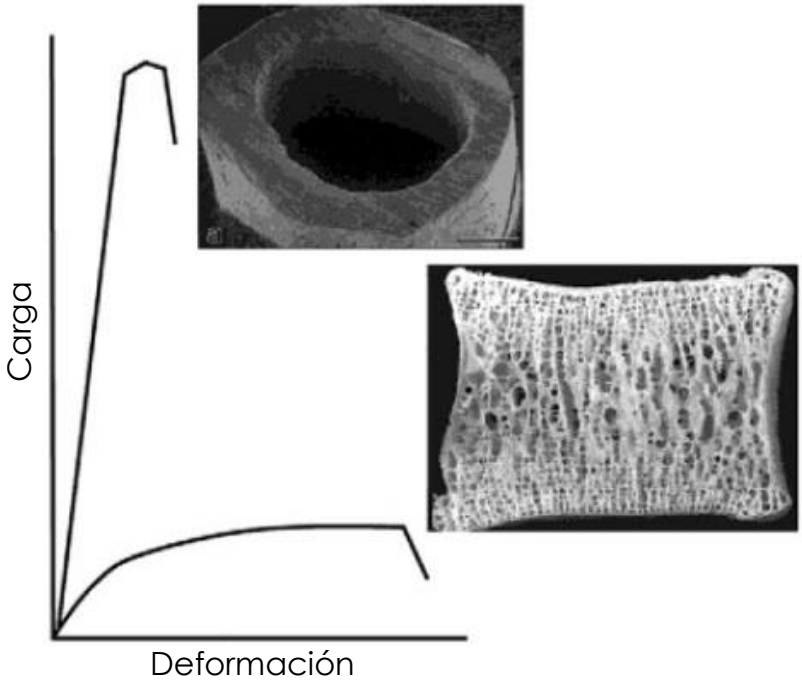


Figura 1. Estructura y propiedades mecánicas del tejido óseo compacto y esponjoso
Adaptada del libro "Dynamics of Bone and Cartilage Metabolism" (4)

Esta diferente disposición les confiere distintas propiedades mecánicas como se puede observar en la figura 1. El hueso compacto es capaz de soportar altas cargas sin romperse; sin embargo, el hueso trabecular es más flexible pudiendo someterse a mayores deformaciones. Cada hueso tiene una proporción y disposición de estos tejidos específica de manera especializada para la función que vaya a desempeñar. Estructuralmente se pueden clasificar en huesos largos como el fémur, cortos como el calcáneo, planos como la escápula e irregulares como las vértebras.

La parte central de un hueso largo se denomina diáfisis y consiste en un cilindro de hueso compacto en cuyo interior se sitúa la médula ósea. Los dos extremos de los huesos largos se denominan epífisis y tienen un mayor contenido de hueso trabecular. La superficie exterior del hueso está protegida por una membrana fibrosa llamada periostio, mientras que la membrana que cubre la cavidad medular es el endostio. En la figura 2 se pueden apreciar estas estructuras.

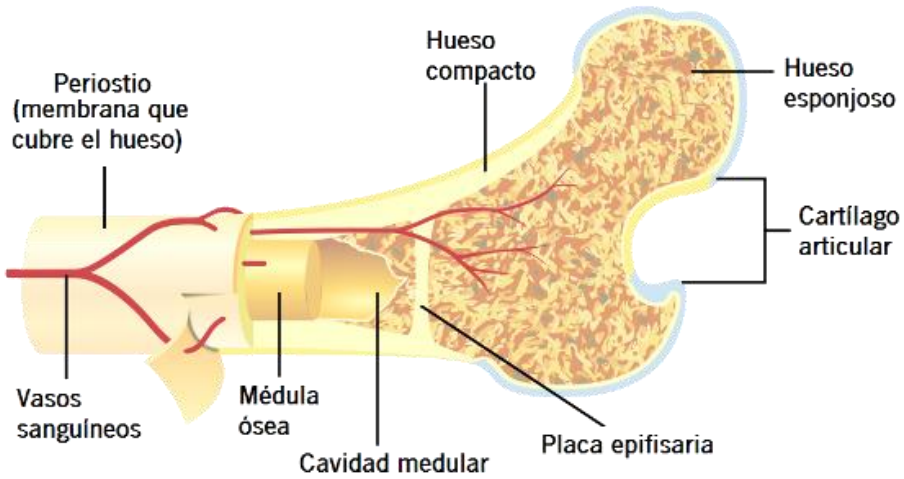


Figura 2. Sección transversal de un hueso largo

Traducida de la imagen orignal de Pbroks, con licencia CC BY 3.0, disponible en: https://commons.wikimedia.org/wiki/File:Bone_cross-section.svg

Las principales funciones del tejido óseo son las siguientes (5):

- Sostener y dar forma al cuerpo.
- Proteger los tejidos subyacentes como los órganos vitales y el sistema nervioso central.
- Servir como palanca mecánica en la producción de movimiento y de fijación para los músculos.
- Contribuir a la formación de las células sanguíneas desde la médula ósea roja.
- Actuar como depósito de sales minerales, siendo el mayor reservorio de calcio y fósforo.

Para desempeñar de una manera eficiente estas funciones, el hueso tiene que cumplir distintas características, siendo ligero para facilitar el movimiento, rígido para resistir las cargas y, al mismo tiempo, flexible para deformarse sin agrietarse (6). Como hemos visto, esta resistencia del hueso a las cargas externas no está determinada exclusivamente por su cantidad de contenido mineral, sino que la estructura es responsable de entre un 20% y un 40% de la misma (7).

1.2 Evolución de los parámetros óseos a lo largo de la vida

1.2.1 Remodelado óseo

Como ya se ha mencionado, el tejido óseo no se encuentra en un estado estático, sino que está sometido constantemente a variaciones en su tamaño, forma y composición mediante los procesos de modelado (formación de nuevo tejido) y remodelado (sustitución del tejido existente).

Un ciclo de remodelado consta de cuatro fases, como se representa en la figura 3: resorción, inversión, formación y mantenimiento. Hay tres tipos de moléculas involucradas en este proceso: los osteoclastos, encargados de la destrucción del tejido óseo, los osteoblastos que proceden a la remineralización y los osteocitos, que se encuentran atrapados en la matriz ósea y cumplen una función mecanorreceptora (8).

Durante un ciclo estándar, la fase de resorción ocupa entre 7 y 10 días creando una laguna en la que se instalarán los osteoblastos para proceder a la fase de formación, que necesita dos o tres meses para completarse (9). La velocidad de estos procesos puede verse modificada por la carga mecánica o por causas hormonales (10).

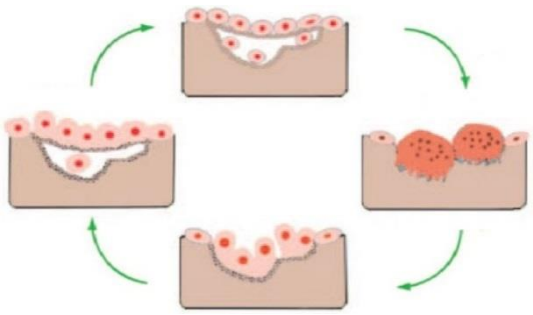


Figura 3. Etapas del proceso de remodelado óseo
Adaptada del artículo "Biochemical markers of bone turnover – uses and limitations" (11)

En el transcurso de estos procesos se generan residuos, generalmente derivados de las fibras de colágeno fusionadas o seccionadas. La medición de los niveles en sangre u orina de estos componentes, denominados biomarcadores del metabolismo óseo, puede ayudar a estimar la tasa metabólica de formación y resorción. Sin embargo, hay que tomar una serie de precauciones ante su interpretación ya que estos marcadores pueden verse afectados por la dieta, el ritmo circadiano o la estación del año. Asimismo, hay alguno de estos marcadores que no es exclusivo del metabolismo óseo o que sufre procesos de degradación dentro del organismo.

La Fundación Internacional de Osteoporosis ha publicado unas pautas en colaboración con la Federación Internacional de Química Clínica para el correcto muestreo, almacenamiento, análisis e interpretación de los marcadores del metabolismo óseo (12) en la que se identifican los marcadores de formación y resorción más fiables y se detallan las fuentes de variabilidad a tener en cuenta con cada uno de los metabolitos. Estas entidades recomiendan el uso de la osteocalcina (OC) y del propéptido amino-terminal del procolágeno tipo I (P1NP) como biomarcadores de formación ósea y el telopéptido carboxi-terminal del colágeno tipo I (CTX) como marcador de resorción.

1.2.2 Evolución del contenido mineral óseo

La cantidad de tejido óseo varía constantemente a lo largo de la vida de las personas. El sistema esquelético empieza a formarse durante el desarrollo embrionario donde comienza como moldes de tejido hialino que se irá mineralizando comenzando por la diáfisis (13). A lo largo de la infancia se va acumulando nuevo tejido óseo de una manera similar en ambos sexos (figura 4).

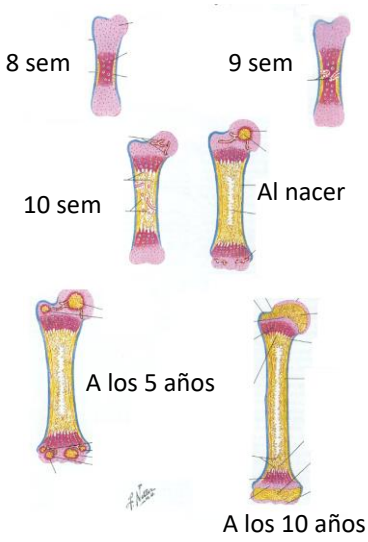


Figura 4. Primeras etapas del desarrollo óseo.
Extraída del libro "Atlas ilustrado de anatomía" (14)

Posteriormente, en la adolescencia se llega a un periodo crítico, el pico de ganancia de masa ósea. Se trata de un repunte en la acumulación de contenido mineral óseo, que se produce antes en las chicas (en torno a los 12,5 años) que en los chicos (alrededor de los 14 años de edad), si bien la ganancia que experimentan los varones es de mayor magnitud (15). La figura 5 representa de manera gráfica este proceso.

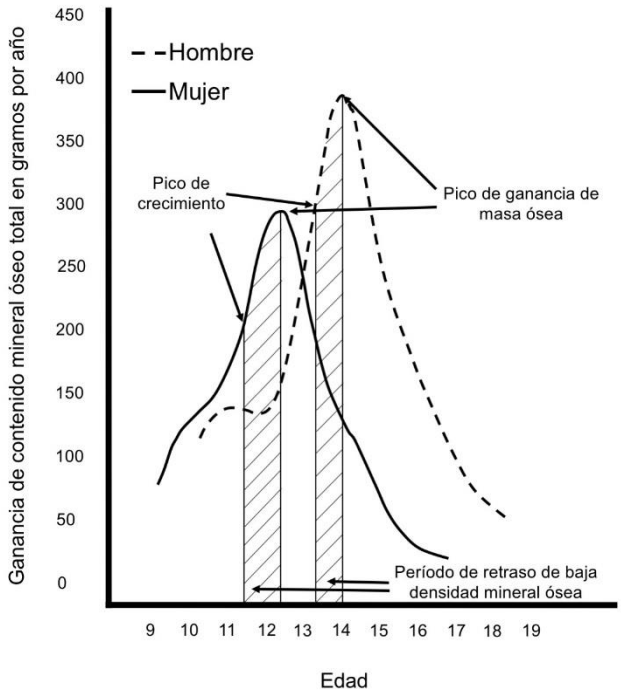


Figura 5. Pico de ganancia de masa ósea en chicos y chicas europeos.
Adaptada del artículo "The National Osteoporosis Foundation's position statement on peak bone mass development and lifestyle factors: a systematic review and implementation recommendations" (16) Licencia CC BY-NC 4.0

Al terminar la pubertad se ha formado ya la mayor parte del hueso adulto; a partir de este momento la formación de nuevo tejido óseo continúa, pero de manera mucho más reducida, hasta alcanzar otro punto crítico del desarrollo: el pico de masa ósea. Este se entiende como el mayor contenido mineral óseo que tendrá una persona a lo largo de su vida, el cual suele darse en torno a la tercera década de la vida (17). En este punto también podemos apreciar dimorfismo sexual, ya que los hombres alcanzan un pico de masa ósea superior a las mujeres. La obtención de un pico de masa ósea lo más elevado posible es uno de los factores que más pueden proteger frente a la aparición de osteoporosis en la etapa anciana, ya que se ha estimado que una ganancia igual a una desviación estándar en el pico de masa ósea supone una reducción del riesgo de fractura a la mitad (18).

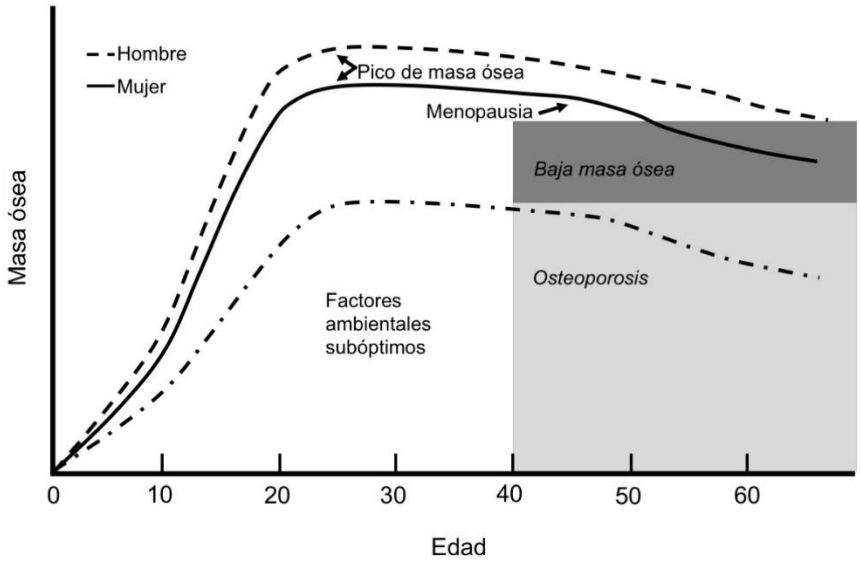


Figura 6. Evolución de la masa ósea durante el ciclo vital en hombres y mujeres.
Adaptada del artículo "The National Osteoporosis Foundation's position statement on peak bone mass development and lifestyle factors: a systematic review and implementation recommendations" (16) Licencia CC BY-NC 4.0

Una vez alcanzado el pico de masa ósea, la ganancia de contenido mineral óseo se detiene y se mantiene un equilibrio dinámico protagonizado por la formación y resorción ósea del proceso de remodelado (19). Con el paso de los años el equilibrio entre estos procesos se deteriora, llegándose a producir una pérdida paulatina de masa ósea. En este periodo de nuevo aparecen diferencias entre los sexos, ya que en el momento de la llegada

de la menopausia las mujeres sufren una pérdida más acentuada de su masa ósea (20). Esta pérdida prolongada de contenido mineral óseo puede dar lugar a la aparición de osteoporosis en las últimas etapas de la vida, especialmente en aquellas personas que no alcanzaron un pico de masa ósea elevado. La figura 6 esquematiza la evolución del contenido mineral óseo a lo largo de la vida.

1.2.3 Evolución de la estructura ósea

Los cambios en el tejido esquelético experimentados a lo largo de la vida no se limitan a la mera ganancia o pérdida de material óseo, sino que el proceso de formación y resorción ósea va moldeando la estructura del hueso con el paso del tiempo, modificando así sus propiedades mecánicas.

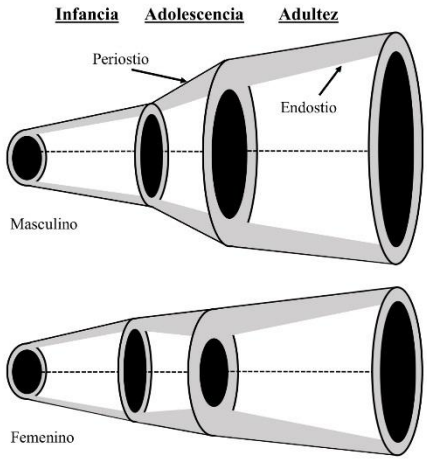


Figura 7. Evolución de la estructura ósea durante el ciclo vital en hombres y mujeres.
Adaptada del artículo "Bone quality: the material and structural basis of bone strength" (21)

El crecimiento óseo durante la etapa infantil y adolescente descrito en el apartado anterior no sólo se produce en el eje longitudinal, a través de las placas epifisiarias, sino que también se produce un aumento en el grosor cortical, puesto que la formación ósea en el periostio excede a la resorción experimentada en el endostio. Durante la etapa adulta, estos procesos se equilibran, por lo que se obtiene un mayor diámetro óseo manteniéndose

el grosor cortical (4). En mujeres el proceso de modelado en el periostio es de menor magnitud que en los hombres, resultando en huesos más pequeños. No obstante, el grosor cortical es similar, ya que también se inhibe el proceso de resorción en el endostio (22). En la figura 7 se muestra una representación esquemática de la evolución en el grosor cortical y el diámetro óseo en hombres y mujeres a lo largo de la vida.

Con respecto al hueso trabecular, durante la etapa de crecimiento no se generan nuevas trabéculas, sino que aumenta la densidad mineral de cada una de ellas (23). Durante el envejecimiento, la pérdida neta de densidad mineral en las trabéculas es similar en hombres y mujeres, sin embargo, el proceso es cualitativamente diferente. En el caso de los hombres se produce una disminución de la densidad de las trabéculas, resultando en un estrechamiento de estas, mientras que en el caso de las mujeres desaparecen trabéculas completas, comprometiendo así la integridad estructural de todo el entramado (24).

1.2.4 Evolución de los marcadores óseos

En el apartado anterior se han explicado los factores que pueden causar variaciones intraindividuales en los niveles de los marcadores de metabolismo óseo en el corto y medio plazo. En este apartado, por el contrario, se describirán las variaciones en estos parámetros a lo largo de los primeros años de vida, puesto que son las poblaciones de interés de los estudios incluidos en la presente Tesis Doctoral.

Los niveles de marcadores bioquímicos de remodelado óseo son muy altos en el momento del nacimiento y van disminuyendo hasta los 3 años de edad aproximadamente, correspondiendo con la ralentización del crecimiento (25). A partir de esta etapa los valores de marcadores se mantienen estables, en niveles superiores a los encontrados en adultos pero inferiores a los encontrados en neonatos o adolescentes. Además, no se observan diferencias entre chicos y chicas en estos parámetros. Con la llegada de la pubertad y los cambios hormonales asociados, aumentan los niveles de varios marcadores, coincidiendo

con el periodo de máximo crecimiento lineal de los adolescentes (26). Este aumento en los niveles de marcadores durante la adolescencia se da más tarde en los chicos que en las chicas y es asimismo de una duración y magnitud mayores (27). Hay que tener en cuenta también que los valores de alguno de los marcadores se ven afectados por los niveles de hormonas sexuales y que el proceso de acumulación mineral ósea durante la pubertad es un mecanismo complejo en el que están involucrados la práctica de actividad física, la composición corporal, y distintos factores hormonales (28).

En la figura 8 se presentan los valores normativos de osteocalcina y CTX en niños y adolescentes europeos, publicados por Rauchenzauer y colaboradores (25).

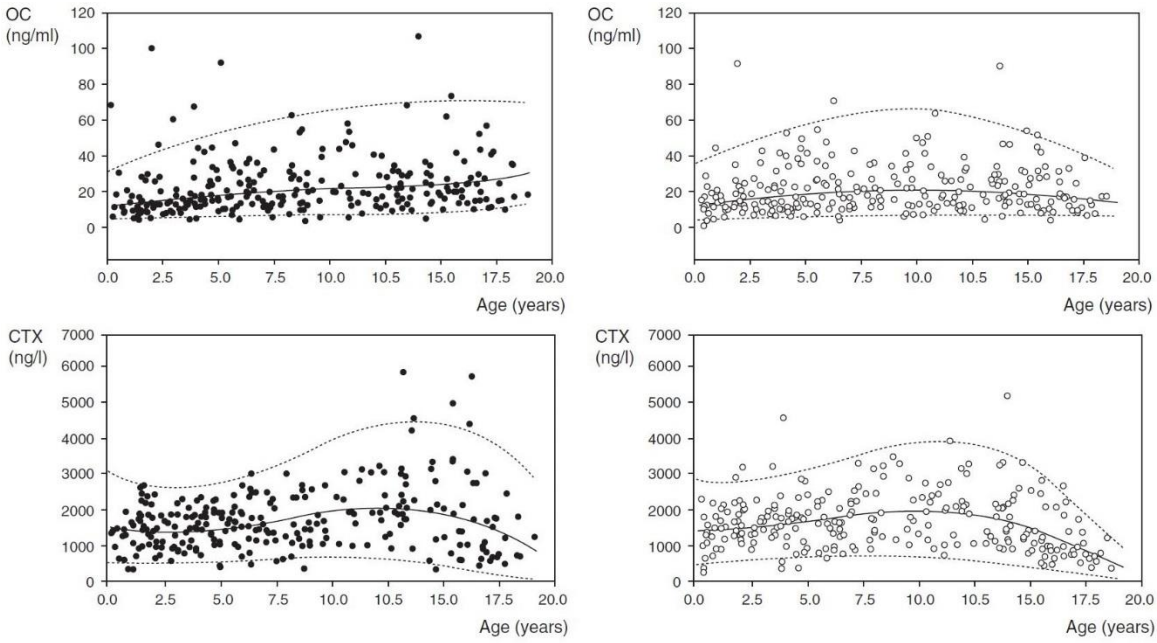


Figura 8. Valores normativos de osteocalcina en chicos (círculos sombreados) y chicas (círculos claros) de entre 0 y 18 años.

Extraída del artículo "Sex- and Age-Specific Reference Curves for Serum Markers of Bone Turnover in Healthy Children from 2 Months to 18 Years" (25) Licencia: 4665800866439

1.3 Osteoporosis

Las personas que han experimentado deterioro grave del tejido óseo, ya sea por una gran pérdida de densidad mineral, por fallos estructurales o por un desequilibrio en la actividad metabólica, presentan una elevada susceptibilidad a sufrir fracturas, sufriendo así de la enfermedad denominada osteoporosis.

La osteoporosis es una enfermedad metabólica ósea que cursa con una reducción de la resistencia ósea, lo que conlleva un aumento del riesgo de fractura ósea, de manera que eventos de impacto de baja intensidad que normalmente no comportarían ningún problema médico para el paciente osteoporótico pueden suponer una fractura (29). Asimismo, en casos más graves también se pueden dar fracturas atraumáticas, es decir, colapsos de la estructura ósea sin necesidad de eventos externos.

Esta enfermedad cuenta con una alta prevalencia entre las personas mayores, afectando a un 6,6% de los hombres y un 22,1% de las mujeres mayores de 50 años dentro de la Unión Europea (30). En nuestro país, según la Encuesta Europea de Salud 2014, el 10,4% de las personas entre 65 y 74 años han recibido un diagnóstico de osteoporosis, mientras que este porcentaje aumenta al 14,4% en el grupo de 75 a 84 años y al 16,6% entre los mayores de 84 años (31). Podemos comprobar que la prevalencia es mayor en los grupos de edad más avanzados, de hecho se espera que el número total de personas afectadas por esta enfermedad crezca en los próximos años, dados los cambios demográficos de nuestra sociedad (32).

Además, la osteoporosis se ha descrito como una enfermedad "silenciosa" ya que no causa dolor ni presenta otros síntomas de manera que, a menos que se realicen pruebas diagnósticas, los afectados no son conscientes de la patología hasta que se produce la fractura ósea (33).

El elevado número de fracturas osteoporóticas que tienen lugar cada año plantean un grave problema para los sistemas sanitarios de los países desarrollados (34), ya que han sido relacionadas con disminuciones de la capacidad funcional y de la calidad de vida, pérdida de independencia, aparición de discapacidad e incluso con un aumento de la mortalidad (35). Al mismo tiempo, a estos costes sociales también hay que añadirles los costes económicos. Concretamente, en el año 2008 el tratamiento de fracturas de cadera supuso un gasto de casi 400 millones de euros al sistema sanitario español público, más del doble del que se registró una década atrás (36).

Por lo tanto, uno de los grandes retos a los que se enfrentan los sistemas sanitarios es el de la prevención temprana de la osteoporosis. Para ello es de vital importancia la estimulación osteogénica durante el periodo de pico de obtención de masa ósea definido anteriormente. Una de las estrategias más recomendadas para conseguir un desarrollo óseo adecuado durante esta etapa es la participación en actividad física de impacto.

1.4 Métodos de medición de la masa ósea

Existen diversas opciones para la evaluación de distintos parámetros relacionados con la salud ósea. A continuación se presentarán brevemente aquellos métodos de utilización más frecuente en los estudios de investigación relacionados con la actividad física y salud en niños y adolescentes.

1.4.1 Absorciometría de rayos X de energía dual (DXA)

El DXA es el método de referencia para la determinación de la densidad mineral ósea. Se trata de un método por imagen basado en la emisión de radiación ionizante, la cual será recogida por un receptor que registrará la proporción de la radiación absorbida tras atravesar el cuerpo del paciente. La radiación utilizada es de dos frecuencias diferentes, de manera que una de ellas es absorbida por el tejido óseo mientras que la otra es absorbida

por el tejido blando. De este modo, se puede realizar un análisis de distintos componentes corporales de manera simultánea, aportando información no sólo sobre el contenido y densidad óseos sino también sobre la masa grasa y magra.

Este método se utiliza para el diagnóstico de osteoporosis, a partir de una comparación de los resultados del paciente con los de una población de referencia de jóvenes adultos (en el pico de masa ósea) de su mismo sexo y etnia. Si el resultado está al menos 2.5 desviaciones estándar por debajo de la media de esta población de referencia, el participante se puede clasificar como osteoporótico (37)

Existen pautas para el correcto posicionamiento de los pacientes, realización de las mediciones e interpretación de las imágenes, establecidas por la Sociedad Internacional de Densitometría Clínica (38). El escaneo se puede realizar tanto de cuerpo completo como de regiones específicas, siendo las de interés más generalizado la columna lumbar, la cadera y el antebrazo.

Es importante recalcar que las imágenes obtenidas mediante este método sólo distinguen dos dimensiones, de manera que los valores de densidad ósea obtenidos son relativos al área del hueso, lo que puede sesgar la comparación entre huesos pequeños y grandes o la interpretación de los datos pertenecientes a pacientes de muy baja estatura. Por esto, en algunos casos es recomendable realizar un ajuste de los valores de densidad encontrados, calculando la densidad mineral volumétrica aparente (39).

Asimismo, los parámetros obtenidos sólo se tratan de variables de composición ósea, sin hacer referencia a su estructura, si bien existen complementos adicionales en el software que permiten obtener información estructural como la puntuación trabecular ósea (40) o el análisis estructural de cadera (41).

1.4.2 Tomografía axial computerizada periférica (pQCT)

El pQCT, un método de imagen que utiliza radiación ionizante al igual que el DXA, está diseñado para el análisis de secciones transversales de radio y tibia. Este método no es dependiente del área del hueso analizado como sucedía en el DXA, ya que a diferencia de este, proporciona valores de densidad mineral ósea volumétrica. Además, ofrece resultados de áreas transversales, contenido mineral y geometría ósea, distinguiendo entre hueso trabecular y cortical. A partir de los valores obtenidos, se puede calcular el comportamiento del hueso frente a fuerzas externas, pudiéndose así conocer su comportamiento mecánico.

Existe un modelo de pQCT más avanzado que ofrece una mayor definición en las imágenes obtenidas: el pQCT de alta resolución. Este permite obtener variables estructurales adicionales, pudiendo incluso llegar a establecer el número de trabéculas óseas y la separación entre ellas, algo que la resolución del pQCT estándar no permite.

1.4.3 Resonancia magnética

La resonancia prescinde del uso de radiación ionizante para obtener sus imágenes, ya que utiliza un campo magnético y ondas de radio. Este método proporciona valores de densidad volumétrica y realiza la distinción entre hueso cortical y trabecular al igual que el pQCT, si bien permite la evaluación de todo el cuerpo al mismo tiempo, al contrario que este.

1.4.4 Ultrasonidos

A través de la emisión de ultrasonidos aplicados de manera directa al hueso se puede medir la velocidad a la que el sonido atraviesa el hueso y la atenuación que sufre al hacerlo, lo que está relacionado con la elasticidad y densidad ósea. Este método también presenta

la ventaja de no utilizar radiación ionizante, además de ser más económico y de utilización más rápida que los métodos descritos hasta el momento.

Hay que destacar que todas las técnicas presentadas a lo largo de este apartado son costosas y requieren tanto personal especializado como material tecnológicamente avanzado. Por estas razones, según las recomendaciones de salud públicas (42), el uso rutinario de estas técnicas para la detección de patologías óseas es exclusivo en personas de edad avanzada, ya que es donde el sistema demuestra ser más costo-efectivo (43). Sin embargo, sería importante contar con herramientas sencillas para el cribado y detección precoz en poblaciones en riesgo de presentar una baja densidad mineral ósea durante edades tempranas.

1.5 Actividad física y masa ósea

Se estima que en torno al 60% de la variabilidad interindividual en la masa ósea es debido a factores endógenos como la genética o la raza, siendo el resto por lo tanto debido a factores controlables (44). En el año 2016, la Fundación Nacional de Osteoporosis publicaba una revisión sistemática que establecía su posicionamiento con respecto a distintas intervenciones dirigidas al aumento del pico de masa ósea (16). En esta revisión se realizaba una evaluación de la utilidad de cada una de estas intervenciones. La práctica de actividad física obtuvo los valores más elevados de entre los distintos tipos de intervenciones, recibiendo la mayor gradación posible con respecto a su utilidad para la mejora del contenido y la densidad mineral óseos y la segunda mejor calificación para la mejora de los parámetros estructurales.

1.5.1 Mecanismos de adaptación ósea

Como ya se ha mencionado anteriormente las primeras descripciones de la relación entre la forma del hueso y su función se remontan al siglo XIX, con la publicación de la conocida como ley de Wolff (2). Siguiendo esta teoría, el profesor Frost describió el "mecanostato óseo" (45,46), según el cual, las cargas realizadas sobre los huesos generan unas deformaciones en estos que pueden ser de naturaleza compresiva, tensional, torsional o combinada. Estas deformaciones estimularían la actividad de las células óseas de la región, produciéndose adaptaciones específicas.

La transformación de las cargas mecánicas en respuestas celulares se denomina "mecanotransducción" y es posible gracias a la labor mecanorreceptora de los osteocitos.

Existen una serie de parámetros que condicionan la respuesta efectiva a dichas deformaciones y son su magnitud, velocidad, frecuencia, novedad, ciclos y periodos de descanso (47). Frost ya contempló la relevancia de la magnitud de la deformación en su propuesta original del mecanostato, definiendo los umbrales que se presentan en la siguiente figura.

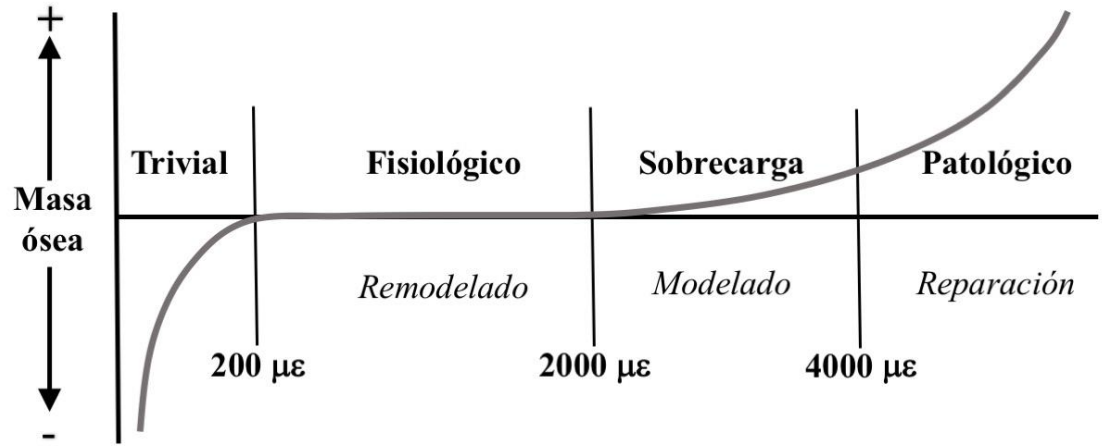


Figura 9. Umbrales de estimulación ósea propuestos por Frost (46)
Traducida del artículo "Skeletal adaptations to mechanical usage: results from tibial loading studies in rats" (48)
Licencia: 4665810036414

En investigaciones posteriores se ha comprobado que esta propuesta explica que la carrera muestre un efecto osteogénico (49) mientras que la marcha tan sólo presente utilidad para el mantenimiento de la masa ósea y aunque no para su mejora (50), ya que sólo las deformaciones encontradas para la carrera sobrepasan el umbral de los 2000 με.

1.5.2 Relación entre actividad física y hueso

Existen numerosas revisiones que han demostrado que la actividad física (51,52), el ejercicio (53,54) y el deporte (55,56) provocan un estímulo osteogénico en sus practicantes siempre y cuando se cumplan los requisitos mencionados anteriormente, lo cual se puede conseguir con cualquier actividad que incluya carreras, saltos, golpeos o cambios repentinos de dirección, de manera que se genere una carga sobre el tejido óseo, activando así el mecanostato.

Como hemos visto, optimizar el desarrollo óseo durante la pubertad es clave en la prevención de la osteoporosis, por lo que la práctica de actividad física durante etapa es fundamental para conseguir este objetivo, especialmente porque se ha visto que la actividad física es capaz de mejorar la densidad ósea incluso en sujetos con una predisposición genética a presentar una mineralización deficiente (57). Además, se ha comprobado que los beneficios a nivel óseo obtenidos a través de la práctica de actividad física durante la adolescencia se conservan en etapas posteriores de la vida (58,59).

Dentro de la práctica de actividad física en general, parece que la actividad vigorosa es la intensidad más efectiva para estimular la osteogénesis (60,61), si bien también se han hallado beneficios a nivel esquelético relacionados con la práctica de actividad ligera o moderada (62,63). Con respecto al volumen de práctica de actividad física necesario para generar un estímulo suficiente para la adaptación ósea, parece que el cumplimiento de las

recomendaciones generales para este grupo de edad no es suficiente (64), por lo que se han propuesto otras alternativas como la práctica semanal de 3 horas de deporte (65) o de 32 minutos de actividad física vigorosa (64). Sin embargo, hay que tener en cuenta que la frecuencia, duración y distribución de los periodos de práctica de actividad física intensa pueden modificar las adaptaciones específicas (66), por lo que es necesario estudiar en mayor profundidad estos parámetros para identificar el patrón óptimo de actividad vigorosa.

1.5.3 Natación y tejido óseo

Los efectos de la natación sobre el tejido óseo fueron estudiados en profundidad en una revisión sistemática, combinando resultados de 64 artículos individuales (67). De manera general se concluyó que los nadadores presentaban una densidad mineral ósea inferior a la de los practicantes de otros deportes y similar a la de controles inactivos, independientemente de la edad. Esto puede explicarse teniendo en cuenta que al tratarse de un deporte practicado en un medio hipogravitatorio, no proporciona los estímulos necesarios para la generación de una deformación dentro del umbral del remodelado óseo.

Por otra parte, en esta misma revisión se mostró que los nadadores presentaron niveles de marcadores de metabolismo óseo más elevados que los de los controles inactivos, sugiriendo que pese a que no se observen mejoras en el contenido y densidad mineral óseos quizá sí que se den a nivel estructural, dando como resultado un hueso más resistente.

En un estudio transversal publicado recientemente se comprobó que al final de la pubertad, los nadadores presentaban valores inferiores de densidad mineral ósea que los atletas y jugadores de hockey hielo en los puntos de soporte del peso corporal (cadera y

cuello femoral), pero no así en otras regiones (68). Tampoco se encontraron diferencias en variables estructurales medidas mediante tomografía computerizada periférica, lo cual concuerda con lo propuesto en la revisión. Asimismo, los autores de este estudio transversal hipotetizan que como la adaptación osteogénica se limita a la región que es sometida a tensión durante la práctica deportiva, quizá la natación sea beneficiosa también para la densidad mineral ósea, tan sólo que está limitado a regiones que no son escaneadas habitualmente (68). El análisis de los niveles de biomarcadores del metabolismo óseo podría dar respuesta a esta incógnita.

1.5.4 Entrenamiento con plataformas vibratorias

A principios del siglo XX, Clinton Rubin mostró que la aplicación de un estímulo mecánico vibratorio introducido de manera no invasiva era capaz de producir una respuesta osteogénica tanto en animales como en humanos (69,70). Desde ese momento la vibración de cuerpo completo se convirtió en un tema de principal interés entre la comunidad científica.

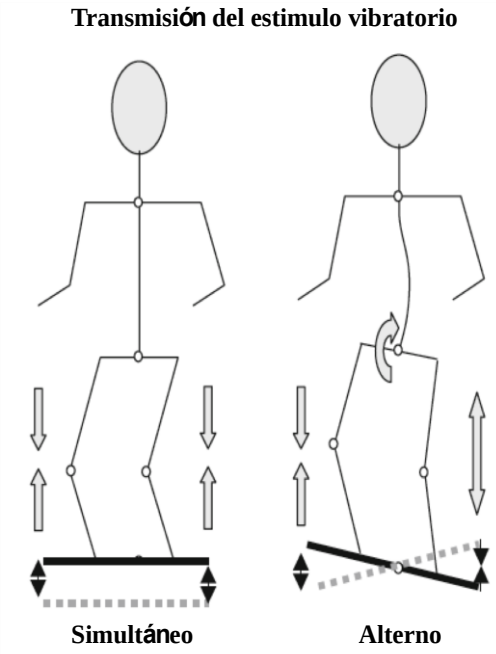


Figura 10. Modalidades de transmisión de la vibración en plataformas vibratorias
Adaptada del artículo "Whole body vibration exercise: are vibrations good for you?" (71) Licencia: 4665810036414

La vibración de cuerpo completo se basa en la misma teoría del mecanostato, aplicando la carga osteogénica mediante vibraciones de elevada frecuencia y baja amplitud en una plataforma sobre la que se coloca el participante. Existen dos tipos principales de plataformas para suministrar la vibración, esquematizados en la figura 10. En la vibración simultánea el estímulo vibratorio se transfiere a ambos pies al mismo tiempo, mientras que la vibración alterna se produce de manera contralateral; es decir cuando uno de los laterales se encuentra en su punto más alto el otro se encontrará en su punto más bajo, lo que reduce la propagación del estímulo vibratorio al tronco, permitiendo tolerar mayor intensidad de la vibración (72). Las plataformas no sólo difieren en el tipo de vibración suministrada, sino que también varían en la frecuencia y la amplitud de su vibración, obteniéndose así un gran rango de aceleraciones disponibles.

Como se ha mencionado, la vibración de cuerpo completo ha suscitado el interés investigador y existen un gran número tanto de estudios individuales como de revisiones narrativas y sistemáticas centradas en este tema. Sin embargo, todavía existen discrepancias entre las revisiones con respecto a la eficacia real de este tipo de entrenamiento en las distintas poblaciones, lo que dificulta su aplicación práctica

Capítulo 2

Hipótesis y objetivos

Hipótesis

Los preescolares con buena condición física general presentarán parámetros óseos más saludables que sus pares con una condición física pobre.

La práctica de actividad física vigorosa en tandas de al menos cinco minutos continuados se correlacionará positivamente con la densidad y el contenido mineral óseos en adolescentes sanos. También se espera encontrar una relación dosis efecto, de manera que la participación en periodos más largos de actividad vigorosa conllevará una mayor mejoría de la salud ósea.

Un periodo de entrenamiento sobre plataforma vibratoria de cuerpo completo de 6 meses será suficiente para incrementar los niveles de marcadores de formación ósea en nadadores adolescentes.

Hypothesis

Fit preschoolers will present better bone mineral parameters than their unfit counterparts.

The participation in vigorous physical activity in periods of at least five consecutive minutes will be positively correlated with bone mineral content and density in healthy adolescents. Additionally, higher increases in these parameters are expected with longer periods of vigorous physical activity, following a dose-response effect.

A 6-month whole-body vibration protocol will increase the levels of bone formation markers in adolescent swimmers.

Objetivos

El *objetivo general* de la presente Tesis Doctoral es el de ampliar el conocimiento científico al respecto del papel que juegan la práctica de actividad física y la condición física en la mineralización y estructura óseas durante las primeras etapas de la vida, poniendo particular atención en los adolescentes practicantes de natación, al tratarse de un grupo de población más susceptible de presentar una deficiente mineralización ósea.

Más concretamente, los *objetivos específicos* de cada uno de los seis artículos que componen esta Tesis Doctoral son:

Artículo I. Determinar la relación entre distintos parámetros estructurales del hueso con la condición física en su conjunto, así como con cada uno de sus componentes individuales en niños y niñas en edad preescolar.

Artículo II. Analizar la relación entre los distintos patrones de práctica de actividad física y la densidad ósea de adolescentes sanos, poniendo especial interés en la distribución de la actividad vigorosa.

Artículo III. Examinar el efecto de distintas trayectorias de participación en actividad física sobre el crecimiento óseo en adolescentes sanos, considerando la actividad física actual y pasada.

Artículo IV. Resumir la literatura científica existente al respecto del entrenamiento con plataforma vibratoria de cuerpo completo como herramienta para la mejora de la salud ósea.

Artículo V. Analizar los efectos de 6 meses de entrenamiento sobre plataforma vibratoria de cuerpo completo sobre los niveles en suero de los marcadores de metabolismo óseo en nadadores adolescentes.

Artículo VI. Desarrollar un método de cribado para la identificación temprana de nadadores adolescentes en riesgo de presentar una baja densidad mineral ósea, partiendo de variables cuya medición sea accesible para los centros deportivos.

Aims

The *general aim* of the present Thesis is to enlarge the scientific knowledge regarding the role of physical activity and fitness on bone mineralization and structure during the first stages of life. An especial focus is put on adolescent swimmers, since it is a sector of the population at high risk of suffering from poor bone mineralization.

More concretely, the *specific aims* of each of the six articles that constitute this Doctoral Thesis are:

Manuscript I. To determine the relationship between several bone structural parameters and physical fitness and its components in preschool children.

Manuscript II. To analyze the relation among different patterns of physical activity participation and bone density in healthy adolescents, focusing on the distribution of vigorous physical activity bouts.

Manuscript III. To examine the effect that different physical activity participation trajectories have on bone growth in healthy adolescents, taking into consideration both present and past physical activity participation.

Manuscript IV. To summarize the available scientific literature regarding the use of whole-body vibration training as a tool for the improvement of bone health.

Manuscript V. To analyze the effects of 6 months of whole-body vibration training on the serum levels of bone turnover markers in adolescent swimmers.

Manuscript VI. To develop a screening method for the early detection of adolescent swimmers that are susceptible of suffering from low bone mineral density, based on easily measurable variables on a regular training setting.

Capítulo 3

Material y métodos

En esta sección se detallarán los procedimientos generales utilizados para obtener los resultados que dan lugar a la presente Tesis Doctoral. Al darse la particularidad de que estos estudios se engloban en tres proyectos de investigación diferentes, se dedicará un apartado específico para la metodología relativa a cada uno de estos proyectos. Por otra parte, aspectos más generales de estos proyectos, como pueden ser las consideraciones éticas y el tratamiento estadístico de los datos se detallarán en apartados independientes que incluirán información de los tres proyectos. La metodología concreta de cada estudio individual puede consultarse en el manuscrito correspondiente en la sección de Resultados y Discusión de este documento.

3.1 Consideraciones éticas y legales

Durante el desarrollo de los tres proyectos se siguieron los *Principios Éticos para las Investigaciones Médicas en Seres Humanos* reconocidas por la *Declaración de Helsinki de 1975* según su revisión más actual en el momento de conceptualización del proyecto, es decir, de acuerdo con la revisión de la 59ª Asamblea General de Seúl 2008, Corea (73) para los proyectos de Estonia y RENACIMIENTO y la revisión de las 64 Asamblea General en Fortaleza 2013, Brasil (74) para el proyecto PREFIT. Asimismo, se acató la normativa legal española en materia de investigación con seres humanos (ley 14/2007, de 3 de Julio, de Investigación Biomédica).

Todos los proyectos fueron revisados y aprobados por los comités de ética de las regiones correspondientes. Así pues, el proyecto RENACIMIENTO recibió un *dictamen favorable* (referencia CP08/2012) tras la reunión del 18 de abril de 2012 del *Comité de Ética de la Investigación de la Comunidad Autónoma de Aragón* (CEICA). El proyecto PREFIT, por su parte, recibió el visto bueno del *Comité de Ética en Investigación Humana*

de la Universidad de Granada (referencia 845), mientras que el proyecto realizado en la Universidad de Tartu (Estonia) fue aprobado por el *Human Ethical Committee* de dicha universidad (referencia 179/T-4).

Los detalles específicos del proyecto RENACIMIENTO y la investigación adicional realizada con la cohorte de Zaragoza de PREFIT se encuentran disponibles en el repositorio público ClincalTrials.gov bajo los números de referencia NCT02380664 y NCT02457754 respectivamente.

Antes del comienzo de los estudios se realizaron reuniones informativas con los responsables de los centros educativos o clubes deportivos en los que se realizaría posteriormente el reclutamiento de los participantes. Asimismo, se llevó a cabo una charla explicativa del proyecto destinada a los padres de los candidatos a participar en el proyecto. Previamente al comienzo de las evaluaciones e intervenciones, los padres o tutores legales proporcionaron un consentimiento firmado tras recibir información escrita detallando tanto las características del proyecto como los resultados esperados y potenciales riesgos, así como la posibilidad de retractarse de la participación en cualquier momento. Además, los participantes expresaron verbalmente su acuerdo de formar parte de los estudios.

3.2 Proyecto PREFIT

3.2.1 Diseño y participantes

El proyecto PREFIT es un estudio multicéntrico con un diseño transversal cuyo principal objetivo es la evaluación de la condición física de una muestra representativa de preescolares españoles. Para ello se contó con más de 3000 niños y niñas de diez ciudades españolas (Almería, Cádiz, Castellón, Cuenca, Granada, Las Palmas de Gran Canaria, Madrid, Palma de Mallorca, Vitoria y Zaragoza) entre enero de 2014 y noviembre de 2015.

El estudio que forma parte de la presente Tesis Doctoral se centra exclusivamente en la cohorte de Zaragoza, lo que elimina la representatividad de la muestra con respecto al territorio nacional, pero gracias al proyecto adicional que se llevó a cabo en esta ciudad se pudo evaluar la masa ósea de los participantes por medio de una tomografía computerizada periférica, algo que sólo se realizó en este nodo.

Así pues, la muestra perteneciente al estudio se compuso de un total de 139 preescolares de entre 3 y 5 años de edad (77 niños y 62 niñas) de distintos centros de enseñanza públicos y concertados de la ciudad de Zaragoza y pueblos circundantes. Si algún candidato presentara una enfermedad o condición que impidiera el correcto desempeño de las pruebas sería retirado del estudio, así como aquellos que se encontraran tomando medicación que afecte a la masa ósea. A los participantes en el estudio se les realizaron mediciones antropométricas básicas, un escaneo de la estructura ósea de la tibia y una batería de pruebas de condición física. Por otra parte, los padres cons Las imágenes radiográficas que presentaron artefactos debido al movimiento de los participantes durante la prueba (pese a las medidas tomadas para minimizar estos posibles movimientos, que se detallan a continuación) fueron eliminadas del estudio, dejando así un total de 92 particpantes con las mediciones completas (50 niños y 42 niñas).

3.2.2 Mediciones antropométricas y evaluación de la masa ósea

Todos los participantes fueron medidos con un estadiómetro con una resolución de 0,1 cm (SECA 213, Hamburgo, Alemania) y pesados en una báscula equipada con bioimpedancia (TANITA BC420 SMA, Hamburgo, Alemania).

El contenido y estructura óseos de la tibia no dominante fueron evaluados utilizando un escáner de tomografía computerizada periférica (XCT-2000L, Stratec Medizintechnik,

Pforzheim, Alemania). Este aparato permite obtener valores no sólo de composición corporal como el contenido o la densidad mineral sino también valores estructurales como el grosor cortical o la densidad trabecular, que permiten calcular el comportamiento mecánico del hueso. Estas variables se evaluaron en la diáfisis de la tibia, al 38% de su longitud, tras medirse manualmente la longitud de la tibia y tomando como referencia el extremo distal de la tibia identificado con un escaneo preliminar. Los coeficientes de variación de este aparato dentro de nuestro laboratorio al utilizar el fantoma de calibración fueron inferiores al 1%, mientras que para las variables evaluadas en vivo en esta región de la tibia oscilaron entre el 0.49% (densidad cortical) y el 5.31% (área cortical) (75). El aparato se sometía a una calibración completa al inicio de cada día de mediciones, siguiendo las instrucciones del fabricante.

Para el correcto desarrollo de la prueba es vital que el participante permanezca inmóvil durante la misma, puesto que los movimientos distorsionan la imagen obtenida, por lo que su realización se complica para niños en edad preescolar, si bien ya se ha demostrado que esta técnica se puede utilizar en este rango de edad (76). Con el objetivo de minimizar los movimientos durante la prueba, los participantes se colocaban en un asiento ajustable y se restringía la movilidad de la pierna mediante una fijación al aparato. Además, se distraía su atención utilizando dibujos animados.

3.2.3 Pruebas de condición física

La evaluación de la condición física de los preescolares se realizó mediante una serie de test físicos diseñados específicamente para este proyecto, denominada batería PREFIT. Esta batería tiene en cuenta distintos componentes de la condición física y se ha demostrado que es válida y aplicable para la valoración de la condición física en preescolares (77). La batería PREFIT incluye las siguientes pruebas.

- Fuerza de prensión manual

Los participantes ejercen la mayor fuerza posible sobre un dinamómetro manual (TKK 5001, Takei Corp., Tokio) manteniendo el brazo extendido y ligeramente separado del tronco. Se utilizó un dinamómetro analógico para sortear la limitación de los modelos digitales cuyo umbral inferior de registro es de 5 kg, por lo que no son capaces de detectar las pruebas de algunos participantes. La prueba se realiza un total de dos veces con cada brazo.

- Salto de longitud

En esta prueba que valora la fuerza explosiva de las extremidades inferiores, los participantes deben realizar un único salto despegando y aterrizando con los dos pies simultáneamente intentando recorrer la mayor distancia horizontal posible. Se realizan tres intentos y se registra el mejor de ellos.

- Equilibrio

Esta prueba consiste en conservar durante el mayor tiempo posible un equilibrio estático manteniendo un pie elevado del suelo, es decir, a la pata coja. Los movimientos con los brazos para reestablecer el equilibrio están permitidos, la prueba concluye cuando el participante es incapaz de mantener la postura. Se realiza un intento con cada pierna.

- Agilidad

Para la evaluación de la agilidad, los participantes deben completar un recorrido de 10 metros un total de cuatro veces (realizando dos idas y dos vueltas) en el menor tiempo posible. Esta prueba se realiza por duplicado, con un periodo de al menos un minuto de descanso entre los dos intentos.

- Condición cardiorrespiratoria

Esta prueba consiste en una adaptación del test de Leger (78). Los participantes deben recorrer de ida y vuelta un tramo de 20 metros a una velocidad que se indica con una señal acústica al final de cada trayecto. La prueba inicia a una velocidad de 6,5 km/h y se incrementa en 0,5 km/h cada minuto. Los participantes Estuvieron acompañados en todo momento por dos investigadores, que ayudan a mantener el ritmo indicado. La prueba finaliza cuando el participante es incapaz de completar el trayecto en el tiempo indicado en dos veces consecutivas o se retira debido a la fatiga.

3.3 Proyecto internacional

Como ya se ha mencionado, durante el transcurso de dos estancias internacionales, el doctorando tuvo la ocasión de colaborar en un proyecto de la Universidad de Tartu (Estonia), cuyas características se detallan en este apartado.

3.3.1 Diseño y participantes

Un total de 264 chicos de entre 11 y 13 años de edad, reclutados en las escuelas de la ciudad de Tartu y sus alrededores, formaron parte de este estudio que tiene como objetivo analizar distintos factores que puedan afectar a la adquisición de masa ósea durante toda la etapa puberal, con especial énfasis en la actividad física. De acuerdo con el diseño del proyecto, el cual continúa en activo, los participantes serán evaluados en repetidas ocasiones durante su periodo de desarrollo madurativo si bien se mantiene un enfoque observacional, sin realizarse ninguna intervención sobre los participantes. Las variables principales sobre las que se centra este proyecto son la densidad mineral ósea, obtenida a partir de una densitometría de rayos-X de energía dual y la participación en actividad física de distintas intensidades, registrada mediante acelerometría.

Uno de los artículos pertenecientes a este proyecto que forman parte de la presente Tesis Doctoral utiliza únicamente los datos relativos a la evaluación inicial, siguiendo por lo tanto un diseño transversal, en el que un total de 180 participantes suministraron registros de acelerometría completos. Por otra parte, el segundo estudio incorpora los datos obtenidos en la evaluación de seguimiento, llevada a cabo un año después de la inicial. Tras este periodo, fueron 140 los participantes que proporcionaron datos suficientes para ser incluidos en el análisis.

3.3.2 Mediciones antropométricas y evaluación de la masa ósea

Para la sesión de evaluación antropométrica y de la composición corporal, los participantes permanecían en ropa interior y descalzos. La talla de los participantes se midió utilizando un antropómetro metálico (GPM Anthropological Instruments, Zurich, Suiza) con una precisión de 0.1 cm y el pesaje se realizó con una báscula médica (A&D Instruments Ltd., Abingdon, Reino Unido) de resolución 0.05 kg.

Para la medición de la masa ósea se realizaron escaneos con DXA (DX-IQ, Lunar Corp., Madison, Wisconsin, Estados Unidos) en el cuerpo completo, la columna lumbar (de L2 a L4) y la cadera no dominante, obteniéndose valores de contenido y densidad mineral óseos. Se siguieron las instrucciones del fabricante tanto para el posicionamiento del participante como para el posterior análisis de las imágenes obtenidas. El mismo técnico realizó las mediciones antropométricas y el análisis de los datos de la densitometría en ambas evaluaciones. El coeficiente de variación del aparato se obtuvo duplicando las mediciones (con reposicionamiento) de 20 de los participantes, resultando inferior al 2% para todas las variables analizadas.

Por otra parte, el estado madurativo se determinó mediante una prueba de rayos-X en la muñeca izquierda, siguiendo el método propuesto por Groulich y Pyle (79,80).

3.3.3 Registro de la práctica de actividad física

Para la evaluación de la práctica de actividad física, los participantes llevaron en su cadera derecha durante una semana un acelerómetro uniaxial (GT1M Actigraph, Monrovia, California, Estados Unidos), exceptuando los periodos de sueño y de ducha o actividades acuáticas.

Los datos en bruto, originalmente de una frecuencia de 30 Hz, se condensaron en periodos (epoch) de 15 segundos para facilitar su tratamiento. Una vez obtenidos los epoch, se eliminaron de los registros semanales todos aquellos periodos iguales o superiores a 20 minutos sin registrar aceleración alguna, al considerarse tiempo no válido (periodos en los que el participante no ha llevado el acelerómetro consigo). Tan sólo se aceptaron para el análisis definitivo aquellos registros semanales que incluyeran al menos tres días con 10 o más horas de tiempo válido, siendo además festivo al menos uno de esos días.

La intensidad de la actividad física se dividió en las categorías de sedentarismo, actividad ligera, moderada y vigorosa de acuerdo con los puntos de corte propuestos por Evenson y colaboradores (81). Además, dado que los estudios se orientaron hacia el análisis de los patrones de participación en actividad física vigorosa, se registraron todas aquellas tandas (bouts) superiores a cinco minutos continuados de actividad vigorosa, con una tolerancia de un epoch. Se analizó tanto el número de estas tandas diarias como su duración media y máxima.

3.4 Proyecto RENACIMIENTO

Dentro de este proyecto se enmarcan tres de los manuscritos que componen la presente tesis doctoral. No obstante, uno de ellos, el artículo IV, presenta una metodología particular al tratarse de un artículo de revisión sistemática, por lo que se le dedicará un apartado independiente.

3.4.1 Diseño y participantes

El proyecto RENACIMIENTO es un ensayo clínico aleatorizado realizado entre septiembre de 2012 y diciembre de 2015. Si bien el desarrollo efectivo del proyecto no se pudo ajustar completamente al diseño inicialmente concebido y descrito (75), los principales componentes de este se mantuvieron, contando con una intervención de entrenamiento vibratorio de 6 meses y cuatro puntos de evaluación; uno antes de comenzar la intervención, otro al final de esta para observar los cambias inducidos por la intervención y otros dos en los dos años posteriores a la finalización de la intervención para comprobar la perdurabilidad de los efectos.

El proyecto contó inicialmente con 75 controles normoactivos y 98 nadadores, los cuales fueron aleatoriamente distribuidos en dos grupos, uno de los cuales continuaría con su entrenamiento habitual de natación y otro incorporaría además el entrenamiento sobre plataforma vibratoria.

Los participantes tenían que ser caucásicos de una edad comprendida entre los 10 y los 18 años en el momento del comienzo del estudio, además de no sufrir ninguna enfermedad ni tomar medicación que afectase al hueso. Para optar a unirse al estudio dentro del grupo de nadadores, los participantes debían acumular al menos 6 horas de entrenamiento semanal de natación, así como tener 3 o más años de experiencia en competición a nivel regional o superior.

Tras aplicar estos criterios de inclusión y excluir a los participantes con datos faltantes, la muestra final incluida fue de 68 nadadores (40 chicos y 28 chicas) y 41 controles (27 chicos y 14 chicas) en el artículo V que sigue un enfoque longitudinal, centrándose en los dos primeros puntos de evaluación. Por otra parte, el artículo VI, que se centra exclusivamente en el grupo de nadadores y sólo tiene en cuenta la evaluación inicial pudo contar con un total de 78 nadadores (40 chicos y 38 chicas).

3.4.2 Protocolo de entrenamiento vibratorio

Las características de la intervención de entrenamiento de vibración de cuerpo completo se detallan en la tabla 1. Como se puede apreciar, se optó por un protocolo incremental, en el que el aumento paulatino de la frecuencia y amplitud de la vibración conforme avanza la intervención resulta en aceleraciones progresivamente mayores.

Los participantes tenían que asistir la sesión con calzado deportivo y realizaban por duplicado una serie de ejercicios sobre la plataforma: sentadilla estática a 120º, sentadilla estática a 90º, sentadilla dinámica (oscilando entre las dos posiciones anteriores) y fondos con cada uno de los pies al frente (figura 11). Las sesiones eran supervisadas en todo momento por un licenciado en Ciencias de la Actividad Física y el Deporte que velaba por la seguridad de los participantes y se aseguraba de la correcta ejecución de los ejercicios. Los participantes acudían a las sesiones por parejas, alternándose los periodos de descanso y ejercicio.

	Mes 1	Mes 2	Mes 3	Mes 4	Mes 5	Mes 6
Sesiones	12	12	12	12	12	12
Frecuencia (Hz)	30	30	32	34	36	38
Amplitud (mm)	1	2	2	2	2	2
Duración (s)	45	45	45	60	60	60
Descanso (s)	45	45	45	60	60	60
Tiempo total de vibración (min)	7.5	7.5	7.5	8	8	8
Duración de la sesión (min)	15	15	15	16	16	16
Aceleración resultante (g)	3,6	7,2	8,2	9,3	10,4	11,6

Tabla 1. Parámetros del protocolo de vibración

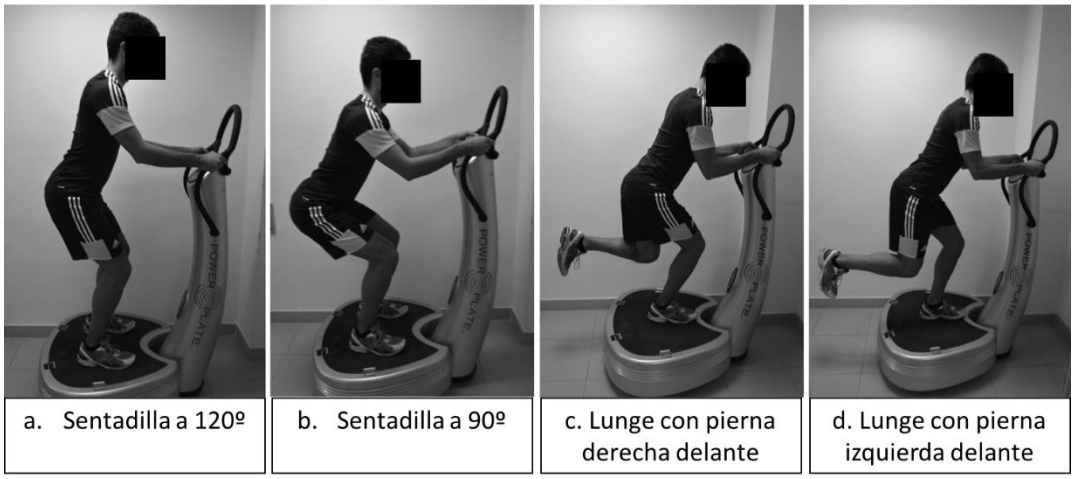


Figura 11. Ejercicios realizados durante la sesión de entrenamiento vibratorio
Adaptada del artículo "Do 6 monthe of whole-body vibration training improve lean mass and bone mass acquisition of adolescent swimmers?" (82) *Licencia: 4665810667489*

3.4.3 Mediciones antropométricas y evaluación de la masa ósea

Al igual que en los proyectos descritos con anterioridad, los participantes fueron medidos (estadiómetro SECA 225, SECA, Hamburgo, Alemania, resolución 0,1 cm) y pesados (báscula electrónica SECA 861, SECA, Hamburgo, Alemania, resolución 0,1 kg) mientras se encontraban en ropa interior y descalzos.

Posteriormente se procedió a la evaluación de su masa ósea (área, contenido y densidad mineral ósea) utilizando un aparato de DXA (Hologic Corp., software versión pediátrica 12.4, Bedford, Massachussetts, Estados Unidos), realizando escaneos del cuerpo completo, columna lumbar (L1-L4) y cadera no dominante. Todas las mediciones y análisis de las imágenes fueron realizadas por el mismo operador entrenado. Los coeficientes de variación del DXA en nuestro laboratorio son del 2,3% para el contenido mineral óseo y del 1,3% para la densidad mineral ósea (83).

3.4.4 Biomarcadores de metabolismo óseo

Para la determinación de los niveles en sangre de los marcadores de metabolismo óseo se siguieron las indicaciones de la *Fundación Internacional de Osteoporosis* y la *Federación Internacional de Química Clínica* (12). Se escogieron dos marcadores de

formación ósea: la osteocalcina (OC) y el propéptido amino-terminal del procolágeno tipo I (P1NP). Como marcador de resorción ósea se analizó el telopéptido carboxi-terminal del colágeno tipo I (CTX). Con el objetivo de reducir la variación circadiana intra-individual, las muestras sanguíneas se extrajeron tras un periodo de ayuno en el mismo momento del día (entre las 8:00 y las 10:00 de la mañana).

Tras su extracción, la muestra se dejaba reposar durante media hora y se procedía al centrifugado a 10.000 revoluciones por minuto durante 15 minutos y su posterior almacenamiento en una cámara frigorífica a -80ºC. La determinación de los niveles de marcadores presentes en el suero se llevó a cabo en un laboratorio independiente, mediante inmunoensayos de electroquimioluminiscencia.

3.4.5 Pruebas de condición física

Dentro del proyecto RENACIMIENTO se llevaron a cabo diversas pruebas de condición física. No obstante, en este apartado se describen únicamente aquellas pruebas incluidas en los análisis realizados en los artículos pertenecientes a la presente Tesis Doctoral.

Las pruebas de fuerza se realizaron siguiendo el mismo procedimiento descrito en el proyecto PREFIT, evaluando la fuerza de los miembros superiores mediante la prueba de dinamometría manual y la fuerza de las extremidades inferiores con la prueba de salto de longitud a pies juntos. Para la evaluación de la resistencia cardiorrespiratoria se empleó el test de Leger original (78). Por otra parte, para la medición de la velocidad se cronometró el tiempo transcurrido en realizar un sprint de 30 metros en línea recta utilizando células fotoeléctricas.

3.4.6 Otras variables

Dentro del proyecto RENACIMIENTO se tuvieron en cuenta diversas variables que pueden afectar al proceso de crecimiento óseo durante la adolescencia y por lo tanto se registraron para poder controlar su efecto como variables de confusión o mediadoras.

Para la determinación del estado de desarrollo puberal se facilitó a los participantes una planilla en la que se representa gráficamente las etapas descritas la escala de Tanner y Whitehouse (84) de manera que pudieran proceder a su autoevaluación.

La ingesta diaria de calcio se calculó a partir de un cuestionario validado de frecuencia de consumo de alimentos ricos en calcio como el queso o el pan (85).

Asimismo, los participantes completaron un cuestionario en el que se recogía la práctica de actividad física tanto en el presente como en el pasado. Además, para los participantes nadadores se recogió su volumen de entrenamiento (en horas por semana), su experiencia en competición (en años) y su rendimiento en competición, comprobando los tiempos oficiales en competición y la puntuación otorgada por la Federación Internacional de Natación.

3.4.7 Revisión de revisiones sistemáticas

Como se ha mencionado, en este apartado se describirán las pautas generales seguidas para la realización de la revisión sistemática perteneciente a esta Tesis Doctoral. Se puede encontrar una descripción detallada de todos los pasos seguidos durante su elaboración en el apartado de metodología del artículo IV del capítulo *Resultados y discusión.*

La primera particularidad que se debe resaltar con respecto al trabajo de revisión llevado a cabo es que su objetivo principal no es el de recopilar información recogida en distintos artículos individuales, sino que pretende recoger los resultados obtenidos en

distintas revisiones, tratándose por tanto de una revisión de revisiones sistemáticas, también denominada *overview* en lengua inglesa.

Como tal, hay algunos aspectos de su procedimiento metodológico que son similares a los de una revisión tradicional, como la sistematización de la recogida de información, pero hay otros aspectos que difieren, al tener que tratar con dos capas de fuentes de información, esto es, tanto las revisiones sistemáticas como los estudios individuales que las componen. Por lo tanto, para la realización de esta revisión de revisiones se siguió una metodología diseñada específicamente para este tipo de investigación (86,87).

Así pues, tras definir los términos de búsqueda dentro de tres bases de datos electrónicas (PubMed, Cochrane Library y SportDiscus), dos investigadores examinaron de manera independiente los artículos encontrados seleccionando únicamente los documentos que consistieran en revisiones sistemáticas que analizaran los efectos del entrenamiento en plataforma vibratoria de cuerpo completo sobre parámetros óseos, ya fueran relativos a su composición, estructura o metabolismo. No se realizó ninguna restricción respecto a la población objetivo de la revisión ni a la fecha de publicación de esta.

Una vez recopiladas las 17 revisiones sistemáticas que terminaron siendo incluidas en la revisión de revisiones, se procedió a la evaluación de la calidad metodológica de estas, siguiendo la escala AMSTAR (88). Asimismo, se llevó a cabo una recopilación sistemática de las características principales y resultados obtenidos en cada una de las revisiones, decidiéndose realizar una división de los resultados en tres categorías en función de la población estudiada: mujeres postmenopáusicas, adultos jóvenes y niños y adolescentes con baja densidad mineral ósea.

También se tuvo en cuenta la posible duplicidad de la información recogida por las distintas revisiones, por lo que se recogió una lista de los estudios individuales que componían cada revisión sistemática y se realizó una comprobación cruzada de aquellos estudios individuales que figuraban en más de un documento, calculando además el grado de solapamiento de la información según la fórmula propuesta por Pieper y colaboradores (89). En el caso de detectarse conflictos entre distintas revisiones a la hora de realizar la interpretación de los resultados de algún estudio individual, se procedió a consultar la fuente original.

3.5 Análisis estadísticos

Dado que algunos de los métodos estadísticos utilizados son comunes a varios de los artículos que componen la presente Tesis Doctoral, se ha optado por describir las distintas pruebas estadísticas utilizadas en este apartado conjunto, en el que también se incluyen procedimientos más específicos que no tienen por qué ser utilizados en todos los manuscritos. No obstante, en cada uno de los artículos incluidos en el siguiente capítulo de *Resultados y discusión* se encuentra disponible una descripción detallada de las técnicas estadísticas empleadas.

El software empleado para realizar la mayoría de las pruebas estadísticas fue el *Statistical Package for the Social Sciences* (SPSS Inc., versión 22.0, Chicago, Illinois, Estados Unidos), si bien tato el análisis de los datos de acelerometría como la elaboración de los árboles de decisión del manuscrito VI se realizaron utilizando el lenguaje de programación estadística *R* (90), utilizando distintos paquetes para ampliar su funcionalidad. El nivel de significación estadística se fijó en $\alpha<0,05$.

Antes de proceder con pruebas estadísticas paramétricas, se comprobó que no se violara la asunción de normalidad mediante pruebas de Kolmogorov-Smirnov, además de realizarse una exploración de posibles casos extremos u *outliers* univariante y multivariante según la comprobación de la distancia de Mahalanobis. También se realizó una estadística descriptiva de las variables de interés de cada artículo.

Las distintas pruebas paramétricas incluyen test de chi-cuadrado para comprobar la homogeneidad entre grupos en la distribución de variables de clasificación (como el sexo o la etapa de desarrollo madurativo), pruebas t de muestras relacionadas para el análisis longitudinal de la evolución de las variables dentro de un mismo grupo y pruebas t de muestras independientes para evaluar las diferencias entre dos grupos. En el caso de que se requiriera realizar comparaciones entre más de dos grupos, se llevó a cabo un análisis de varianza (ANOVA) con ajuste de Bonferroni para las pruebas post-hoc. También se utilizaron ANOVA de medidas repetidas para comparar la evolución longitudinal entre distintos grupos, analizando la interacción grupo por tiempo. Se calcularon coeficientes de correlación de Pearson para evaluar la relación entre distintas variables y para la obtención de la contribución específica de distintas variables independientes sobre la variable de interés se realizaron modelos de regresión lineal. En caso necesario, durante la realización de estas pruebas estadísticas se introdujo un ajuste con respecto a las potenciales variables de confusión identificadas.

Dentro de los procedimientos de análisis estadístico más específicos cabe destacar la construcción de curvas de características receptor-operador (curvas ROC) para la detección de puntos de corte, análisis de clúster para identificar agrupaciones de sujetos definidos por poseer una serie de características similares y la elaboración de árboles de regresión y decisión para identificar secuencias de variables que permitan identificar una característica clave dentro de la variable de interés.

Capítulo 4

Resultados y discusión

Los resultados y la discusión de la presente Tesis Doctoral se han estructurado en distintos apartados relacionados con cada uno de los proyectos incluidos. El capítulo 4.1 se centra en la relación entre condición física y parámetros estructurales óseos en preescolares. En los capítulos 4.2 y 4.3 se analizan de manera transversal y longitudinal respectivamente las asociaciones entre la práctica de actividad física vigorosa y el estado óseo en adolescentes. El apartado 4.4 consiste en una revisión sistemática de trabajos de revisión centrados en los efectos del entrenamiento vibratorio de cuerpo completo en la salud ósea de distintas poblaciones. En el capítulo 4.5 se exponen los efectos sobre el metabolismo ósea de los nadadores adolescentes producidos por el protocolo de entrenamiento vibratorio y finalmente en el apartado 4.6 se presenta un modelo computacional para la detección temprana de nadadores adolescentes con riesgo de presentar un baja densidad mineral ósea.

The results and discussion of this Doctoral Thesis have been subdivided in different sections, according to various aspects of the included projects. Chapter 4.1 is focused on the relationship between physical fitness and structural bone parameters in preschoolers. Sections 4.2 and 4.3 show respectively a cross-sectional and longitudinal analysis of the associations between vigorous physical activity and bone status in adolescents. Section 4.4 features an overview of systematic reviews regarding the effects of whole-body vibration on bone health in different populations. In chapter 4.5 the effects of vibration training on the bone turnover markers of adolescent swimmers are shown. Finally, Chapter 4.6 introduces a computer model for the detection of adolescent swimmers at risk of having low bone mineral density.

4.1 Associations between physical fitness and bone strength and structure in 3 to 5-year-old children

ABSTRACT

Background: The positive association between physical fitness and bone structure has been widely investigated in children and adolescents with no studies evaluating this influence in young children, i.e. preschoolers.

Hypothesis: Fit children will present improved bone variables when compared to unfit children and no sex differences will emerge in the sample.

Study design: Cross-Sectional Study

Level of evidence: 2c

Methods: Handgrip strength, standing long jump (SLJ), speed-agility, balance and cardiorespiratory fitness (CRF) were assessed with the PREFIT battery in 92 children (50 boys; ages from 3 to 5). A peripheral quantitative computed tomography scan was performed at the 38% of the non-dominant tibia. Cluster analysis from handgrip strength, SLJ, speed-agility and CRF was developed to identify fitness groups. Bone variables were compared between sexes and between cluster groups. The association between individual physical fitness components and different bone variables was also tested.

Results: Three cluster groups emerged and were named as FIT (high values of all the included physical fitness variables), STRONG (high strength values and low speed-agility and CRF), and UNFIT (low strength, agility and CRF). FIT presented higher values than STRONG and UNFIT for total and cortical bone mineral content, cortical area and polar strength strain index (all $p<0.05$). FIT also presented a higher cortical thickness when compared to UNFIT ($p<0.05$). Handgrip, SLJ and speed-agility predicted all the bone variables except for total and cortical volumetric bone mineral density (vBMD). No differences were found for bone variables between sexes.

Conclusions: These results suggest that global fitness is a key determinant to bone structure and strength, but not vBMD in preschoolers.

Clinical relevance: Physical fitness is determinant to tibial bone mineral content, structure and strength in very young children. Performing physical fitness tests could provide useful information related to bone health in preschoolers.

Keywords: Bone mass, preschool, muscular strength, children, fitness.

INTRODUCTION

Physical activity is a major determinant of body composition (91), cognition (92), bone mass (93), bone strength (94) and quality of life (95) in children. Nonetheless, some studies have suggested that the effect of physical activity on the previously mentioned outcomes is mediated by physical fitness (96). It is clear that both are positively associated and that while registers of physical activity are usually based on a 7-day window, physical fitness is a robust physiological measure that could reflect the amount of physical activity performed during the previous months.

The effects of physical fitness on health have also been widely studied in children (97), finding positive associations with quality of life (95), metabolic risk factors (98) and bone health (98), among others. Focusing on bone health, 12 out of 17 studies included in a meta-analysis developed by Smith et al. (98) showed a positive association between muscular fitness and bone mass. Gracia-Marco et al. (99) evaluated the influence of several components of physical fitness and physical activity on bone mineral content (BMC) in adolescents, finding that lower levels of fitness were associated with lower BMC. An interesting finding of the previous study was that cardiorespiratory fitness had no influence on BMC in boys, suggesting that the development of different fitness components might act unequally on bone mass.

Both BMC and bone mineral density (BMD) measured with dual energy x-ray absorptiometry (DXA) have been the most researched bone-variables. Nevertheless, DXA has several limitations when used in small children (100), and unlike peripheral quantitative computed tomography (pQCT), DXA only provides information about bone quantity without evaluating structure and therefore bone quality. This might be very important

information, as bone strength, which is a fracture risk factor, is determined by both BMD and bone structure (101).

Few studies have measured the association between physical fitness and bone structure in children, finding that muscular fitness predicts adolescent bone strength in 17 year-olds (102), and that active fit 7 to 9 year-old boys present greater cortical area and thickness compared to inactive-unfit boys (103). To the best of our knowledge no studies have evaluated the association between physical fitness and bone structure in very young children as preschoolers. It is important to clarify this effect, as poor bone accrual during growth is associated with increased fracture risk in childhood (104,105) and may influence lifelong fracture risk (106).

Therefore, the aims of the present study were: 1) to determine the association between different physical fitness components and several structural bone parameters in 3 to 5 year olds, 2) to analyze the overall association between physical fitness and bone structure, and 3) to determine if sex differences regarding bone structure have already emerged in 3 to 5 year olds.

It was hypothesized that fit children would present improved bone variables when compared to unfit children and that no sex differences would be present in the sample.

METHODS

Design and participants

This study is part of the PREFIT project (Assessing FITness levels in PREschoolers, http://profith.ugr.es/prefit), a multicentric study developed in 10 Spanish cities, aiming to include 3000 pre-school children to develop physical fitness reference values with the

PREFIT physical assessment battery and also to determine the association of physical fitness and several health outcomes. The present study focused on the sample from Zaragoza (Spain), the only center in which bone structure was measured, and it is not representative of the whole Spanish population of preschoolers. The study protocol was approved by the Review Committee for Research Involving Human Subjects from the University of Granada (nº 845) and by the Ethics Committee of the University of Zaragoza (CEICA CP18/2014), and it adheres to the Helsinki Declaration of 1961 (revision of Fortaleza 2013). Signed informed consent was retrieved from the parents or legal guardians of the participants.

In order to be included, participants had to be: 1) in pre-school years 1, 2 or 3 (starting ages 3, 4 and 5 years respectively), 2) healthy and not taking medications affecting bones. Those participants that did not present complete fitness and bone data, were excluded from final analyses. Bone scans showing any sign of movement were omitted from all analyses. From the initial 139 participants (77 boys and 62 girls), 47 scans (27 boys and 20 girls) were excluded from the analysis due to movement artifact (37%). Therefore, to guaranty high quality data all the analyses for the present manuscript were conducted with 92 participants (50 boys and 42 girls).

Physical fitness assessment

Physical fitness was assessed with the PREFIT battery (107) which is known to be feasible and reliable in preschoolers (108).

The PREFIT battery was performed to measure physical fitness. In order to obtain the highest level of performance participants were verbally encouraged during the duration of all the tests. The performed tests were:

1) Handgrip strength tests (Handgrip): It was measured using a handgrip dynamometer (TKK 5001, grip A, Takei, Tokyo; range 0–100kg; accuracy 0.5 kg). Children were in a standing position maintaining the arm of the tested side straight down with the shoulder slightly abducted (~10° not touching the rest of the body), the elbow in 0° flexion, and the forearm in neutral position and the wrist in 0° flexion. The best value of the four attempts (two trials with each hand) was chosen (kg).
2) Lower limb explosive strength (standing long jump test; SLJ): This test consists of jumping horizontally with both feet at the same time the maximum distance over a non-slippery and hard surface with the feet immediately behind the starting line and separate from each other approximately at the shoulder's width. Children performed three jumps with 1–2 min rest between attempts. The best value of three attempts in centimeters was used for the analysis
3) Balance (standing on one leg test; balance): One-leg stance test was used to evaluate the static balance. An attempt was made on each leg scoring the time it got to keep it there.

   Children were allowed to use their arms if it was necessary to maintain the balance position as long as possible. The timer was activated when the free leg left the ground. The test ended when the children were not able to maintain the required position, i.e. moved the supporting foot, heel or toe of the original position. The mean of two attempts was registered in seconds.
4) Speed-agility (4x10m shuttle run test; agility): Participants ran back and forth four times along a 10-m track at the highest speed possible. At the end of each track section, the participants had to touch the hand of a researcher crossing the limit line with both feet. Children performed the test twice with 1–2 min rest between attempts. The best result (minimum score in seconds) was used for the analysis.

5) Cardiorespiratory fitness (PREFIT 20-m shuttle run test; laps): This test required participants to run back and forth between two lines set 20m apart. Running pace was determined by audio signals emitted from a prerecorded compact disc: the initial velocity was 6.5 km/h, which was increased by 0.5 km/h/min. The test also requires that two researchers ran with the preschool children one in front and another behind them forming an imaginary band in motion that helps to maintain the correct speed. The test was finished when the participant failed to reach the end lines concurrent with the audio signals on two consecutive occasions or the child stopped because of fatigue
6) Relative handgrip strength (Rel_Handgrip) was calculated as the absolute handgrip strength divided by the body weight.

The PREFIT battery also includes anthropometric measurements with weight measured to the nearest 0.1 kg with a body composition analyzer (TANITA BC420 SMA, Hamburg, Germany) and height assessed to the nearest 0.1 cm with a stadiometer (SECA 213, Hamburg, Germany).

Bone measurements

A Stratec XCT-2000 L scanner (Stratec Medizintechnik, Pforzheim, Germany) that has been proved to be a valid device to measure bone in 3 to 4 year-old children (109) was used to evaluate the non-dominant tibia. This device is a translate-rotate, small computed tomography scanner that acquires a trans-axial image and allows the measurement of tibia and radius. In order for the obtained image to be valid for posterior analyses participants must stay totally still for the entire duration of the scans. We have seen that the radius is more sensitive to movement than the tibia (110,111), therefore only the tibia was analyzed. Participants were distracted with a cartoon chapter that was played during the whole scan with the aim of reducing movement.

The coefficient of variation between measurements for the phantom is < 1%. In vivo coefficients of variation for several measures of pQCT in our laboratory have been described elsewhere (112). The non-dominant tibia was selected for measurements as recommended by the International Society for Clinical Densitometry (113). The diaphyseal tibia results (located at the 38% of the total tibia length) were used, always placing the reference line at the tibial endplate. Participants were seated in a stationary chair, adjusted to the appropriate height. The tibia length from the distal end of the medial malleolus to the medial knee joint cleft was measured. A tibial adjustable fasten belt was used to hold the limb and to limit motion during the scans. Every limb was centered in the imagining field. The scanner was positioned on the distal tibia, and a coronal computed radiograph (scout view) was performed to manually locate a reference line on the distal end of the tibia. The measurement sites were located proximal to this reference line by a distance corresponding to 38% (diaphyseal tibia) of the tibia length.

Bone outcomes

Data for total and cortical bone at the 38% of the tibia length were registered. Volumetric BMD (vBMD), BMC and area, were derived for both cortical and total bone. Additionally, cortical thickness was measured. Bone strength was established with respect to torsion (Polar strength strain index: SSIPOL), and bending with respect to the X axis (Frac_X).

Statistical analyses

Power calculation and sample size estimations were computed based on the primary outcome of the multicentric PREFIT project (114). The present study is based on a secondary analysis only using data from a research center from the multicentric project (as only one center collected bone data). Nonetheless, sample size is similar to a previous

important study that also measured bone structure in preschool children that presented a sample of 101 children (53 boys) (109).

Sample characteristics are presented with mean and standard deviations or frequencies. Sex differences for bone variables were evaluated using Analyses of Covariance (ANCOVA) adjusting by age and tibia length.

The association between different fitness variables and bone was evaluated through linear regression models. For each physical fitness variable two models were created, with Model 1 only including age, sex and tibia length (the same model for all physical fitness variables). Model 2 included all the variables from Model 1 plus the physical fitness variable.

Cluster analysis was performed to identify groups of physical fitness and compare bone variables among groups. As no sex-differences for bone variables were found, cluster analyses were performed for the whole sample.

Firstly, Z-scores were calculated for Rel_handgrip, SLJ, agility and laps. As Rel_handgrip and SLJ both express strength, they were grouped into one variable ((SLJ Z-score + Rel_handgrip Z-score) / 2), called Strength. Balance was not included as the linear regression coefficients were all non-significant suggesting that it was not associated with bone structure or strength. Although the linear regression coefficients for cardiorespiratory fitness were also non-significant, we decided to include this variable in the cluster analysis as previous studies suggest that it is an important fitness variable regarding bone mass in children and adolescents (115).

Secondly, hierarchical cluster analysis was performed with the 3-fitness Z-score values (Strength, Agility and Laps), finding that the final cluster solutions were always highly influenced by age, with those classified as FIT mostly 5-year-old participants (from third

grade (5-6-year olds), and those classified as UNFIT mostly 3-4 year olds (from first grade). These age differences also emerged when stratifying by grade (in to 3 groups), as those classified as FIT were always significantly older than those classified as UNFIT. Therefore, in order to control for the important effect of age, and the possible effect of sex, linear regressions were performed with physical fitness components as the dependent variable and age and sex as independent variables. Standardized regression residuals were saved and the cluster analysis was developed from the standardized regression residuals of the three previously described variables: Strength, Agility and Laps.

To be consistent with clustering methods reported in previous studies (116,117), two types of cluster analyses were used: hierarchical clustering and k-means clustering. To reduce the sensitivity of the Ward´s method to outliers, individual outliers and multivariate outliers (those with high Mahalanobis values distance) were investigated. Firstly, hierarchical cluster analysis was initially used as the numbers of clusters in the data were unknown beforehand. Number of clusters was determined by examining dendrograms, which suggested a solution of 3 cluster groups.

K-means cluster analysis was therefore performed with 3 possible solutions. This approach minimizes the within-cluster variance and maximizes the between-cluster distance so that resulting clusters are as homogeneous as possible. K-means cluster analysis is considered superior to hierarchical methods because it is less sensitive to outliers and has been found to result in greater within-cluster homogeneity and between-cluster heterogeneity (118).

ANOVAs with the Z-scores of the fitness variables and the raw fitness variables were performed to classify and name the 3 cluster groups. ANOVAs were performed to evaluate anthropometric differences among groups, and Chi-square tests were developed to compare the gender and school year distribution among groups.

Finally, age, sex and tibia length adjusted ANCOVAs were performed to compare bone variables among the three defined cluster groups.

RESULTS

Participant characteristics and physical fitness

Ninety-two children were included in the study. Regarding physical fitness, data for handgrip, relative handgrip, standing long jump, agility, balance and cardiorespiratory fitness are presented for the whole sample and stratified by sex in Table 1.

Table 1 - Descriptive characteristics of pre-school children (Mean ± SD).

	Whole sample (n=92)	Boys (n=50)	Girls (n=42)
Age (y)	4.81±0.76	4.85±0.69	4.76±0.84
School year (1/2/3)	23/40/29	10/24/16	13/16/13
Weight (kg)	18.6±2.8	18.7±2.3	18.5±3.3
Height (cm)	107.1±6.7	107.3±5.8	106.8±7.7
BMI (kg/m^2)	16.2±1.2	16.2±1.2	16.1±1.3
Tibia length (mm)	227.8±19.3	226.3±16.8	229.4±22.0
Physical fitness			
Mean handgrip (kg)	6.87±2.47	6.89±2.44	6.84±2.53
Rel_handgrip (kg/weight)	0.36±0.10	0.36±0.11	0.36±0.09
Standing long jump (cm)	81.70±20.99	85.06±20.47	77.69±21.12
Speed run time 4x10 (s)	16.49±2.26	16.22±2.06	16.80±2.46
Standing one leg (s)	14.70±12.17	13.76±11.83	15.81±12.61
PREFIT 20m SRT (number of laps)	20.43±11.69	22.44±13.03	18.05±9.51

School year (1/2/3) indicates the number of participants in each group from year 1, year 2 and year 3. BMI=Body mass index, Rel= relative; SRT=Shuttle run test

pQCT variables

From the initial 139 participants, 47 scans presented movement (37%). No differences were found between sexes for any of the bone variables (all p>0.05; Table 2).

Table 2 - Sex differences in bone mass (adjusted by age and tibia length)

Tibia 38% variables	Boys (n=50)	Girls (n=42)
Total BMC (g)	1.188±0.099	1.161±0.097
Total Area (mm^2)	160.366±14.404	156.421±14.433
Total vBMD (mg/cm^3)	741.026±41.571	742.837±41.652
Cortical Thickness (mm)	2.803±0.212	2.718±0.214
Cortical BMC (g)	1.042±0.092	1.015±0.091
Cortical Area (mm^2)	101.081±09.242	97.511±09.261
Cortical vBMD (mg/cm^3)	1031.010±35.560	1042.091±35.631
SSIPOL $(mm)^3$	360.825±45.714	350.453±45.806
Frc.Load X (N)	743.204±92.645	745.426±92.830

No differences were found between boys and girls (all p>.05)
BMC=Bone mineral content; vBMD=Volumetric bone mineral density; SSIPOL=Polar strength strain index; Frc.Load X= Bone strength with respect to the X axis

Influence of physical fitness on bone content, structure and strength

Linear regression analyses showed similar results for Rel_handgrip, SLJ and agility, as they all increased r-square of Model 1 for total and cortical BMC, total and cortical area, SSIPOL and Frac_X (from 2 to 5%, all p<0.05; Table 3). SLJ and agility also increased the cortical thickness r-square of model 1 (both p<0.05; Table 3).

Both total and cortical vBMD were unaffected by all of the fitness components (all p>0.05; Table 3). Balance and Laps did not modify Model 1 predictions (all p>0.05; Table 3).

Cluster analysis

The three physical fitness clusters are presented in Figure 1. Cluster 1 was labelled as STRONG, as it was characterized by high levels of strength, average levels of speed-agility and low levels of cardiorespiratory fitness. Cluster 2 was labelled as FIT, as it presented

Table 3 - Linear regression coefficients for the influence of physical fitness on tibia values

			Tot.BMC	Tot.Area	Tot.vBMD	Crt.Thick	Crt.BMC	Crt.Area	Crt.vBMD	SSIPOL	FRAC_X
Model 1	r^2		0.550*	0.477*	0.067*	0.434*	0.539*	0.536*	0.031	0.502*	0.542*
		Rel_handgrip *(B)*	0.414*	57.20*	-20.58	0.565*	0.366*	37.65*	-35.96	186.30*	371.91*
		Change r^2	0.037*	0.044*	0.002	0.022*	0.033*	0.038*	0.007	0.042*	0.037*
		Total r^2	0.587	0.522	0.068	0.455	0.573	0.574	0.038	0.544	0.570
		Standing long jump *(B)*	0.003*	0.366*	-0.054	0.005*	0.002*	0.271*	-0.373	1.016*	2.427*
		Change r^2	0.043*	0.048*	<0.001	0.039*	0.040*	0.052*	0.019	0.033*	0.042*
		Total r^2	0.592	0.525	0.067	0.473	0.579	0.588	0.050	0.534	0.583
		4x10 (s) (B)	-0.035*	-3.388*	-6.803	-0.071*	-0.033*	-3.189*	0.702	-11.15*	-25.05*
Model 2		Change r^2	0.058*	0.033*	0.036	0.072*	0.057*	0.058*	0.001	0.032*	0.036*
		Total r^2	0.608	0.511	0.102	0.506	0.597	0.594	0.032	0.534	0.578
		Balance (s) *(B)*	<0.001	0.063	-0.270	-0.001	-0.001	<0.001	-0.498	0.208	0.157
		Change r^2	0.001	0.001	0.003	0.001	0.001	<0.001	0.016	0.001	<0.001
		Total r^2	0.551	0.478	0.070	0.435	0.540	0.536	0.047	0.709	0.542
		Laps (s) *(B)*	0.001	0.085	-0.067	<0.001	0.001	0.041	0.211	0.279	0.441
		Change r^2	0.001	0.002	<0.001	<0.001	0.002	0.001	0.004	0.001	0.001
		Total r^2	0.551	0.479	0.067	0.434	0.542	0.537	0.035	0.503	0.542

*p<0.05 for the included fitness variable.

Unstandarized Beta (B) coefficients and r-square for each of the fitness predictors adjusting by age and sex.

(B)=Unstandarized Beta; SLJ=Standing long jump; Tot.BMC=Total bone mineral content; Tot.Area=Total area; Tot.vBMD=Total volumetric bone mineral density; Crt.Thick=Cortical thickness; Crt.BMC=Cortical bone mineral content; Crt.Area=Cortical area; Crt.vBMD=Cortical volumetric bone mineral density; SSIPOL=Polar strength strain index; FRAC_X=Fracture load in the X axis.

Table 4 - Anthropometric and fitness differences among cluster groups

	STRONG (n=37)	FIT (n=29)	UNFIT (n=26)
Age (y)	4.88±0.72	4.80±0.71	4.71±0.89
Weight (kg)	18.51±2.85	18.59±2.63	18.73±2.91
Height (cm)	107.27±6.63	107.69±5.25	106.04±8.19
BMI (kg/m^2)	16.01±1.24	15.97±1.27	16.55±0.99
Tibia length (mm)	228.32±18.60	228.52±17.33	226.08±22.78
Sex males (%)/females (%)	19 (51%)/18 (49%)	16 (55%)/13(45%)	15 (58%)/11 (42%)
School year (1/2/3)	6/19/12	8/11/10	9/10/7
Physical fitness			
Mean handgrip (kg)	7.46±2.329*	7.19±2.42*	5.68±2.39
Relative handgrip (kg/weight)	0.40±0.10*	0.38±0.09*	0.30±0.10
Standing long jump (cm)	87.19±20.02*	85.48±19.99*	69.65±19.14
Speed run time 4x10 (s)	16.33±1.92*	15.36±1.76*#	17.98±2.46
Standing one leg (s)	16.00±12.42	15.03±10.40	12.48±13.73
Laps (number of laps)	16.84±8.09	31.31±11.21*‡	13.42±7.40

*Significant difference with the UNFIT group ($p<0.05$)

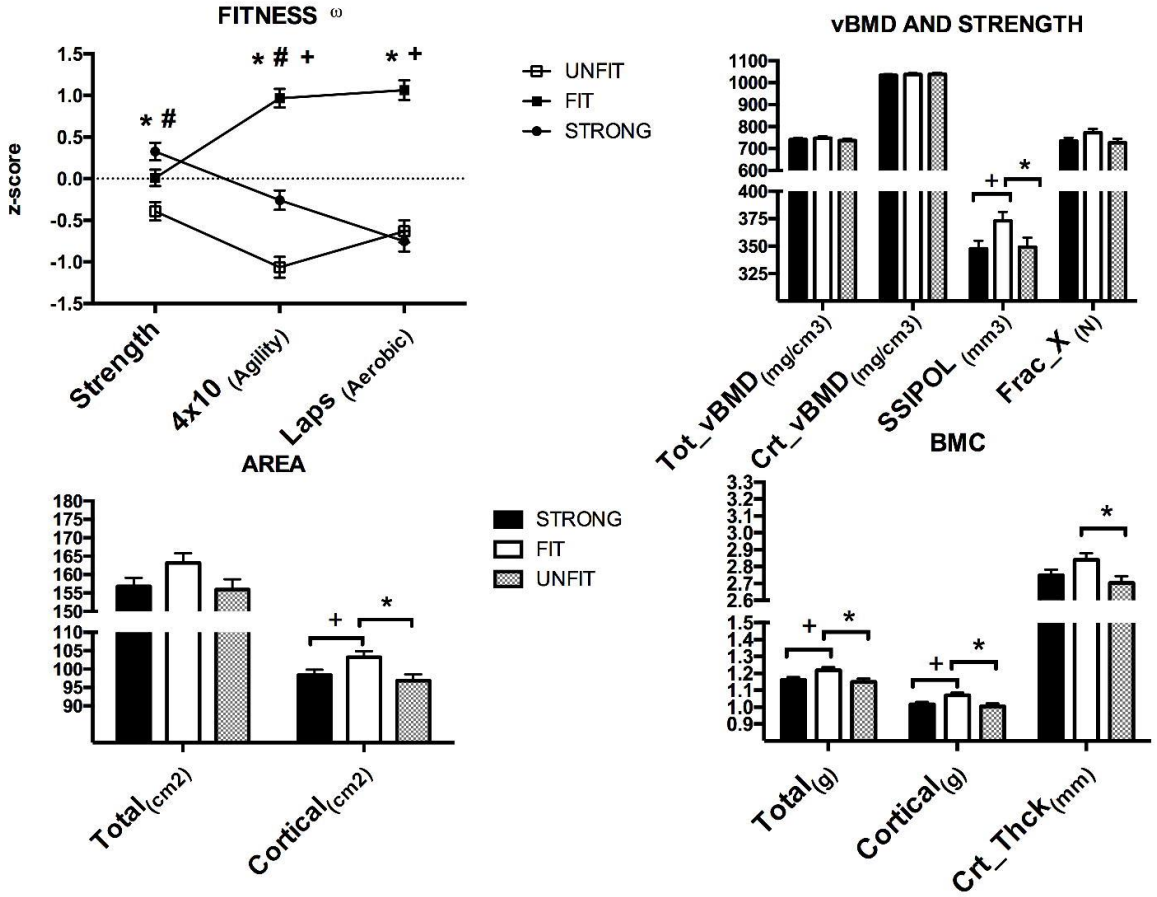
‡Significant difference between the STRONG and FIT groups ($p<0.05$)

#Tendency towards a difference between the STRONG and FIT groups ($p=0.06$)

School year (1/2/3) indicates the number of participants in each group from year 1, year 2 and year 3.

BMI=Body mass index

Figure 1 - Physical fitness z-scores and adjusted by tibia length and age bone content, structure and strength according to cluster group



ω= Agility z-scores were inverted with lower z-scores representing low agility values

*****=Significant difference between FIT and UNFIT groups

#=Significant difference between STRONG and UNFIT groups

+=Significant difference between FIT and STRONG groups

similar values than the STRONG group for the strength variables, and also presented high values for both speed-agility and Laps. Finally, Cluster 3 was labelled as UNFIT as it showed the lowest values for strength and agility and similar values for Laps than the STRONG group. Statistical differences among the groups are described in Figure 1. Descriptive characteristics and group composition are displayed in Table 4.

Regarding bone variables, adjusted age and tibia length results are presented in Figure 1. No differences were found among groups for total and cortical vBMD, total area and FRAC_X. The FIT presented higher values than the STRONG and UNFIT for the SSIPOL, cortical area and total and cortical BMC (all $p<0.05$; Figure 1). Additionally, FIT also presented higher values than the UNFIT for cortical thickness ($p<0.05$; Figure 1). No differences were found between the STRONG and UNFIT for any of the measured bone variables.

DISCUSSION

Bone sexual dimorphism

No bone sexual dimorphism, as reported later in life by previous studies (119,120), was found in our study. Very few studies have evaluated bone structure in the same age range sample, with Moon et al. (121) who measured at the 38% of the non-dominant tibia also finding no differences between 6 year-old boys and girls. Binkley and Specker (109) in a study aiming to evaluate the validity of pQCT measures in 3 to 4 year olds reported no differences between boys' and girls' tibial values with the same device used in the present study (Stratec XCT 2000). These findings, in line with ours, suggest that BMC, vBMD and bone structural sexual dimorphism could emerge later in life, although further research is needed in this age range population to confirm our results.

Associations between physical fitness and bone

The findings of the present study suggest that physical fitness might modify bone structure without stimulating vBMD, which is in line with previous studies. For example, Schöenau (122) evaluated the association of handgrip and bone strength in 6 to 13 year-old children finding age-dependent increases in bone strength indexes, cross-sectional and cortical areas without increases in vBMD, and more importantly, finding positive associations between handgrip and structural parameters without associations between handgrip and vBMD. Similar results can be found in older populations, for example when comparing the dominant radius to the non-dominant radius of tennis players (mean age 30 years). In this regard, Haapsalo et al. (123) found significant differences between both limbs for most of the structural variables without finding differences in vBMD. These findings, in line with ours, suggest that physical fitness stimulates bone structure and not vBMD. Increases in cortical thickness and in cross-sectional areas entail increases in areal BMD as measured with DXA, which would explain why so many studies have found benefits of exercise interventions on areal BMD (53,124).

The negative results of the balance test were expectable as Cadenas-Sanchez et al. (108) recently published a study evaluating the reliability of the tests included in the PREFIT battery showing a low reliability for the one leg balance test, suggesting that this test should be eliminated from the battery. Nonetheless, because data for the present study were already collected before the publication of the aforementioned study it was decided to present all the collected physical fitness data and then explore possible associations with bone variables. Similarly, results from the adapted shuttle run test, which is performed to test cardiorespiratory fitness, did not predict any of the measured bone variables. Cardiorespiratory fitness has shown controversial results when evaluating its association with bone variables in previous studies. Some researchers have found positive associations

between cardiorespiratory fitness and bone variables measured with DXA in cross-sectional studies evaluating adolescents (125,126), while others in longitudinal studies found no association between cardiorespiratory fitness and the enhancement of bone mass (127). Data from long follow-up studies suggest that only neuromotor fitness (muscular strength and speed), is related to BMD at adulthood (128,129).

Although there is no clear explanation for the controversial results found when examining literature regarding cardiorespiratory fitness and bone, it is possible that if, instead of analyzing each fitness component separately, a holistic analysis was performed clearer results would emerge. The cluster analysis results support this idea, as those classified as FIT who presented higher cardiorespiratory levels than both the STRONG and UNFIT, presented enhanced bone variables. These results are similar to those found by Duckham et al. (103) who evaluated 7 to 9 year-old children finding that those classified as fit presented better structural bone variables than those classified as unfit. Summarizing, these results suggest that although when independently assessed, muscular fitness and agility are determinant to bone mass, cardiorespiratory fitness is also important in preschool children. The fact that no differences were found between the STRONG and UNFIT groups for any of the measured bone variables suggests that for preschool children global fitness (high levels of all the fitness variables), is more important than just high muscular strength.

Similar results have been found in children (103) and adolescents (99), where physical fitness has been shown to be critical to bone health. Additionally, previous studies have shown that differences in physical fitness during adolescence can lead to differences in BMD during adulthood[2]. This might suggest that the differences in bone health found already at the preschool stage could be retained through childhood and adolescence or that those who are active at young ages are more likely to stay active during adolescence and

consequently present an improved bone mass during their entire life. Further, long-term longitudinal studies are needed to confirm this hypothesis.

It is important to notice that similarly to previous studies developed in preschool children (130) and older children and adolescents (131), we found a high number of pQCT blurred scans. When comparing our results to previous studies it seems like the percentage of moved scans decreases with an increase of participants' age, as in studies developed with younger participants the percentage of loss was found to be even higher (51% of scans presented poor quality) (130), while in studies including older participants the percentage of moved scans seems to decrease as Moon et al. (121) and Cole et al. (132) found a 20.5% and 16% of moved scans respectively when measuring the diaphyseal tibia in 6 to 7 year-olds. It is important to acknowledge this loss of scans in our study and in previous ones due to movement so that further studies aiming to evaluate bone structure with pQCT in young children take this sample loss into account for sample size calculations.

Strengths and limitations

Although the study presents several strengths such as the measurement of tibia structure with a pQCT and the assessment of physical fitness with a validated battery it is not exempt of limitations. Firstly, this is a cross-sectional study, and therefore we cannot conclude that an increase in any of the physical fitness variables would improve bone structure or strength, secondly, physical activity and nutrition that could affect bone variables were not registered and finally, some physical fitness tests like the balance test and the long jump test have shown poor reliability in previous studies developed in similar age-samples (108).

Conclusions

Regarding the influence of fitness on bone, relative upper- and lower-muscular strength, and speed-agility predicted all the measured bone variables except for vBMD. Although

balance and cardiorespiratory fitness did not directly influence any of the measured bone variables, high cardiorespiratory fitness was one of the main characteristics of the FIT group that presented higher bone values than the STRONG and UNFIT groups. These results suggest that global fitness is a key determinant to bone structure and strength, but not to vBMD in preschool children. Consequently, performing physical fitness tests could provide useful information related to bone health in preschoolers. Bone mass sexual dimorphism has still not emerged in 3 to 6 year olds. Further similar studies with preschoolers are needed in order to corroborate the present results.

4.2 Frequency and duration of vigorous physical activity bouts are associated with adolescent boys' bone mineral status: A cross-sectional study

ABSTRACT

Purpose: Vigorous physical activity (VPA) has been proven to promote osteogenesis in adolescents; however, the specifics of the optimal pattern of frequency and duration of VPA are unknown. The main goal of the present study was to analyze the associations of different length of VPA bouts with bone health.

Methods: 180 healthy male adolescents (11-13 years) had their bone mineral content and density assessed by dual-energy X-ray absorptiometry scans at the whole body, femoral neck (FN) and lumbar spine and their physical activity measured by an accelerometer during one week.

Results: VPA was the intensity with the strongest associations with bone mineral parameters especially at the FN. Subjects whose longest VPA bout was 5 minutes or above had higher FN bone mineral density (BMD) than those who did not complete any 5-minute bout and these differences were greater with participants who reached 15 consecutive minutes of VPA (>15': 0.977 ± 0.020g/cm2; 5'-15': 0.907 ± 0.009g/cm2; <5': 0.876 ± 0.009g/cm2; all $p<0.05$). When comparing the relevance of VPA bouts and volume of physical activity, the group with low volume and having a VPA bout had better FN BMD compared to the group with high volume but no VPA bout. Additionally, the group with both high volume and VPA bout showed better FN BMD than the rest of the groups.

Conclusions: VPA may be the most effective activity intensity to improve bone mineral density and content of adolescent boys, with greater benefits if VPA periods either long or frequent.

Keywords: Puberty; Osteoporosis; Osteopenia; Prevention; DXA, Physical activity bouts

INTRODUCTION

Osteoporosis, a disease characterized by a low bone mineral density (BMD), shows a prevalence for people above 50 years of age of 6.6% for men and 22.1% for women in the European Union (30) and 16% and 29.9% for men and women respectively in the United States (32). This bone fragility results in a reduced tolerance to stress and an increased fracture risk (29). Osteoporotic fractures can, in turn, lead to reduced functionality, disability and even death (35). Osteoporosis is already an economical burden for healthcare systems which is expected to rise in the coming years due to demographic changes (30). Therefore, osteoporosis management and prevention is a main concern for developed countries (34,133).

One of the key strategies for preventing osteoporosis relies on maximizing the peak BMD (44,134) reached in early adulthood, since an increase of only 10% on this parameter has been shown to delay osteoporosis up to 13 years (135). Adolescence is a critical stage for bone development, as 40% of adult bone mass is accumulated during the pubertal years (18). It is estimated that around 60% of the interindividual variability in bone mass is due to endogenous factors such as race, sex or genetics (44). Within the controllable factors that affect bone mass accrual, weight-bearing physical activity (PA) is among the ones with the highest impact and therefore an adequate management of this behaviour is crucial for a healthy bone development (65).

There are several reviews that show strong and consistent positive effects on bone development of sports practice (55,56), exercise interventions (54,136) and even participation in unstructured weight-bearing PA (51,52) during childhood and adolescence. Moreover, structural benefits obtained during puberty are retained later in life (58,59,137).

High-impact PA has been proven to be effective for improving BMD even in subjects with a worse genetic predisposition for bone development (57).

Vigorous weight-bearing physical activity (VPA) seems to be the most effective intensity for bone growth (60,61), even though some associations have been found among bone mineral parameters and total, light or moderate PA as well, whereas the negative influence of sedentary time has been observed (62,63). Apparently, complying with the general recommendations of PA during childhood and adolescence may not be enough to guarantee an adequate bone development (64) and hence, specific recommendations of daily VPA have been proposed (62,138). However, frequency, duration and distribution of VPA may alter the effectiveness of the specific adaptations (66). Nonetheless, to our best knowledge, this has only been studied in animals (139) and in bone strength parameters at the tibia (66). A better understanding of the optimal pattern of VPA for the improvement of adolescent bone health may be decisive for the development of PA recommendations and exercise interventions aimed for the prevention of osteoporosis.

Therefore, the main goal of the present investigation was to analyze the repercussion of objectively measured physical activity patterns on bone mass in healthy adolescents, focusing on the analysis of vigorous physical activity bouts and the overall volume of physical activity.

METHODS

Participants and study design

A total of 264 healthy Caucasian boys between 11 and 13 years of age from different schools in Tartu and its surroundings participated in this cross-sectional study between October 2010 and March 2011 (140–142). The tests included anthropometry and bone mineral measurements, sexual maturation evaluation and physical activity registration.

Participants and parents completed a questionnaire of about the child´s general health and development. Boys who had their participation in physical education classes restricted by a clinician (due to either chronic illness or medication contraindications) were excluded from the study, as well as those participants taking medications known to affect bone. No participant reported smoking or participating in weightlifting training.

The Human Ethical Committee of the University of Tartu, Estonia, approved the study protocol (179/T-4), which complied with the Declaration of Helsinki (revised in Fortaleza 2013). All the participants and their parents were given a full written description of the study characteristics and provided signed informed consent prior to the beginning of the tests. The subjects of the present study were part of a longitudinal study cohort where boys were followed until they reached pubertal maturity.

Anthropometry, maturation and bone mineral measurements

Height was measured to the nearest 0.1 cm using Martin's metal anthropometer and body mass to the nearest 0.05 kg with a medical scale (A&D Instruments Ltd; Abingdon; UK) while wearing underwear and no shoes. All anthropometric measurements were performed by the same technician and following the International Society for the Advancement of Kinanthropometry protocol. Body mass index was calculated dividing the body mass by the height squared (kg/m2). In order to assess maturation status, skeletal age was determined with an X-ray scan at the left hand and wrist, according to the procedure of Greulich and Pyle (79,80).

Bone mineral content (BMC, g) and density (BMD, g/cm2) were measured at the whole body (WB), lumbar spine (L2 to L4, LS) and femoral neck (FN) using dual-energy X-ray absorptiometry (DPX-IQ densitometer, Lunar Corporation, Madison, WI, USA). The measurements were performed in the medium scan mode, with standard positioning and

participants wearing light clothing. The same examiner evaluated all the scans and results using the extended analysis option from the proprietary software, version 3.6. Coefficients of variation were established by duplicate measurement following repositioning of 20 boys and were below 2% for bone mineral measurements.

Physical activity assessment

In order to evaluate physical activity (PA) participation, participants wore a uniaxial accelerometer (GT1M Actigraph, Monrovia, CA, USA) during one week on their right hip. Data were aggregated into 15-second epoch periods and all intervals of 20 or more minutes with zero counts were excluded from the register (143,144). Days in which the total duration of registered data was shorter than 10 hours were considered invalid. Finally, only registers with a minimum of three valid days (at least one of them during weekend) were included in the final analysis.

The intensity of the PA was established according to the Evenson cutpoints (sedentary ≤100 counts/min, light >100 counts/min, moderate ≥2296 counts/min and vigorous ≥4012 counts/min) (81,145). According to the original study in which this specific cutpoints were described, one example of sedentary activity would be sitting in a chair, slow walking would be classified as light PA, stair climbing or brisk walking as moderate PA, and bicycling or running as VPA (81). In order to analyze the distribution of VPA, the presence of VPA bouts was evaluated for each participant. To analyze the distribution of VPA over time, the consecutive periods (bouts) of VPA were analyzed. In order to be included in the analysis, the minimum duration of a bout was set at five consecutive minutes of VPA, with a tolerance of one epoch.

If participation in light and moderate PA was taken into consideration along with the VPA bouts, four groups can be defined. Participants were classified as either high or low volume

according to the median value of light and moderate PA participation and as "VPA bout" or "no VPA bout" regarding whether or not they had at least one 5-minute bout of VPA during the measurement period (Supplementary table 1).

Statistical analysis

Statistical analyses were carried out with SPSS v22.0 for Windows (Chicago, IL, USA). After confirming the normality assumption with Kolmogorov-Smirnov tests, variables were explored for the presence of outliers. Descriptive statistics were obtained for anthropometric, physical activity and bone mineral variables and presented as mean ± standard deviation. Partial Pearson's correlation coefficients among bone mineral and physical activity variables were calculated after adjusting by body mass and skeletal age. Receiver-operator characteristics (ROC) curves analysis was performed to evaluate the adequacy of physical activity parameters for predicting bone mineral outcomes. Analysis of covariance was applied to assess differences in bone mineral status between different PA participation profiles, with Bonferroni post-hoc tests. Statistical significance was set at $p<0.05$.

RESULTS

From the original sample of 264 subjects, a total of 180 participants completed all body composition measurements and provided valid accelerometry data to fulfill inclusion criteria. The anthropometric characteristics, physical activity participation and bone mineral parameters of the sample are presented in table 1. No differences in height, weight, BMI and both skeletal and chronological age were found between excluded and included boys (data not shown).

Table 1 – Descriptive characteristics of the sample (n=180) [a]

Anthropometry	
Chronological age (y)	12.07 ± 0.69
Skeletal age (y)	11.87 ± 1.06
Height (cm)	154.6 ± 7.7
Body mass (kg)	47.1 ± 12.5
BMI (kg/m^2)	19.5 ± 4.1
Physical activity	
Sedentary time (min/d)	544.3 ± 76.0
Light PA (min/d)	237.6 ± 47.5
Moderate PA (min/d)	40.3 ± 14.2
Vigorous PA (min/d)	19.5 ± 13.6
Total PA (counts/min)	486.3 ± 149.1
Bone mineral parameters	
WB BMC (g)	1702 ± 346
WB BMD (g/cm^2)	0.976 ± 0.066
FN BMC (g)	4.067 ± 0.642
FN BMD (g/cm^2)	0.899 ± 0.092
LS BMC (g)	26.87 ± 6.22
LS BMD (g/cm^2)	0.822 ± 0.091

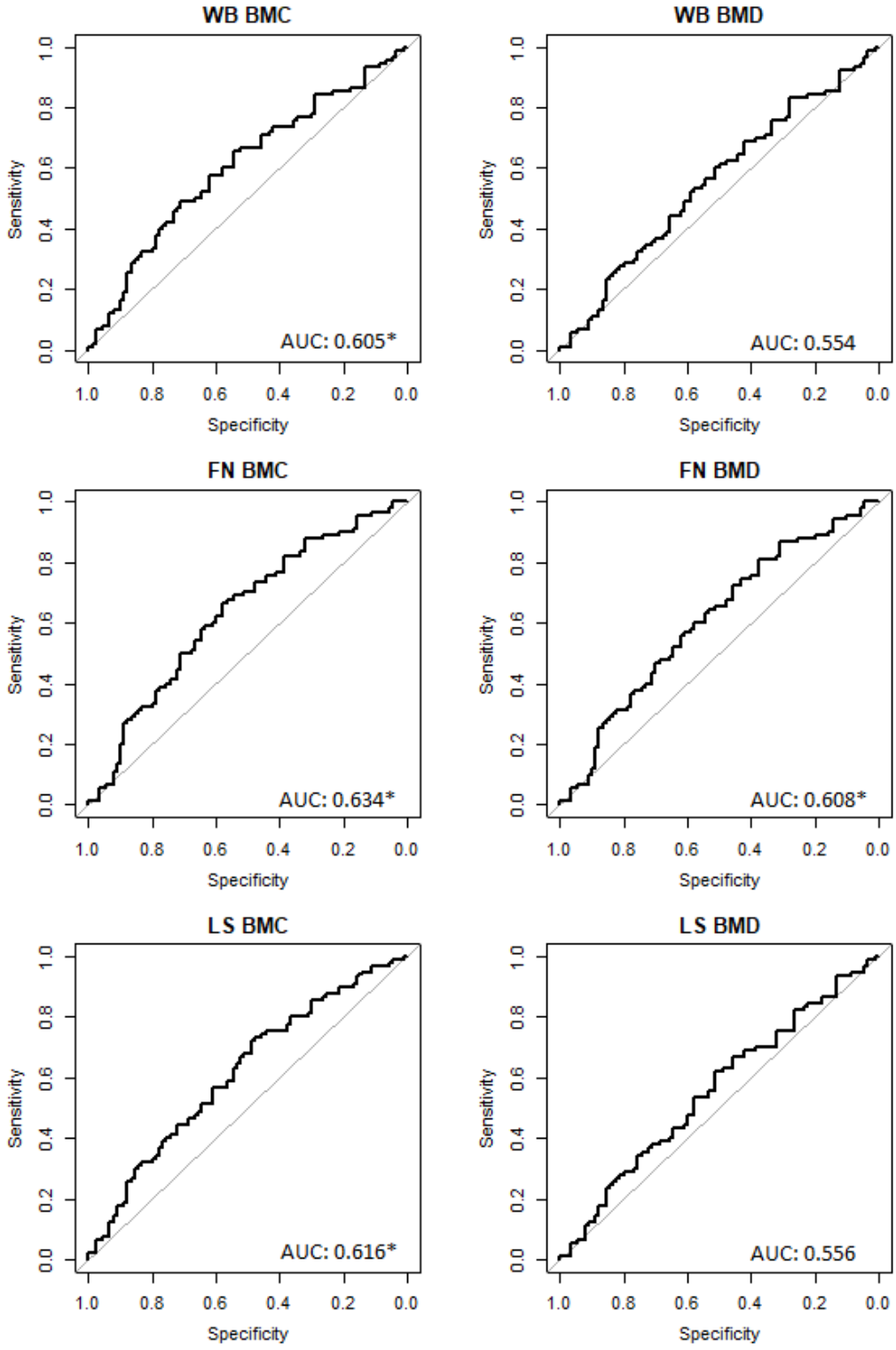
BMI: Body mass index; PA: Physical activity
WB: Whole body; FN: Femoral neck; LS: Lumbar spine
BMC: Bone mineral content; BMD: Bone mineral density
[a] Values are expressed as mean ± standard deviation

Table 2 – Partial correlation coefficients between physical activity intensities and bone mineral parameters, adjusted by body mass and skeletal age (n=180) [a]

	Sedentary (min/d)	Light PA (min/d)	Moderate PA (min/d)	Vigorous PA (min/d)	Total PA (counts/min)
WB BMC (g)	-0.045	-0.049	0.094	**0.234**	**0.193**
WB BMD (g/cm^2)	-0.089	-0.081	0.087	0.141	**0.168**
FN BMC (g)	-0.127	0.042	**0.254**	**0.364**	**0.337**
FN BMD (g/cm^2)	-0.108	0.039	**0.238**	**0.317**	**0.308**
LS BMC (g)	0.041	-0.047	0.065	**0.201**	0.138
LS BMD (g/cm^2)	0.042	-0.133	0.000	0.096	0.074

[a] Bold characters indicate significant correlations ($p<0.05$)
PA: Physical activity; WB: Whole body; FN: Femoral neck; LS: Lumbar spine
BMC: Bone mineral content; BMD: Bone mineral density

Figure 1 - ROC curves for VPA (min/day) as a predictor of bone mineral parameters



*AUC significantly greater than 0.5 ($p<0.05$)
ROC: receiver operator characteristics VPA: vigorous physical activity
WB: Whole body; FN: Femoral neck; LS: Lumbar spine
BMC: Bone mineral content; BMD: Bone mineral density

Table 2 shows the relationships among physical activity, BMC and BMD at different sites, after adjusting by body mass and skeletal age. The skeletal site that showed the highest correlation with PA participation was the femoral neck, where both BMC and BMD presented significant correlations with moderate and VPA as well as with the total PA (all $p<0.05$). Moreover, VPA was positively correlated with whole body and lumbar spine BMC and total PA was related to whole body BMC and BMD (all $p<0.05$).

Body mass and maturation-adjusted median bone mineral values were used to classify participants according to their current bone health status. After performing ROC curves analysis for all activity intensities in each skeletal site, only VPA yielded significant results for whole body, femoral neck and lumbar spine BMC and femoral neck BMD (area under the curve (AUC) of 0.605, 0.634, 0.616 and 0.608 respectively, figure 1). Daily time spent at different physical activity intensities was compared between those subjects who were above the median for all bone mineral parameters and those who were below the median in all of them. Particpants who had bone mineral parameters above the median at all regions (categorized as "good overall status") spent more time in VPA and had a higher overall physical activity participation compared to those labelled as "bad overall status", who were consistently below the median in all six bone variables ($p<0.05$; table 3).

Adolescents who engaged in VPA for five consecutive minutes at least once during the testing period had higher BMC and BMD at the femoral neck and the whole body compared to those who didn't ($p<0.05$; Table 4). However, if the frequency of the participation in these 5-minute VPA bouts was less than once for every three days, the differences disappeared. Additionally, further improvements were observed in those boys who extend their practice of VPA for at least 15 consecutive minutes (all $p<0.05$; table 4).

Table 3 – Physical activity profile comparison between bone mineral status categories [a]

	Bad overall status (n= 66)	Good overall status (n= 63)
Sedentary time (min/d)	551.6 ± 69.6	540.1 ± 75.2
Light PA (min/d)	234.2 ± 43.2	241.5 ± 56.0
Moderate PA (min/d)	38.5 ± 13.4	41.2 ± 14.1
Vigorous PA (min/d)	17.0 ± 12.2	22.5 ± 14.3*
Total PA (counts/min)	456.0 ± 127.8	516.9 ± 168.6*

[a] Values are expressed as mean ± standard deviation
*Significant differences between the groups ($p<0.05$)
PA: Physical activity

Table 4 – Bone mineral parameters after adjustment by weight and biological age in relation with VPA bouts occurrence, frequency and duration [a]

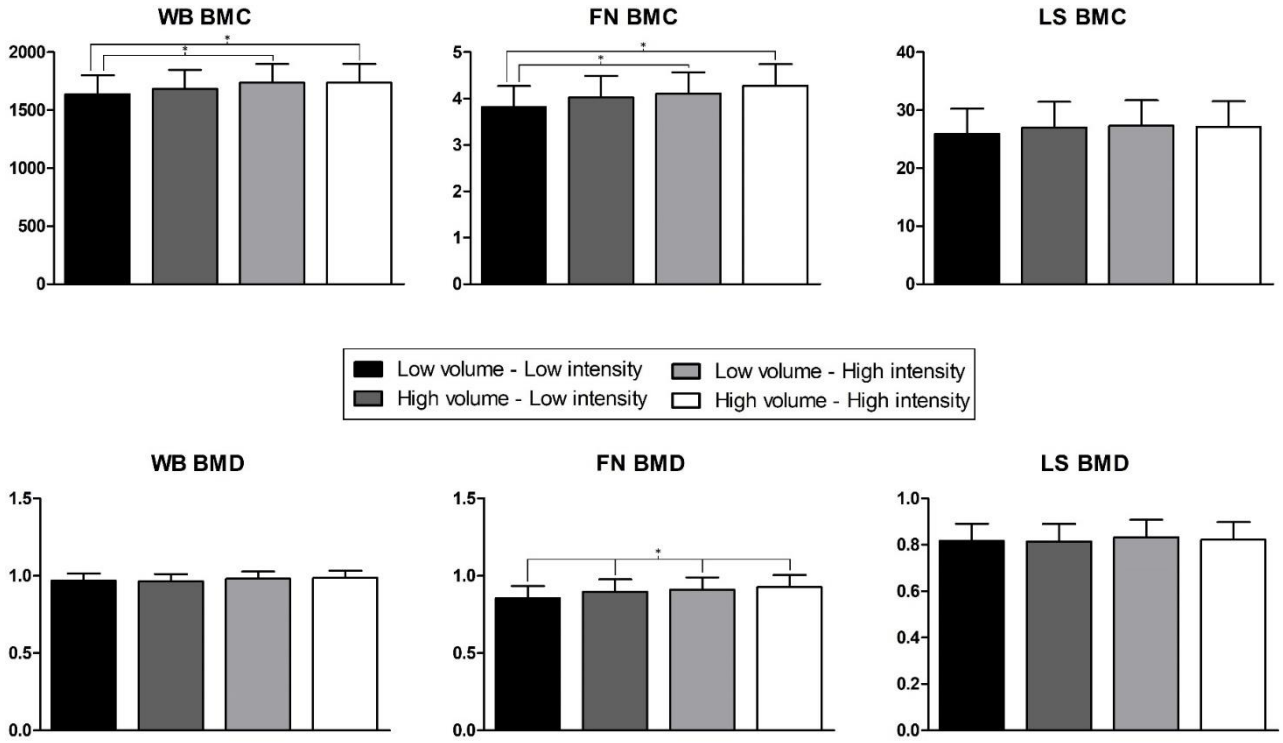
Existence of any 5-min VPA bout	No (n=83)	Yes (n=97)
WB BMC (g)	1662 ± 18	1737 ± 17*
WB BMD (g/cm^2)	0.967 ± 0.005	0.984 ± 0.005*
FN BMC (g)	3.92 ± 0.05	4.19 ± 0.05*
FN BMD (g/cm^2)	0.876 ± 0.009	0.918 ± 0.008*
LS BMC (g)	26.46 ± 0.49	27.24 ± 0.45
LS BMD (g/cm^2)	0.816 ± 0.008	0.828 ± 0.008

Frequency of 5-min VPA bouts	No bout (n=83)	Less than once every 3 days (n=41)	At least once every 3 days (n=56)
WB BMC (g)	1662 ± 18	1723 ± 25	1748 ± 22*
WB BMD (g/cm^2)	0.967 ± 0.005	0.979 ± 0.007	0.988 ± 0.006*
FN BMC (g)	3.92 ± 0.05	4.07 ± 0.07	4.28 ± 0.06*
FN BMD (g/cm^2)	0.876 ± 0.009	0.903 ± 0.012	0.93 ± 0.011*
LS BMC (g)	26.46 ± 0.49	26.8 ± 0.69	27.56 ± 0.59
LS BMD (g/cm^2)	0.816 ± 0.008	0.826 ± 0.012	0.83 ± 0.01

Maximum VPA bout duration	No bout (n=83)	Less than 15 min (n=81)	At least 15 min (n=16)
WB BMC (g)	1662 ± 18	1730 ± 18*	1773 ± 41*
WB BMD (g/cm^2)	0.967 ± 0.005	0.981 ± 0.005	1.000 ± 0.011*
FN BMC (g)	3.92 ± 0.05	4.14 ± 0.05*	4.46 ± 0.12*†
FN BMD (g/cm^2)	0.876 ± 0.009	0.907 ± 0.009*	0.977 ± 0.020*†
LS BMC (g)	26.46 ± 0.49	27.04 ± 0.49	28.24 ± 1.10
LS BMD (g/cm^2)	0.816 ± 0.008	0.827 ± 0.008	0.832 ± 0.019

[a] Values are expressed as mean ± standard error
* Significant differences compared to the group with no bouts ($p<0.05$)
† Significant differences compared to the group "less than 15 min" ($p<0.05$)
VPA: Vigorous physical activity WB: Whole body; FN: Femoral neck; LS: Lumbar spine
BMC: Bone mineral content; BMD: Bone mineral density

Figure 2 - Body mass and biological age-adjusted bone mineral content (g) and density (g/cm^2) across different PA patterns



* Significant differences between groups ($p<0.05$)

WB: Whole body; FN: Femoral neck; LS: Lumbar spine BMC: Bone mineral content; BMD: Bone mineral density

Four different groups were defined according to the volume and intensity of PA. The comparison among these groups is presented in table 5, which indicates that there were significant differences in femoral neck BMD among all four groups showing that the higher the volume and the intensity the higher the bone parameters (all $p<0.05$, figure 2). Additionally, both "VPA bout" groups showed higher values of whole body and femoral neck BMC compared to the "low volume - no VPA bout" group (all $p<0.05$; table 5).

Table 5 – Body mass and biological age-adjusted bone mineral parameters across different PA patterns [a]

	Low volume No VPA bout (n=41)	High volume No VPA bout (n=42)	Low volume VPA bout (n=49)	High volume VPA bout (n=48)
WB BMC (g)	1640 ± 25	1683 ± 25	1737 ± 23*	1738 ± 24*
WB BMD (g/cm^2)	0.969 ± 0.007	0.965 ± 0.007	0.981 ± 0.006	0.987 ± 0.007
FN BMC (g)	3.81 ± 0.07	4.03 ± 0.07	4.11 ± 0.07*	4.28 ± 0.07*
FN BMD (g/cm^2)	0.855 ± 0.012	0.897 ± 0.012*	0.91 ± 0.011*†	0.926 ± 0.011*†‡
LS BMC (g)	25.9 ± 0.7	27 ± 0.7	27.3 ± 0.6	27.1 ± 0.6
LS BMD (g/cm^2)	0.817 ± 0.012	0.815 ± 0.012	0.833 ± 0.011	0.823 ± 0.011

* Significant differences compared to the "low volume – no VPA bout" group ($p<0.05$)
† Significant differences compared to the "high volume – no VPA bout" group ($p<0.05$)
‡ Significant differences compared to the "low volume – VPA bout" group ($p<0.05$)
PA: Physical activity; WB: Whole body; FN: Femoral neck; LS: Lumbar spine
BMC: Bone mineral content; BMD: Bone mineral density
[a] Values are expressed as mean ± standard error

DISCUSSION

The main finding of the present study was the acknowledgement of the importance of accumulating VPA activity in continuous periods of at least 5 minutes for the improvement of whole body and femoral neck bone health in male adolescents.

As expected, among the different intensities of PA, VPA presented the strongest relation with bone mineral parameters; being the only intensity with statistically significant relationships with whole body and lumbar spine BMC values and the highest correlation coefficients with femoral neck BMC and BMD. These results are in agreement with the

current evidence regarding the effect of PA on bone health, which points towards VPA or moderate-to-vigorous PA as the ideal intensities to elicit bone growth (60,146,147). It should be noted that overall PA also indicated significant relationships with whole body and femoral neck BMC and BMD, suggesting that PA volume may also play a role in bone development. Similar results have been reported in other studies (83,148) and this relationship may be exclusive to boys (148).

The skeletal region that seemed to be more affected by all moderate, vigorous and overall PA was the femoral neck. This site is of special importance for osteoporosis prevention, since it is one of the most prevalent locations of osteoporotic fractures (30). Moreover, the detection of osteoporosis is based on the measurement of femoral neck BMD (149). These results may be due to the highest responsiveness of cortical bone in contrast to trabecular bone (150,151). However, this cannot be inferred from our results, as the methods used for bone mineral assessment do not allow for the differentiation between cortical and trabecular bone.

Regarding the ROC curves analyses, even though VPA reached statistical significance for the classification of bone mass status, the correspondent AUC were low and therefore the accuracy of VPA to stratify the sample according to bone mineral parameters was limited. It has been proposed that VPA and moderate-to-vigorous PA are best fit to identify those individuals with excellent bone health (>2 SD above the mean) rather than those with poor bone mineral status (>1 SD below the mean) (64). Unfortunately, we were not able to test these results within our sample, since only 5 participants presented BMC values corresponding to the excellent category. However, when grouping the sample according to the global bone mass status, higher levels of vigorous and total PA were found among those subjects who were consistently above the median value for BMC and BMD at every

skeletal site than those participants that were consistently below the median. This finding further highlights the importance of VPA for bone mass of male adolescents.

It has been suggested that not only the time spent in VPA but also the pattern of this activity might be relevant for bone accretion during puberty (66). In order to test that, accelerometry registers were scanned and all periods of at least five consecutive minutes of VPA were retrieved for each subject. Both the quantity and the maximum duration of these periods showed a positive association with whole body and femoral neck BMC and BMD within the sample. More specifically, it seems that it is necessary to participate in VPA bouts at least once every three days for 5 consecutive minutes in order to benefit from this improvement. Additionally, those participants who engaged in VPA for longer periods (15 consecutive minutes or more) had even higher bone mass and density at the femoral neck and at the whole body. These results suggest that in order to optimize the beneficial effects of PA on bone health when developing PA recommendations or designing exercise interventions, VPA practice should be aggregated in periods of at least 5 minutes, taking into account that if it is possible to extend the duration or frequency, better results should be expected.

To our knowledge, one study has analyzed the relative importance of light and moderate to VPA and found that those subjects with a higher proportion of moderate-to-vigorous PA presented higher values of subtotal BMC, BMD and bone area (152). In order to analyze the importance of volume and intensity of physical activity the sample was split according to the presence of at least one 5-min VPA bout and then both groups were further subdivided using the median score of the daily minutes of light and moderate PA. The relative homogeneity of the sample sizes of the different groups should be highlighted, since it shows that PA volume and intensity may be considered as independent entities. According to our results, all four groups differed in their femoral neck BMD content with

the “high volume – VPA bout” group presenting the highest values, followed by the “low volume – VPA bout” category. It is interesting to note that in comparison to the “low volume – no VPA bout” group, only those adolescents in the “VPA bout” groups showed higher values of whole body and femoral neck BMC, whereas the “high volume – no VPA bout” category did not present these improvements. According to these results, an increase in both volume and intensity of PA can be beneficial for the least active subjects, but the focus should be put in participating in VPA.

This study has some limitations that should be acknowledged. The cross-sectional design of the study did not allow to infer causal relationships. Also, the lack of a nutritional assessment made controlling the calcium intake impossible. Moreover, as it has been mentioned, even though a reference standard method was used for the bone mineral assessment it does not allow to differentiate between cortical and trabecular tissue and therefore bone structure was not analyzed. Additionally, the impossibility of analyzing the raw acceleration data directly hindered the precise evaluation of the number of impacts at different intensity levels and its effects on bone structure, as suggested by Vainionpää et al. (153). It should also be taken into account that these results are only applicable for early pubertal boys. Research is needed to clarify if these effects are also present in girls, since they follow a different maturation process. Further research could also clarify the specific effects of VPA bouts on cortical and trabecular bone and longitudinal studies could analyze the evolution of BMC and BMD in relation to diverse VPA patterns.

Conclusions

Taking into account that both volume and intensity of PA are relevant, VPA seems to be the most effective activity intensity to improve bone mineral density and content of adolescent boys, especially at the femoral neck. These benefits appear when VPA is

accumulated in uninterrupted periods of 5 minutes for at least once every three days and can be even greater if the duration is extended to 15 minutes of consecutive VPA.

Funding

JMP received a Grant FPU014/04302 from 'Ministerio de Educación Cultura y Deportes'. The present study was funded by the Estonian Ministry of Education and Science (grant number IUT 20-58).

4.3 Vigorous physical activity patterns affect bone growth during early puberty in boys

ABSTRACT

Purpose: The main purpose of the study was to analyze the effects of different patterns of vigorous physical activity (VPA) on bone development.

Methods: One-week accelerometry registers and dual-energy X-ray absorptiometry scans at the whole body, hip and lumbar spine of 140 healthy male adolescents (11-13 years, at baseline) were performed twice with a one-year interval between measurements.. Four patterns of VPA evolution ("low-low", "low-high", "high-low" and "high-high") and three patterns of bone growth ("optimal", "mean" and "reduced") were defined according to the median participation in VPA and a cluster analysis of the longitudinal changes in BMC and BMD in all sites measured, respectively. Bone mineral parameters were adjusted for skeletal age and body weight prior to statistical comparison among groups.

Results: Participants in the "low-high" group had greater adjusted BMD increases at both the femoral neck and lumbar spine when compared to the "low-low" group (estimated mean (95%CI): 0.066 (0.047-0.085) vs. 0.034 (0.021-0.047) g/cm2 and 0.074 (0.054-0.093) vs. 0.049 (0.035-0.062) g/cm2 respectively, both $p<0.05$). Femoral neck BMD adjusted increase was also different between the "high-high" and the "high-low" groups (0.053 (0.041-0.066) vs. 0.030 (0.011-0.049) g/cm2, $p<0.05$). Additionally, a higher percentage of "optimal" growth was found in the "low-high" group than in the "low-low" and "high-low" categories (36.3, 12.5 and 13.6% respectively, $p<0.05$).

Conclusions: Engaging in VPA as well as maintaining high levels of VPA during puberty are associated with greater gains in bone mass, which can have an impact in future bone health.

Keywords: Adolescence, accelerometry, growth profile, cluster analysis

INTRODUCTION

Osteoporosis is one of the most prevalent metabolic skeletal diseases, affecting an estimate of 27.5 million people in the European Union (30) and 10.2 million older adults in the United States (154). The osteoporotic patients suffer a decrease in bone mineral density (BMD) that implies a reduced tolerance to stress, and consequently, even a low-magnitude impact could cause a bone fracture (29). These fractures can result in reduced functionality, disability and even death (35). The management and prevention of osteoporosis is one of the main challenges for healthcare systems in developed countries (34).

Even though approximately 60-80% of bone mass is determined by genes or other unmodifiable factors (44,155), lifestyle choices play a crucial role in bone mass accrual and the optimization of its influence is vital for bone health (65). Maximizing the peak BMD attained in early adulthood (18) is an important strategy aiming osteoporosis prevention, as it is estimated that an increase of one standard deviation in peak bone mass could reduce later fracture risk up to 50% (156).

In a systematic review, the National Osteoporosis Foundation identified and graded the evidence of the effect of various lifestyle factors on peak bone mass and strength (16). Participation in physical activity (PA) and exercise showed the highest level of evidence for increasing peak bone mass and density (16). Additionally, there are several reviews that indicate that participation in unstructured weight-bearing PA and sports during childhood and adolescence has strong and consistent positive effects on bone development (52,157,158). It has been observed that mechanical stress stimulates an osteogenic response which can be the result of both muscle contraction and gravitational impact forces (159) both present in weight-bearing PA. This mechanical stress is required to have certain characteristics, and in order to maximize its effects on bone it should be dynamic, intense,

brief, intermittent and including unusual loading (160), suggesting that physical activity should be vigorous and varied.

Early puberty represents an optimal window of opportunity in order to increase of bone mass and strength that might not be present later in life (158). Moreover, it has been stated that PA participation undertaken during puberty may have greater positive effects than pharmacological interventions in adults with osteoporosis (158). It is important to highlight that PA practice during puberty may have long term positive effects, since benefits in bone mass and structure obtained during this stage are retained later in life (134,161,162).

In a recent review from Poitras et al. (52), 12 cross-sectional and eight longitudinal studies regarding the influence of PA on bone health during growth were described and analyzed. In an evaluation of more than 300 children in a 10-year longitudinal study it was observed that vigourous PA (VPA) correlated with bone mineral content (BMC) at weight bearing sites througought all the evaluations and therefore it was concluded that the main focus should be put in VPA interventions (146). Regarding the necessary amount of physical activity to achieve an osteogenic response it has been indicated that 3 hours per week are enough to elicit an increase in bone mass (65), whereas 32 minutes per day of VPA participation have shown benefits for the femoral neck BMD (64).

Only one of the previously mentioned studies longitudinal studies took into consideration the PA trajectories (163), understood as the longitudinal evolution of the PA participation. In this paper, moderate to vigorous PA trajectories were examined and related with changes in BMC, BMD and strength at various sites. It was found that a high participation in moderate to vigorous PA during childhood led to bone strength benefits in late puberty. A more detailed analysis of VPA evolution could help understanding the relationship between

PA activity and bone growth, since VPA is the activity intensity that is associated with higher changes in bone parameters (60,164).

The main objective of this investigation was, thus, to analyze the effects of different longitudinal PA participation patterns on bone growth in healthy, early pubertal boys. More specifically, the link between bone development and both present and previous particpation in VPA will be analyzed. This could help determining if bone response to VPA is dependent on previous activity history.

METHODS

Participants and study design

From the initial sample of 264 healthy boys between 11 and 13 years of age at baseline from different schools in Tartu (Estonia) that took part in this longitudinal study, 140 completed all measurements at both time points and were included in this study (figure A1). Participants were required to have no restriction from participating in PA by their physicians. The initial tests included anthropometry and bone mineral measurements, skeletal maturation evaluation and physical activity registration. These tests were repeated one year after the original measurements. The study protocol followed the ethical guidelines of the Declaration of Helisinki (revised in Fortaleza 2013) and was approved by the Human Ethical Committee of the University of Tartu, Estonia (179/T-4). Signed informed consent was retrieved from all the participants and their parents prior to the beginning of the tests. They were also given a full written description of the study characteristics before signing the informed consent.

Anthropometry and bone mineral assessment

Participants entered the anthropometric and bone mineral evaluation wearing light clothing and no shoes. Height was measured to the nearest 0.1 cm and body mass to the nearest 0.05

kg using Martin's metal anthropometer and a medical scale (A&D Instruments Ltd; Abingdon; UK) respectively.

Dual-energy X-ray absorptiometry (DPX-IQ densitometer, Lunar Corporation, Madison, WI, USA) was used to assess BMC (g) and BMD (g/cm2) at the whole body (WB), lumbar spine (L2 to L4, LS) and femoral neck (FN). The measurements were performed in the medium scan mode, with standard positioning. All scans and result evaluations at both baseline and follow-up were carried out by the same examiner, using the extended analysis option from the proprietary software (enCORE, version 3.6). Coefficients of variation for bone mineral measurements were below 2%. Bone mineral apparent density (BMAD) was calculated following the formulas provided by Katzman et al. (165) and Carter el al. (39).

In order to assess the maturation status, participants had their skeletal age assessed by X-ray scans at the left hand and wrist, according to the method set by Greulich and Pyle (79,80).

Physical activity evaluation

PA participation was assessed by means of a uniaxial accelerometer (GT1M Actigraph, Monrovia, CA, USA) that the participants wore on their right hip during one week. After aggregating data into 15-second epoch periods, all intervals of zero counts lasting 20 minutes or more were removed from the analysis. All registers with less than 3 days without at least 10 hours of valid data were excluded from posterior analyses. The intensity of the physical activity was established according to the Evenson cutpoints (sedentary activity ≤100 counts/min, light activity >100 counts/min, moderate activity ≥2296 counts/min, vigorous activity ≥4012 counts/min) (81,145). In order to analyze the distribution of VPA, the presence of VPA bouts was evaluated for each participant. To analyze the distribution of VPA over time, the consecutive periods (bouts) of VPA were analyzed. In order to be

included in the analysis, the minimum duration of a bout was set at five consecutive minutes of VPA (166). In order to assess the evolution of the PA participation over time, both the mean time and the difference of time spent in each activity intensity between both years was calculated.

Participants were divided according to their participation in VPA at baseline. Since the analysis of VPA distribution did not expose a clear cut-off point, the median value of the 140 participants (15.5 minutes per day) was used in order to obtain two groups with the same sample size. Then, the participation in VPA during the follow-up measurement (median: 17.75 minutes per day) was used to further divide the original groups, thus obtaining four categories ("low-low", "low-high", "high-low" and "high-high") by taking into account the involvement in VPA at both time points.

Statistical analysis

Cluster analyses were used to generate bone growth profiles based on the variation of BMC and BMD (adjusted for change in body mass and bone age). Hierarchical and nonhierarchical clustering methods were combined in a two-step analysis (167). A hierarchical cluster analysis, using Ward's method based on squared Euclidian distances, was used as a preliminary step to identify the cluster solutions. These extracted cluster centers served as non-random starting points in an iterative, non-hierarchical k-means clustering procedure that formed the definitive clusters.

Normality assumption was confirmed with Kolmogorov-Smirnov tests and the outlier presence was explored for all variables. Partial Pearson's correlation coefficients among change in bone mineral parameters and PA variables were calculated after adjustment by change in body mass and skeletal age. Analysis of covariance was used for the intergroup comparisons (VPA evolution and bone growth clusters), as well as repeated measures

Table 1 – Baseline characteristics of the sample

Age and body composition	All participants (n=140)	Low-Low (n=48)	High-Low (n=22)	Low-High (n=22)	High-High (n=48)
Skeletal age (years)	11.85 ± 1.07	11.92 ± 0.73	12.00 ± 0.61	12.33 ± 0.72	12.04 ± 0.65
Height (cm)	154.3 ± 7.5	153.1 ± 7.3	155.2 ± 6.0	155.9 ± 9.1	154.5 ± 7.6
Body weight (kg)	47.0 ± 12.5	49.4 ± 14.6	46.4 ± 11.8	49.7 ± 13.6	43.6 ± 9.2
Fat mass (kg)	11.4 ± 8.4	14.4 ± 9.7	9.8 ± 6.7	12.5 ± 9.2	8.5 ± 5.9*
Lean mass (kg)	33.0 ± 5.7	32.3 ± 5.8	34.0 ± 5.3	34.5 ± 7.4	32.7 ± 4.8
Physical activity					
Sedentary time (min/day)	545.2 ± 75.6	574.8 ± 89.3	528.1 ± 47.5	559.1 ± 78.4	517.1 ± 57.0*
Light PA (min/day)	234.4 ± 47.0	228.3 ± 51.7	241.9 ± 43.3	224.9 ± 38.8	241.5 ± 47.0
Moderate PA (min/day)	39.4 ± 14.5	32.1 ± 10.8	44.0 ± 13.5*	35.0 ± 11.2	46.5 ± 15.5*¬
Vigorous PA (min/day)	18.7 ± 13.2	8.3 ± 3.7	23.2 ± 7.4*	10.9 ± 3.8†	30.7 ± 13.2*†¬
Overall PA (counts/min)	476.2 ± 151.3	367.7 ± 89.5	534.0 ± 125.2*	401.7 ± 86.5†	591.3 ± 139.7*¬

VPA cutpoint at baseline: 15.5 min/day; VPA cutpoint at follow up: 17.75 min/day

* Significant differences compared to Low-Low group ($p<0.05$)

† Significant differences compared to High-Low group ($p<0.05$)

¬ Significant differences compared to Low-High group ($p<0.05$)

Values are expressed as mean ± standard deviation

ANCOVA to test the group by time interaction. Changes in body weight and skeletal age were used as covariates for this analysis. Cross tabulation of the VPA and bone growth groups was performed and the chi-square statistic was calculated to assess the homogeneity of the group distributions. All statistical analyses were carried out with SPSS v22.0 for Windows (Chicago, IL, USA). Statistical significance was set at $p<0.05$.

RESULTS

Results are shown only for those subjects who attended both bone mineral evaluations and provided enough valid data at both the baseline and the follow-up measurements (figure A1), which comprised a total of 140 participants. A full description of the sample characteristics at baseline can be found in table 1.

The relationship between PA evolution with BMC and BMD change, after adjustment for changes in body mass and skeletal age, is presented in table A1. Both the mean and the variation of the time spent practicing VPA intensities showed a small positive correlation with the change in BMD at the WB (mean: 0.214; variation: 0.198, both $p<0.05$, table 1) and the FN BMD (mean: 0.245; variation: 0.254, both $p<0.05$, table 1). When looking at the mean of the overall PA participation we can only find significant correlations with the FN BMD (0.168, $p<0.05$, table A1). It should be mentioned that even though these results are statistically significant, the correlation coefficients are very small, with the highest R^2 being 6.5% (VPA difference – FN BMD).

Figure 1 and table 2 show the baseline and follow-up values of BMC and BMD (after adjustment for body mass and skeletal age) at all sites measured, whereas table 3 presents the change in these variables over time. All the groups showed statistically significant increases over time for BMC and BMD at all regions measured. All the differences found at baseline (WB, FN and LS BMC and FN BMD) disappear at follow-up for the “low-high”

Figure 1 – Baseline and follow-up bone mineral parameters according to vigorous physical activity groups

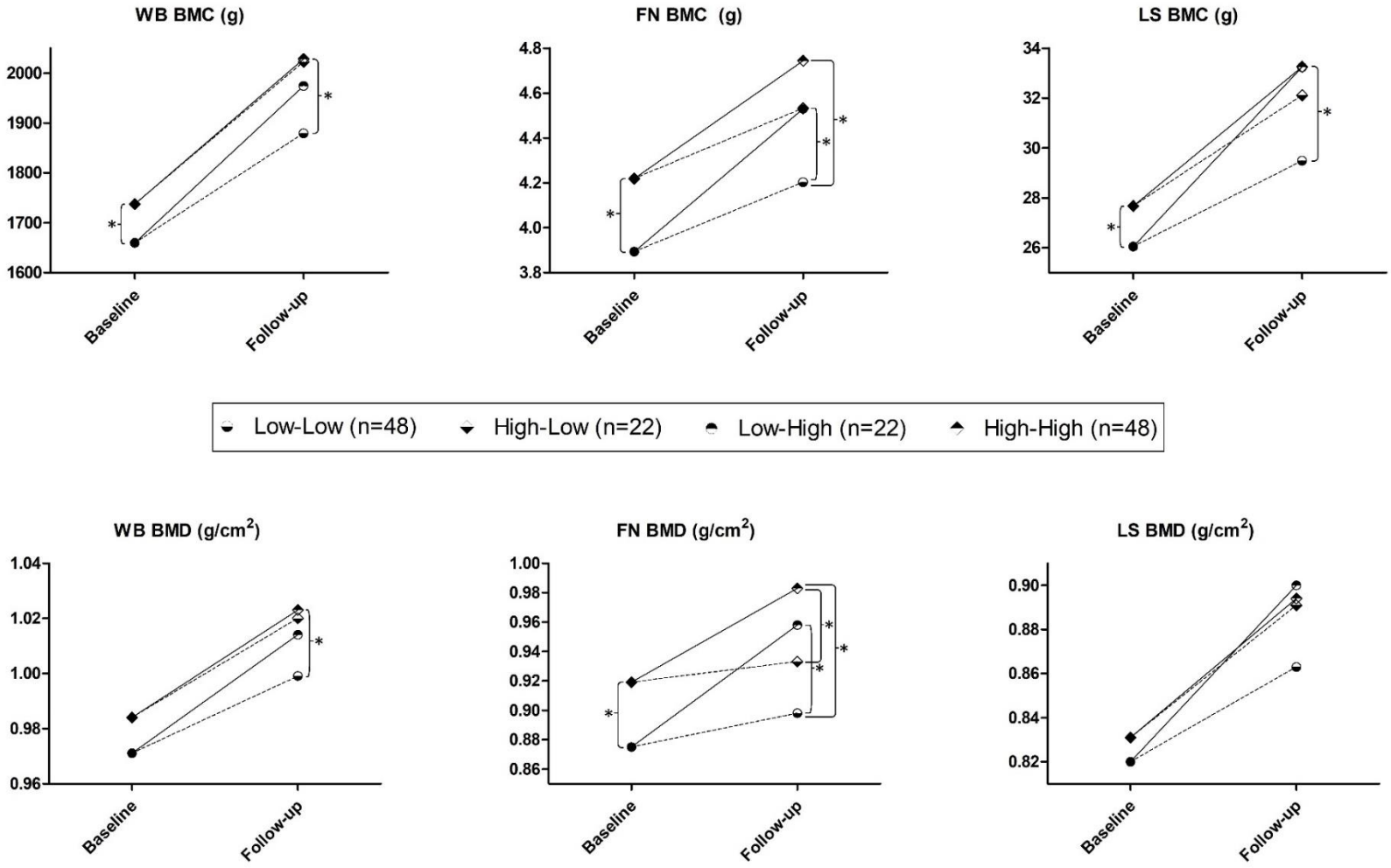


Table 2 – Bone mineral parameters (after adjustement for skeletal age and body weight) according to different vigorous activity evolution patterns

	Baseline		*Follow up*			
Bone mineral parameters	Low (n=70)	High (n=70)	Low - Low (n=48)	High - Low (n=22)	Low - High (n=22)	High - High (n=48)
WB BMC (g)	**1660 ± 20**	**1738 ± 20**	1879 ± 32	2023 ± 46*	1974 ± 47	2028 ± 32*
WB BMD (g/cm^2)	0.971 ± 0.006	0.984 ± 0.006	0.999 ± 0.008	1.014 ± 0.012	1.020 ± 0.012	1.023 ± 0.008*
WB BMAD (g/cm^3)	0.089 ± 0.001	0.088 ± 0.001	0.086 ± 0.001	0.085 ± 0.001	0.087 ± 0.001	0.086 ± 0.001
FN BMC (g)	**3.89 ± 0.06**	**4.22 ± 0.06**	4.203 ± 0.081	4.533 ± 0.118*	4.530 ± 0.119*	4.745 ± 0.081*
FN BMD (g/cm^2)	**0.875 ± 0.010**	**0.919 ± 0.010**	0.898 ± 0.013	0.933 ± 0.019	0.958 ± 0.019*	0.983 ± 0.013*†
FN BMAD (g/cm^3)	0.197 ± 0.003	0.202 ± 0.003	0.194 ± 0.003	0.196 ± 0.005	0.204 ± 0.005	0.206 ± 0.003*
LS BMC (g)	**26.05 ± 0.55**	**27.67 ± 0.55**	29.49 ± 0.82	32.12 ± 1.20	33.25 ± 1.20*	33.25 ± 0.82*
LS BMD (g/cm^2)	0.820 ± 0.009	0.831 ± 0.009	0.863 ± 0.013	0.891 ± 0.019	0.900 ± 0.019	0.894 ± 0.094
LS BMAD (g/cm^3)	0.147 ± 0.002	0.144 ± 0.002	0.149 ± 0.002	0.150 ± 0.003	0.149 ± 0.003	0.148 ± 0.002

VPA cutpoint at baseline: 15.5 min/day; VPA cutpoint at follow up: 17.75 min/day

Bold characters indicate significant differences between groups at baseline

* Significant differences compared to Low-Low group ($p<0.05$)

† Significant differences compared to High-Low group ($p<0.05$)

WB: Whole body; FN: Femoral neck; LS: Lumbar spine

BMC: Bone mineral content; BMD: Bone mineral density; BMAD: Bone mineral apparent density

Values are expressed as mean ± standard error

Table 3 – Bone growth parameters according to different vigorous activity evolution patterns

	Low-Low (n=48)	High-Low (n=22)	Low-High (n=22)	High-High (n=48)	Group by time interaction
Δ WB BMC (g)	245 ± 16 (14.9 ± 0.8%)	272 ± 24 (15.7 ± 1.2%)	278 ± 24 (15.6 ± 1.2%)	288 ± 16 (16.5 ± 0.8%)	0.027
Δ WB BMD (g/cm^2)	0.030 ± 0.003 (3.2 ± 0.3%)	0.028 ± 0.005 (2.8 ± 0.5%)	0.044 ± 0.005*† (4.3 ± 0.5%)	0.039 ± 0.003 (3.9 ± 0.3%)	0.059¬
Δ WB BMAD (mg/cm^3)	-2.73 ± 0.54 (-2.9 ± 0.6%)	-3.46 ± 0.80 (-3.7 ± 0.8%)	-0.55 ± 0.80*† (-0.9 ± 0.8%)	-2.18 ± 0.54 (-2.4 ± 0.5%)	0.053
Δ FN BMC (g)	0.361 ± 0.044 (9.4 ± 1.0%)	0.370 ± 0.065 (9.1 ± 1.5%)	0.562 ± 0.065*† (13.7 ± 1.6%)	0.481 ± 0.044 (11.6 ± 1.0%)	0.061¬
Δ FN BMD (g/cm^2)	0.034 ± 0.007 (4.1 ± 0.7%)	0.030 ± 0.010 (3.4 ± 1.1%)	0.066 ± 0.010*† (7.3 ± 1.1%)	0.053 ± 0.006*† (5.6 ± 0.7%)	0.080¬
Δ FN BMAD (mg/cm^3)	0.50 ± 1.93 (0.5 ± 1.0%)	-3.00 ± 2.85 (-1.4 ± 1.5%)	2.95 ± 2.86 (1.4 ± 1.5%)	1.17 ± 1.92 (0.7 ± 1.0%)	0.017
Δ LS BMC (g)	4.23 ± 0.44 (16.6 ± 1.5%)	4.56 ± 0.65 (16.9 ± 2.2%)	6.12 ± 0.65* (21.1 ± 2.1%)	5.22 ± 0.44 (18.2 ± 1.4%)	0.047
Δ LS BMD (g/cm^2)	0.049 ± 0.007 (6.0 ± 0.8%)	0.056 ± 0.010 (6.7 ± 1.2%)	0.074 ± 0.010* (8.7 ± 1.2%)	0.062 ± 0.007 (7.4 ± 0.8%)	0.035
Δ LS BMAD (mg/cm^3)	1.52 ± 1.14 (1.1 ± 0.9%)	3.04 ± 1.67 (2.1 ± 1.3 %)	4.46 ± 1.68 (3.1 ± 1.3%)	4.06 ± 1.13 (3.2 ± 0.9%)	0.024

VPA cutpoint at baseline: 15.5 min/day; VPA cutpoint at follow up: 17.75 min/day * Significant differences compared to Low-Low group ($p<0.05$)
† Significant differences compared to High-Low group ($p<0.05$) ¬Significant group by time interaction ($p<0.05$)
WB: Whole body; FN: Femoral neck; LS: Lumbar spine; BMC: Bone mineral content; BMD: Bone mineral density
BMAD: Bone mineral apparent density. Values are expressed as mean ± standard error

group. Moreover, significant differences with the "low-low" were found at follow-up for femoral neck BMC (low-low: 4.203, low-high: 4.530 g; $p<0.05$) and BMD (low-low: 0.898, low-high: 0.958 g/cm2; $p<0.05$). Additionally, when comparing BMC and BMD changes, it was observed that the "low-high" group shows higher increases for all bone mineral variables except WB BMC than the "low-low" group and also greater growth than the "high-low" group for WB BMD and FN BMC and BMD (table 3, all $p<0.05$). For the change in BMAD, the only significant differences were found at the whole body (low-low: -2.73, high-low: -3.46, low-high: -0.55, $p>0.05$).

The cluster analysis and the posterior categorization created three clearly defined groups according to bone growth, as illustrated in table 4. When comparing the PA participation of the different bone growth groups, it could be observed that the only differences between the optimal and the reduced growth groups were found in the VPA and its maximum bout duration (table 4). Regarding the relative distribution of the bone growth and VPA groups, shown in figure 2, a trend for an heterogeneous distribution of the groups was found ($\chi 2$: 10.76, $p=0.096$). If the "mean growth" group is excluded from the analysis, statistical significance is reached ($\chi 2$: 9.26, $p=0.026$), with the "low-high" group showing the highest percentage of subjects with optimal growth (36.3%) and the "low-low" (12.5%) and "high-low" (13.6%) showing the lowest proportion of "optimal growth" participants.

DISCUSSION

The main finding of the present study was that engaging in VPA during puberty stimulates bone growth especially in subjects who were in the low-VPA group at baseline. This implies that adolescents engaging in VPA during early puberty are still able to reach BMD values that are similar in comparison with previously active children. The high responsiveness of the bone tissue to VPA during puberty was indicated in most sites by

Table 4 – Physical activity participation (mean of baseline and follow up) and bone mineral parameters (difference follow up - baseline) according to different bone development patterns

Physical activity	Optimal growth (n=29)	Mean growth (n=58)	Reduced growth (n=53)
Sedentary time (min/day)	568.0 ± 61.8	547.8 ± 69.4	563.4 ± 68.1
Light PA (min/day)	210.6 ± 38.8	227.1 ± 38.1	218.1 ± 39.7
Moderate PA (min/day)	36.1 ± 12.9	37.8 ± 12.2	37.8 ± 13.0
Vigorous PA (min/day)	23.2 ± 14.5	21.3 ± 14.2	17.1 ± 9.1*
Overall PA (counts/min)	468.7 ± 159.1	483.3 ± 151.4	445.2 ± 111.6
Time in vigorous PA bouts (min/day)	4.72 ± 4.64	3.70 ± 4.49	2.88 ± 5.32
Maximum bout duration (min)	10.38 ± 6.18	8.09 ± 4.59	6.98 ± 5.49*
Difference in bone mineral parameters [a]			
WB BMC (g)	441 ± 78	260 ± 75*	184 ± 61*†
WB BMD (g/cm^2)	0.065 ± 0.010	0.037 ± 0.009*	0.017 ± 0.008*†
FN BMC (g)	0.897 ± 0.153	0.429 ± 0.160*	0.189 ± 0.125*†
FN BMD (g/cm^2)	0.106 ± 0.016	0.048 ± 0.017*	0.008 ± 0.013*†
LS BMC (g)	9.82 ± 1.39	4.75 ± 1.52*	2.43 ± 1.20*†
LS BMD (g/cm^2)	0.124 ± 0.015	0.060 ± 0.016*	0.020 ± 0.013*†

Values are expressed as mean ± standard deviation

[a] Adjusted for difference in skeletal age and body weight

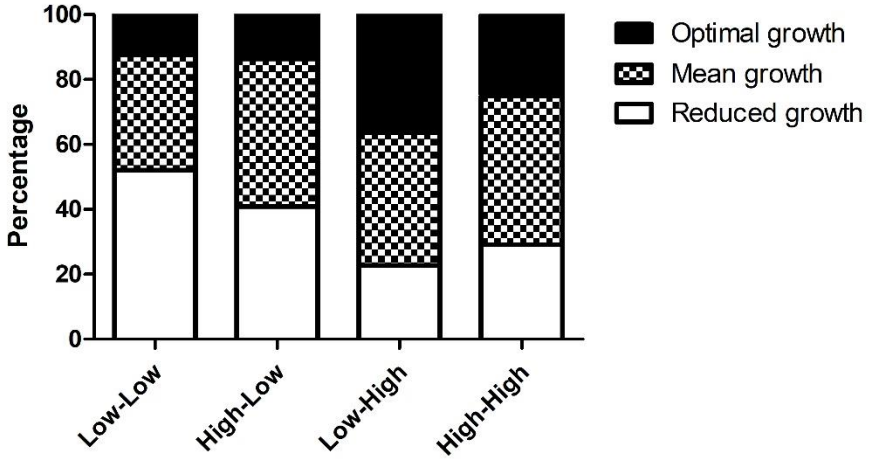
* Significant differences compared to optimal growth group ($p<0.05$)

† Significant differences compared to mean growth group ($p<0.05$)

PA: Physical activity; WB: Whole body; FN: Femoral neck; LS: Lumbar spine

BMC: Bone mineral content; BMD: Bone mineral density

Figure 2 – Relative distribution of growth profiles across physical activity groups



VPA cutpoint at baseline: 15.5 min/day; VPA cutpoint at follow up: 17.75 min/day

higher bone growth in subjects who increased their participation in VPA compared to those subjects who were vigorously active at baseline but were below the median at follow-up.

The analysis of the correlations between mean PA and bone mineral acquisition complements previously published data (141). The previous analyses focused solely in the changes in PA participation and consequently did not discriminate those subjects who remained physically active throughout the study from those who showed low levels of PA participation during the same period. The longitudinal associations among overall and VPA with BMC and BMD are in accordance with previous studies (168). It is important to notice that not only the mean participation in VPA was related to bone growth but its variation as well, indicating that both engaging into and maintaining high levels of VPA participation can positively affect bone accretion. This is further supported by the results obtained in the cluster comparison, with the "optimal growth" group having the highest levels of VPA participation. The rest of the activity intensities did not correlate with bone growth, which is in accordance with previous studies, especially in boys (147,169,170). However, as it has been mentioned, it should be taken into account that the correlation coefficients found are very small and the highest variance explained was only 6.5%, between VPA difference and FN BMD. It should be taken into account that the current one-year study period might have been too short for detecting the influence of lower intensity activities to bone mineral parameters. The results found are in accordance with the mechanostat theorem (45), which states that the mechanical load exerted on bone during PA can stimulate bone remodeling, affecting bone mineral content and structure.

The FN is the skeletal site that showed the highest association with PA parameters, which could be due to the highest responsiveness of cortical bone to physical stimulus (150,151). Despite being unable to test this in our sample, given that the method used does not allow

for cortical and trabecular bone discrimination, the responsiveness of FN bone to continued PA is relevant, since a high proportion of osteoporotical fractures occur in this region (30).

The evolution of the overall PA and the moderate to vigorous PA trajectories have already been related to bone mass and structure (163). The division of the sample into different PA patterns was done following the method used by Janz et al. (163) with the difference that it was applied using VPA instead of overall activity. At baseline, those subjects who were above and below the median value of VPA differred in their BMC at all sites and the FN BMD. At follow-up, however, all these differences disappeared for the subjects who started participating in VPA ("low-high" group). This finding further indicates the importance of intense PA for bone accrual and the resulting peak bone mass. Incorporating vigorous PA to daily routine might help optimizing bone accrual to previously inactive subjects. Particularly, at the FN, statistically significant differences emerged from the "low-high" group and the "low-low" group. The "low-high" group showed greater growth in bone mass and density than both groups who were below the median VPA at follow-up. In contrast, subjects who stopped practicing VPA ("high-low" group) reported lower BMD values at the FN compared to "high-high" group. Nonetheless, the differences with the "low-low" group remained significant for the WB and FN BMC. This benefits, however seem to be limited to areal BMD, greater increases of BMAD were only found for the "low-high" group at the whole body. This may be caused by an increase in bone size along the increase in areal BMD.

When analyzing growth profiles, three clearly defined groups emerged. The main difference between the "optimal" and the "reduced" growth groups was observed in the VPA and its maximum bout duration. As it has been shown, the "low-high" group had the greatest percentage of participants who experienced an improved gain of BMC and BMD at all measured sites.

These results point to an enhancing effect of VPA on bone development during puberty. As it has been shown, bone formation is stimulated by the physical stress produced by VPA practice (171) and early puberty is a stage in which bone is particularly responsive to this stimulus (56,158). It seems that osteocytes play a crucial role in the physiological response of bone, stimulating osteoblastic activity in the presence of mechanical loads while increasing bone resorption in the absence of mechanical strain (172). According to our results, participation in unstructured VPA seems to provide a mechanical load that is sufficient to elicit an osteogenic response following the mechanostat theorem (45).

This increase in BMC and BMD has been observed in our sample with participants who engaged in VPA during the follow-up independently of their previous activity. However it should be pointed out that initial VPA led to a higher bone mineral status and these benefits were maintained after activity cessation, as it has been suggested by previous research (158,162).

The one-year follow-up design, as well as the approach used in the analysis of the evolution of PA participation are among the main strengths of this study, alongside with the employment of objective measures for the PA evaluation and gold standard reference methods for the bone mineral assessment. The lack of a PA intervention and a control group with no exercise does not allow to infer causal relationships and is one of the main limitations that should be taken into account. Also, bone structure was not evaluated due to the bidimensional nature of the bone assessment method. It should also be acknowledged that no nutritional status evaluation was performed and therefore the possible effects of diet on bone parameters were not controlled in the present study. The analysis of longitudinal changes in bone structure or specific adaptations in cortical or trabecular bone related to PA could be the scope for future research.

As a conclusion, both engaging and maintaining high levels of VPA participation during puberty are associated with greater gains in bone mass and density, especially at the femoral neck, where the increase in BMD for the "low-high" group almost doubled the increase found in both "high-low" and "low-low" groups. An interesting conclusion that can be drawn from our results is that engaging in VPA during early puberty can result in increased bone growth, regardless of previous activity levels. Additionally, these benefits could be preserved even after activity cessation.

Funding

JMP received a grant from 'Ministerio de Educación Cultura y Deportes' (FPU014/04302). This study was funded by the Estonian Ministry of Education and Science (IUT 20-58).

Conflict of Interest

Jorge Marin-Puyalto, Jarek Mäestu, Alba Gomez-Cabello, Evelin Lätt, Liina Remmel, Priit Purge, Jose A. Casajús, German Vicente-Rodríguez and Jaak Jürimäe declare that they have no conflict of interest.

4.4 Is Vibration Training Good for Your Bones? An Overview of Systematic Reviews

ABSTRACT

Whole-body vibration (WBV) intervention studies and reviews are increasing lately. However, the results regarding its effects on bone tissue in different populations are still inconclusive. The goal of this overview was to summarize systematic reviews assessing the effects of WBV training on bone parameters. Three electronic databases were scanned for systematic reviews and meta-analyses evaluating the effects of WBV on bone tissue. The search had no time restrictions and was limited to articles written in English. Vibration protocols and the main bone parameters included in each review were extracted. Methodological quality was assessed and analyses were conducted stratifying by age. 15 reviews and meta-analyses fulfilled the inclusion criteria. No increase or small improvements in bone mineral density (BMD) after WBV interventions were observed in reviews regarding postmenopausal women. One intervention study regarding young adults was included and reported no bone-related benefits from WBV. Most reviews including children and adolescents with compromised bone mass showed an improvement of BMD at lower limbs, lumbar spine and whole body. In conclusion, WBV interventions seem to help children and adolescents with compromised bone mass to increase their BMD, but these improvements are limited in postmenopausal women and there is insufficient evidence for young adults. Further research is also needed to identify the ideal parameters of WBV training focused on bone health.

INTRODUCTION

Osteoporosis has been defined by the World Health Organization as a skeletal disease characterized by "low bone density and microarchitectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture" (173). Osteoporosis and its related fractures are becoming an important public health concern, since they affect both quality of life and mortality of individuals (174) and generate health costs (175). Even though this disease is more common among postmenopausal women, men can also suffer from it and, in some cases, its origin can be traced back to a low bone mass during adolescence and adulthood (37). Therefore, the accrual of bone mineral density (BMD) is crucial in order to prevent or retard osteoporosis (176).

It has been widely tested that physical activity (PA) through lifetime as well as specific training programs have a beneficial influence on bone mass (177). For this reason, the promotion of regular PA has been advocated as one of the main non-pharmacological measures for improving bone health (178).

Since Rubin et al. showed that low-magnitude high-frequency mechanical accelerations may produce a strong osteogenic response in animals (70) and humans (69), whole-body vibration (WBV) has become a topic of interest. Indeed, this marked increase in the use of WBV has led to the apparition of both narrative (179–182), systematic (50,183–196) and also state-of-science (197) reviews focusing on different aspects, outcomes or populations within this exercise training modality, as well as its safety for clinical practice.

WBV training uses high-frequency mechanical stimuli generated by a vibrating platform and transmitted through the body (198). The platforms vary in the type of vibration produced (vertical or side-alternating) and the range of amplitudes and frequencies

available (199). The exact nature of the mechanism by which WBV training stimulates osteogenesis is still not certainly known (199).

The large number of studies published in the last years concerning the effects of WBV in different aspects and populations allows gathering the currently existing knowledge about the effects of this type of training on bone mass. However, there are still discrepancies among the reviews regarding the actual efficacy of this type of training for the improvement of bone mass throughout the diverse stages of life. Therefore, the main objective of this overview is to provide a global and summarized perspective of all current evidence regarding the effects of WBV training on bone mass.

METHODS

Data sources and search strategy

This study followed the overview methodology proposed by Smith et al. (86) and the framework provided by the Cochrane network (87).

Reviews were identified by searching electronic databases, scanning reference lists of reviews and consultation with experts in the field. This search was applied to PubMed, SportDiscus and the Cochrane Library. The search had no time restrictions set and was conducted up to and including 1 October 2018. The database-specific search terms were the following:

- PubMed: “vibration” [Title/Abstract] AND ((Meta-Analysis[ptyp] OR Review[ptyp]) AND "humans"[MeSH Terms]).
- Cochrane Library: "vibration" in Title, Abstract, Keywords (Word variations have been searched) (only Cochrane Reviews and Other Reviews sections).
- SportDiscus: vibration AND review (only academic papers).

Bone-related search terms were not included in order to obtain a generic overview of all the reviews published concerning WBV, and therefore have the certainty that no relevant articles were missing.

Review selection

Two reviewers independently examined titles and abstracts. Relevant articles were obtained in full and assessed against the inclusion and exclusion criteria described below. Inter-reviewer disagreements were resolved by consensus. Arbitration by a third reviewer was used for unresolved disagreements.

Inclusion criteria

1. Types of study: Systematic reviews and meta-analyses concerning the effects of WBV training on bone mass. Within each systematic review, only the controlled trials measuring the outcomes later described were taken into consideration.
2. Types of participants: Children, adolescents, adults and elderly populations (no age nor condition restrictions).
3. Types of outcome measured: Bone mineral content (BMC) or BMD of whole body, lumbar spine, arm, hip (femoral neck, trochanter, intertrochanter or Wards triangle subregions), bone architecture (from peripheral quantitative computed tomography (pQCT)), ultrasound parameters (Broadband Ultrasound Attenuation (BUA), Speed of Sound (SOS), stiffness index) or metabolic biomarkers.

Exclusion criteria

1. Reviews in languages other than English.
2. Non-systematic reviews.
3. Unpublished data.
4. Reviews of studies with animals.

5. Reviews focusing only on number of fractures, with no mention to variables obtained by imaging techniques.
6. Meta-analyses that do not feature independent sets of effect sizes.

Assessment of methodological quality

The evaluation of the methodological quality of the reviews was carried out using the AMSTAR tool (88), which has been validated as a mean to specifically assess the methodological quality of systematic reviews.

The overlapping of the included reviews was also considered by calculating the corrected covered area, a metric proposed by Pieper et al. (89), that measures the degree of overlap within a group of systematic reviews.

RESULTS

Search summary

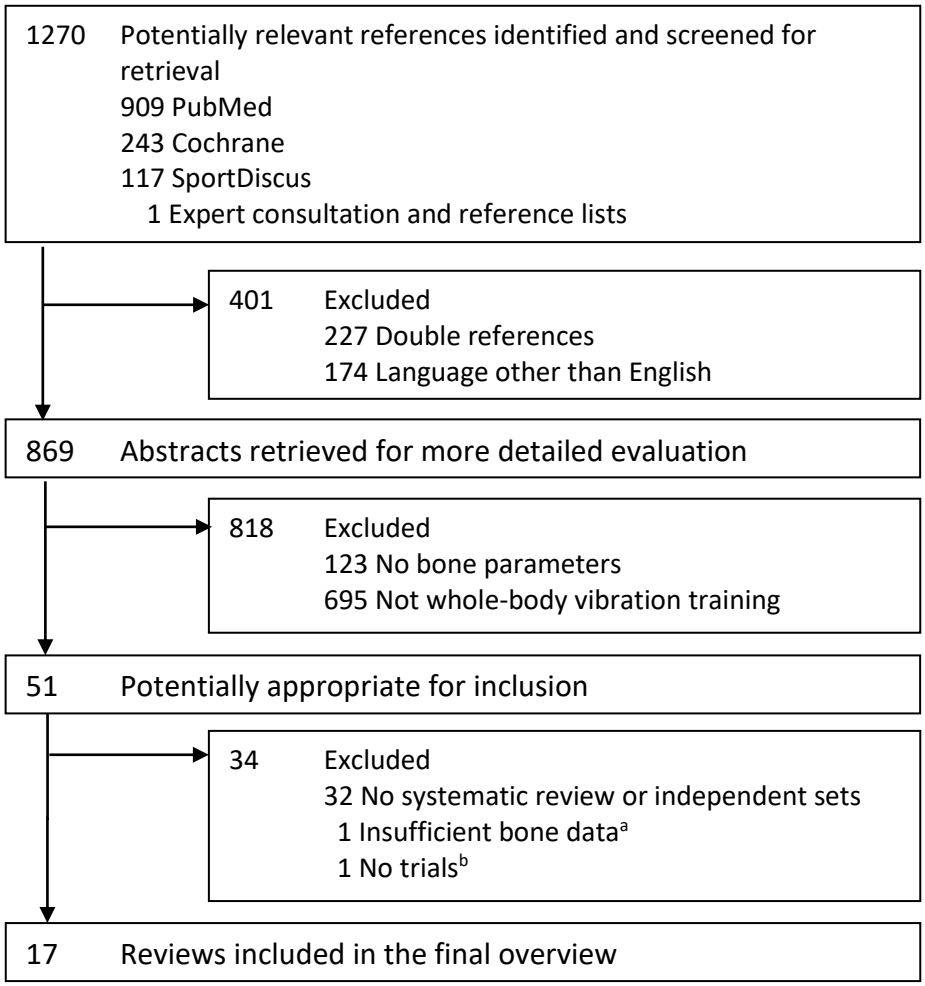
1270 potentially relevant articles were retrieved. After reviewing the titles and abstracts, this total was reduced to 51. Of those reviews, 19 met the inclusion criteria and were included in a primary analysis.

Individual papers included in each review were listed. Studies related to bone variables were further examined and compared among reviews. This analysis allowed us to identify one review (184) that based its BMD results solely on one single trial (which was already covered by eleven of the other reviews) and another one (200) that included data solely from two abstracts. Therefore, the above-mentioned reviews were considered unlikely to add relevant data and were excluded from the final analysis (Figure 1).

Summary of review characteristics

The characteristics of the seventeen reviews included in the study are summarized in Table 1. In this table, the aim of the reviews and the search strategy followed by the authors are

Figure 1 - Flow chart diagram of the review selection.



[a] Chanou et al., [16] obtained its BMD data from a single RCT that was already covered by eleven other reviews.
[b] Bell et al., [36] featured data from two abstracts and no full article was included.

described. The number of studies included in these reviews, the total number of participants, the comparison interventions considered and the duration range of these interventions are also reported. Finally, the main variables of interest for each review are listed.

Table 1 - Summary of review characteristics

Author, year	Aim	Search strategy	Number of WBV bone-related trials included (participants)	Comparison interventions	Duration of the interventions	Bone-related outcomes for which data were reported
Older adults						
Merriman, 2009	To assess the effectiveness of WBV on bone density, muscle performance, balance and functional mobility	MEDLINE (1950-2007), CINAHL (1982-2007) No language restriction Intervention of at least 6 weeks	5 (251)	Control, placebo device, fitness exercise (<=40 min, 3/week), resistance exercise, physical therapy, walking	6-52 weeks	BMD (whole body, total hip, proximal femur, lumbar spine, cortical tibia) Bone turnover markers
Mikhael, 2010	To examine the effect of WBV on muscle or bone morphology and function	Medline, SPORTDiscus, AusportMed, CINAHL, AMED, WoS, Scopus English language Until 2009 (most databases)	3 (153)	Control, resistance, exercise, placebo device	6-52 weeks	BMC (lumbar spine) BMD (lumbar spine, tibia) Bone turnover markers
Lau, 2011	To determine whether WBV improves BMD and leg muscle strength	MEDLINE, CINAHL, EMBASE, PEDro, PubMed, Science Citation Index English language Exclusion criteria: Subjects diagnosed with a primary condition	6 (398)	Control, active exercise	6 weeks - 18 months	BMD (total hip, lumbar spine)
Pollock, 2012	To assess studies which have investigated the outcome of repeated exposure to WBV	WoS, PubMed, EMBASE, CINAHL, PEDro, Cochrane Library English language Case reports and abstracts from conference presentations excluded	10 (719)	Control, placebo device, fitness exercise, walking, resistance training, wellness program	6-52 weeks	BMD (whole body, total hip, proximal femur, radius, lumbar spine)

Gómez-Cabello, 2012	To assess the effects of different training programmes in bone mass	MEDLINE & CENTRAL English and Spanish languages	10 (740)	Control, aerobic training, strength training and multi-component training	6-18 months	BMC (whole body) BMD (lumbar spine, hip, femoral neck, distal radius, tibia)
Sitjá-Rabert, 2012	To analyze the efficacy and safety of WBV training	MEDLINE, EMBASE, CENTRAL, CINAHL, PeDro, PsychInfo English, French and Spanish languages	5 (247)	Control, exercise group (various modalities)	6-52 weeks	BMD (femoral neck, lumbar spine)
Ma, 2016	To evaluate the musculoskeletal effect of WBV	EMBASE, PubMed, Cochrane Central Register of Controlled Trials, ISI Web of Science, CNKI No date nor language restrictions Intervention of at least 6 months	8 (1014)	Control, resistance training, placebo device, walking	6-18 months	BMD (femoral neck, lumbar spine)
Oliveira, 2016	To analyze clinical trials that verified the effects of WBV on BMD in postmenopausal women	PubMed, Web of Science, LILACS, The Cochrane Library, PEDro No date or language restrictions Unpublished studies searched at clinicaltrials.gov	17 (1833)	Control, exercise group (various modalities)	6-18 months	BMD (lumbar spine, total hip, femoral neck, trochanter, Ward's area, tibia, radius)
Dionello, 2016	To review recent literature and highlight novel findings on the effect of WBV exercise on the BMD in women with postmenopausal osteoporosis without medications	PubMed, PEDro English language	11 (922)	Control, placebo device, wellness program, resistance training	2-18 months	BMD (whole body, lumbar spine, total hip, femoral neck) Bone turnover markers

Luo, 2016	To conduct a comprhensive quantitative analysis of WBV in patients with postmenopausal osteoporosis	MEDLINE, CINAHL,PEDro, CENTRAL	9 (625)	Control, wellness program, walking, resistance training	12-48 weeks	BMD (lumbar spine, femoral neck, total hip, Ward's triangle) Bone turnover markers
Jepsen, 2017	To address if WBV in adults over 50 years of age could affect the estimates of bone mass, architecture and turnover biomarkers	PubMed, EMBASE, Cochrane English language	12 (1618)	Control, placebo device, exercise, wellness program	3-24 months	BMD (femoral neck, total hip, lumbar spine) Volumetric BMD (radius, tibia) Bone turnover markers
Children and adolescents with disabilities						
Matute, 2014	To summarize the current literature regarding the effects of WBV on health-related fitness parameters (children and adolescents with disabilities)	MEDLINE (PubMed), SPORTDiscus, EMBASE English language Published studies only	8 (338)	Control	8-52 weeks	BMC (whole body, lumbar spine) BMD (whole body, femoral neck, lumbar spine, tibia, femur) Stress endurance parameters Bone turnover markers
Saquetto, 2018	To verify the effects of WBV training on the muscle strength, bone mineral parameters, balance and body composition of children and adolescents with Down's Syndrome	MEDLINE, LILACS, SciELO, PEDro, Cochrane No language restriction	1 (25)	Control	20 weeks	BMC (whole body, lumbar spine) BMD (whole body, lumbar spine) Volumetric BMD, cortical thickness (radius, tibia)

No population restriction						
Madou, 2008	To critique the research that has used WBV with special populations (elderly, postmenopausal women and neurological patients)	ProQuest, IngentaConnect, Meditext, MEDLINE, Proquest5000, PubMed, SPORTDiscus, Wos, Health and Medical Complete, Google Scholar English, German or Dutch languages (English abstract) Published in peer-reviewed journal	2 (99)	Control, resistance training	6-8 months	BMD (whole body, total hip, lumbar spine)
Rehn, 2008	To systematically review controlled studies that have explored the effects of WBV on BMD in humans	PubMed, Cinahl, Embase, Pedro, Amed Only controlled studies English language	8 (383)	Control, walking, placebo device, strength training	3-52 weeks	BMD (total hip, femoral neck, lumbar spine, tibia)
Slatkovska, 2010	To analyze the effects of WBV on BMD	MEDLINE, EMBASE, CINAHL, Cochrane, SportDiscus, ProQuest Dissertations, Theses Canada Portal No language restrictions Unpublished trials searched	8 (328)	Control, resistance training, placebo device, walking	6-52 weeks	BMD (total hip, lumbar spine)
Wysocki, 2011	To provide an overview of WBV therapy for the prevention and treatment of osteoporosis	MEDLINE, Cochrane Library, ACMD, CINAHL, CSA Physical Education Index, WoS, Physiotherapy Evidence Database, Academic Search Premier + gray literature 2000-2011; English language Exclusion criteria: Population without low-BMD risk, subjects diagnosed with a primary condition	9 (617)	Control, walking, resistance training, wellness program, placebo device,	8-72 weeks	BMD (whole body, lumbar spine, total hip, femoral neck, trochanter, radius, tibia)

WBV: Whole body vibration; BMC: Bone mineral content; BMD: Bone mineral density

Table 2 - Methodological quality of the included reviews according to the AMSTAR tool

	"A priori"design	Duplicate study selection	Comprehensive search	Status of literature as inclusion criterion	List of studies (included and excluded)	Characteristics of studies	Scientific quality assessed	Scientific quality considered in conclusions	Methods to combine findings	Publication bias assessed	Conflict of interest	Total (out of 11)
Rehn, 2008	Y	Y	Y	Y	N	Y	Y	Y	NA	N	N	**7**
Madou, 2008	Y	CA	Y	Y	N	Y	Y	N	NA	N	N	**5**
Merriman, 2009	Y	CA	Y	N	N	Y	Y	Y	NA	N	N	**5**
Slatkovska, 2010	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	**10**
Mikhael, 2010	Y	CA	Y	Y	N	Y	Y	N	NA	N	Y	**6**
Lau, 2011	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	**9**
Wysocki, 2011	Y	Y	Y	Y	N	Y	N	CA	NA	N	Y	**6**
Pollock, 2012	Y	CA	Y	Y	N	Y	Y	Y	NA	N	N	**6**
Gómez-Cabello, 2012	Y	Y	Y	Y	N	Y	N	CA	NA	N	Y	**6**
Sitjá-Rabert, 2012	Y	Y	Y	Y	N	Y	Y	N	Y	N	Y	**8**
Matute, 2014	Y	Y	Y	Y	N	Y	Y	Y	NA	N	Y	**8**
Ma, 2016	Y	Y	Y	Y	N	Y	Y	N	Y	N	Y	**8**
Oliveira, 2016	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	**11**
Dionello, 2016	Y	Y	Y	N	N	Y	N	CA	NA	N	Y	**5**
Luo, 2016	Y	Y	Y	Y	N	Y	Y	Y	Y	N	Y	**9**
Jepsen, 2017	Y	Y	Y	Y	N	Y	Y	Y	Y	N	Y	**9**
Saquetto, 2018	Y	Y	Y	N	N	Y	Y	N	NA	N	Y	**6**

Y: Yes; N: No; CA: Can't answer; NA: Not applicable

Methodological quality

Nine reviews (186,189,193–195,201–204) fulfilled the requirements of seven or more of the eleven items evaluated, with one review (202) obtaining a perfect score and another one (195) achieving ten points. The other eight reviews (50,188,190,191,196,197,205,206) obtained a total score of either five or six points. According to the classification proposed elsewhere (207,208), five of the reviews (186,195,202–204) are considered of high quality and the rest of moderate quality. The complete results of the methodological quality assessment can be checked in detail in Table 2.

Regarding the overlapping among reviews, Table 3 shows the number of individual studies on the subject of bone mass that are repeated in each pair of reviews. The corrected covered area yielded a result of 16.8, which is considered as high. This outcome was expected, since most reviews cover the same target population.

Effects of WBV training on bone mass in different populations

In order to facilitate the comparison between reviews, extracted data were sorted according to the studied population. Two reviews (189,206) focused exclusively on children and adolescents with disabilities, eleven (50,186,190,191,194,196,201–205) on older populations and four (188,193,195,197) included both children with disabilities, young adults and elderly populations. Results from the latter reviews were subdivided into population categories, which included children and adolescents with disabilities, young adults and older adults.

Table 3 - Overlap within reviews

Author, year	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*
1. Madou et al. , 2008	**2**	2	2	2	0	2	2	2	2	2	0	2	2	1	2	1	0
2. Rehn et al. , 2008		**8**	5	8	3	5	6	5	5	5	2	4	5	1	3	2	0
3. Merriman et al. , 2009			**5**	5	3	5	5	5	5	5	0	4	5	1	3	2	0
4. Slatkovska et al. , 2010				**8**	3	5	6	5	5	5	2	4	5	1	3	2	0
5. Mikhael et al. , 2010					**3**	3	3	3	3	3	0	2	3	0	1	1	0
6. Lau et al. , 2011						**6**	6	6	6	5	0	5	6	2	4	3	0
7. Wysocki et al. , 2011							**9**	8	7	5	1	5	6	3	4	3	0
8. Pollock et al. , 2012								**10**	9	5	0	5	7	4	5	4	0
9. Gómez-Cabello et al. , 2012									**10**	5	0	6	8	4	6	5	0
10. Sitjà-Rabert et al. , 2012										**5**	0	4	5	1	3	2	0
11. Matute-Llorente et al. , 2014											**8**	0	0	0	0	0	0
12. Ma et al. , 2016												**8**	8	4	6	5	0
13. Oliveira et al. , 2016													**17**	8	8	9	0
14. Dionello et al. , 2016														**11**	7	6	0
15. Luo et al., 2016															**9**	6	0
16. Jepsen et al., 2017																**12**	0
17. Saquetto et al., 2018																	**1**

The values indicate the number of individual studies that are included in both reviews (row and column)

Bold characters show the number of RCTs for each review

Corrected covered area: 16.8 (Calculated as proposed by Pieper et al.)

Older adults

The vast majority the controlled trials that evaluated the effects of WBV on bone mass included in the present overview involved solely postmenopausal women, with no individual study focusing exclusively in older men. Therefore, the following results depict the effects of WBV on postmenopausal women.

Hip and femoral neck

All of the fifteen reviews that include older adults within their population of interest contain at least one primary study assessing the effects of WBV training on BMD at the hip or, more precisely, at the femoral neck.

All the studies included in the reviews state that WBV training is either positive or neutral regarding BMD at the hip or the femoral neck. Only one negative result from an individual study (209) has been found among all reviews. This article states that there is a slight loss

of hip and femoral neck BMD after 8 months of WBV training. However similar decreases were found in the exercise and control groups, with no group by time interaction, which results in WBV being neutral to BMD at this site.

Positive results predominate over neutral ones in nine of the thirteen reviews (50,188,190,191,193–197), whereas six reviews found more neutral than positive results (186,201–205).

Six meta-analyses(186,194,201–203) have been conducted focusing specifically on the BMD at the hip and femoral neck. In one case (194), WBV training had an overall beneficial result when compared to sedentary controls. On the other hand, five meta-analyses (186,201–204) found WBV to be neutral to hip BMD.

Three reviews (190,193,194) highlighted the positive role that WBV training plays on enhancing BMD at the hip and femoral neck, especially when compared to the results obtained for the lumbar spine. The rest of the researchers define the effects of WBV on BMD at the hip as small or non-significant, pointing out that there is a lack of consistency in the study designs within the literature, and state that WBV training could be useful as a complementary or alternative method to increase BMD at the hip for subjects who have difficulties following a standard training program.

Lumbar spine

The lumbar spine is one of the main locations that have been studied due to its clinical relevance for the likelihood of fracture. All of the reviews include two or more individual studies that assessed BMD at this site.

No study has reported a detrimental effect of WBV training at the lumbar spine; however, neutral results clearly predominate over positive ones. Eight (186,188,191,193–195,203,204) of the fourteen reviews have been unable to find positive results, while

another seven found at least one study showing an enhancement of BMD at the lumbar spine. Nevertheless, all of them have found a greater number of studies reporting a lack of differences in lumbar spine BMD when comparing WBV training with other exercise modalities or controls.

All the authors that conducted a meta-analysis on the effect of WBV training on hip BMD performed the same analysis at the lumbar spine. Four of the meta-analyses (186,194,203,204) showed no differences between WBV training and controls in lumbar spine BMD. Two of the latest meta-analyses included in this overview (201,202), showed instead positive results of WBV training at this site, taking exclusively into consideration those studies with at least 6 months of WBV training.

Other sites

The earliest assessment of cortical and trabecular volumetric BMD at the tibial midshaft was carried out by Russo et al. (210). There have been conflicting interpretations among different authors, so this issue will be addressed in detail during the discussion of this overview.

A recent meta-analysis by Oliveira et al. (202) examined in depth the effects of WBV training on the volumetric BMD in postmenopausal women at both radius and tibia. No differences favouring any of the groups were found in either the primary analysis or the subsequent sensitivity subgroup analyses.

Whole body BMD was assessed in two individual studies (198,211) among the ones included within different reviews. Fjeldstad et al. (211) reported a detrimental effect on whole body BMD for a combination of WBV training with resistance training, so it cannot be determined that these are solely due to the use of WBV. Verschueren et al. (198) showed

no differences in whole body BMD between the WBV and control groups after a 6-month period.

Bone turnover markers

Only five reviews (190,191,203–205) presented data regarding serum levels of bone turnover markers, and extracted the information from six different original studies (198,210,212–215). Four studies (198,210,212,214) found no differences in the serum levels of these metabolic biomarkers among WBV and control groups. The study by Turner et al. (213) reported a reduction in the urinary levels of bone resorption markers (N-telopeptide X normalized to creatinine) following a 2-month WBV intervention. Finally, Corrie et al. found a greater increase in a bone formation marker (procollagen type 1 N-terminal propeptide) in subjects who underwent 12 weeks of WBV training compared to controls.

Ultrasound parameters

Ultrasound parameters have only been considered in the two latest reviews (202,205), since the only study that evaluated the calcaneal region using this methodology was published two years ago by Slatkovska et al. (216). In this study, a small but statistically significant decrease was found in the calcaneal broadband ultrasound attenuation following a 12-month intervention with low-intensity WBV, which implies a negative effect of WBV interventions.

Optimal parameters for WBV training

Several authors of reviews(50,193,195) suggest that more research is needed to establish the optimal parameters of vibration to improve or preserve BMD, but only three reviews(201,202,204) include subgroup meta-analyses to evaluate different vibration protocols.

In the meta-analysis by Ma et al.(201) , the results for lumbar spine BMD favoured the WBV group when pooling the studies with low magnitude vibration (<1 g, as defined by the authors of the meta-analysis), but no differences were found in the studies with high magnitude vibration (≥1 g). However, it is not clear that these effects are due solely to the magnitude of the vibration, given that the studies with low magnitude vibration were as well those with a higher cumulative dose of vibration.

Oliveira et al.(202) carried out various subgroup meta-analysis, and they found significant improvements in lumbar spine BMD for the vibration group in the studies with side-alternating vibration, the studies where the subjects stayed with semi-flexed knees during the WBV and the studies with either high frequency and low magnitude (>20 Hz, <1 g) or low frequency and high magnitude (≤20 Hz, ≥1 g).

In the review by Jepsen et al.(204), side-alternating platforms showed overall better results for lumbar spine BMD when compared to vertical vibration platforms.

Young adults

Two reviews (193,195) included studies regarding the effects of WBV on BMD in young adults, and both of them retrieved the same original study (217). In this randomized controlled trial, subjects that completed an eight-month intervention of WBV training did not show benefits over their control counterparts in BMC at any skeletal site measured (lumbar spine, femoral neck, trochanter, calcaneus, and distal radius). Volumetric BMD at the distal tibia and tibial shaft were also assessed, but no differences between groups were found after the WBV intervention.

Additionally, although the reviews did not analyse the results from bone remodelling markers, these parameters were effectively reported in the original study as not having

changed following the intervention. This study did not include any ultrasound parameters (217).

In summary, the authors of reviews have not been able to find positive results linking WBV training with improved BMC or BMD in young, healthy adults from were reported in the only study found within this population (217).

Children and adolescents with disabilities

A review conducted by Matute-Llorente et al. (189) explored the effects of WBV on bone mass in children and adolescents with disabilities. They claimed that even though the effect that this type of treatment exerts on body composition is not clear yet, it seems to provoke an improvement in bone health, since positive results predominate over neutral and negative ones within the studies they included, especially for lumbar spine BMD. The authors also acknowledge that the minimum dose of exposure to WBV required to elicit an optimal response is a topic that requires further research.

There is another review that explores the effects of WBV on children and adolescents with Down syndrome (206). Different health parameters are analyzed within this review, but only one study regarding bone mineral status is included (218), which showed that WBV training has the potential to generate an increase in subtotal (whole body minus head) BMD above the regular growth in this population.

These are the only two reviews found that focused exclusively on children and adolescents with disabilities, but this population group is also taken into consideration in three other reviews.

On one hand, Rehn et al. (193) found increased volumetric BMD at the proximal tibia following WBV training (219,220). On the other, this improvement remained statistically non-significant at the lumbar spine (220). Slatkovska et al. (195) obtained the same results

for the proximal tibia. However, after performing a meta-analysis pooling both individual studies (219,220), they found significant differences in the lumbar spine as well.

Finally, Wysocki et al. (197) consider that there is insufficient knowledge about the optimal target population for WBV training and therefore they do not reach different conclusions from the ones previously explained when focusing on postmenopausal women. They advise, thus, caution in making claims regarding this intervention, despite the fact that they report significant improvements in lumbar spine BMD when the adherence to WBV training is high in the only controlled trial focusing on children (219) that is included in their review.

All four reviews (189,193,195,197) found positive results following a WBV intervention in BMC or BMD in various sites, including whole body, femoral neck and more consistently at the proximal tibia and lumbar spine. Serum levels of bone formation biomarkers were only included in one controlled trial and had a trend to increase after WBV training (221). Ultrasound parameters have not been reported in any of the reviews.

DISCUSSION

Summary of main findings

Concerning the effectiveness of WBV training for the improvement of BMD in postmenopausal women, the only significant results found are those in the hip or lower body with small effect sizes. The majority of studies that assessed BMD at the level of the lumbar spine found no changes. Several authors reported conflicting results, attributing differences to the variety of protocols used, with the longest training durations leading to changes in BMD (50,193,196,201). The necessity of long training periods in order to obtain results of minor clinic relevance yields WBV training as an ineffective method to improve

bone mass. Nonetheless, it may prove as a valid alternative for subjects unable to perform other types of training.

There is a lack of studies aiming to assess the evolution of BMD after WBV training in young adults, since only one publication (217) has been identified among the 31 papers evaluated by the fifteen reviews. The WBV program applied in that study did not affect BMD nor serum markers of bone turnover. However, more research is needed on this topic before issuing a recommendation for this population.

Improvements in BMD in children and adolescents with compromised bone mass have been found not only at the lower limbs, but also at the lumbar spine and the whole body. Seven out of the nine studies included in the only review focused in this population (189) reported positive results at various sites. This is supported by the meta-analysis carried out by Slatkovska et al. (195), which found significant improvements in trabecular volumetric BMD both at the tibia and the spine following WBV training. The magnitude of the effect observed was higher when compared to postmenopausal women. This review (195), suggested that the growing skeleton of children and adolescents may be more sensitive to WBV training than other populations.

Discrepancies between reviews

There were two papers regarding postmenopausal women that were interpreted differently across researchers. This can in part be the cause of discrepancies among reviews and inaccurate conclusions can be drawn when pooling the results if this is not taken into consideration. Therefore, the original documents of the individual studies were retrieved and further analysed in order to clarify the actual results reported by the original studies.

According to most reviews (50,186,191,195–197,202), the study by Russo et al. (210) did not favour WBV training, since no changes in tibial vBMD were found in the vibration

group after 6 months of intervention. However, the reviews by Merriman et al. and Rehn et al. (190,193) point out that the control group suffered a decrease in the same parameter, and therefore, that WBV training could be considered as a protective agent. The results from the original paper show indeed a significant decline in cortical BMD in controls, whereas it remained unchanged in the intervention group. Nonetheless, when contrasting the loss of BMD over time between the two groups, only a non-significant trend favoring vibration was found. Thus, the results from this paper do not support the claim by the latter reviews.

One of the most repeated studies within the different reviews is the one carried out by Verschueren et al. (198) that was included in a total of eleven reviews (50,186,188,190,193–197,201,203). The reviews certify that this study presented an improvement in hip BMD following 6 months of WBV training, which is in concordance with the results from the original paper. However, when including this study in the context of a meta-analysis focused on the effects of vibration on hip or femoral neck BMD, different conclusions are drawn. In the meta-analysis performed by Lau et al. (186), this article is pooled with the one by von Stengel et al. (222) and in another meta-analysis by Sitjà-Rabert et al. (194) it is analysed along with two other papers (223,224). In both cases, it is reported that the study by Verschueren et al. (198) shows that there are no results favouring vibration training over active exercise or controls.

Agreements and disagreements with other studies or reviews

Research is currently focusing on the different interventions that can help obtaining and maintaining an optimal bone health, and an overview examining the effects of exercise on bone status in female subjects has recently been published (225). Even though it mentions WBV training, it only includes one systematic review (186), but it concludes that this type

of training is not effective for protecting bone loss in postmenopausal women, which is in accordance with the results previously shown.

The findings presented in this overview are consistent with the results found by Gómez-Cabello et al. (226) in a study in which an eleven-week WBV training program was not able to improve BMD nor structure in the elderly. Moreover, a narrative review by Cheung and Giangregorio (227) showed that low-magnitude high-frequency WBV does not improve BMD and bone structure in post-menopausal women.

Similarly, Totosy de Zepetnek et al. (182) stated in their narrative review that the efficacy of WBV training among older adults is somewhat inconclusive, whereas this type of training has shown to be anabolic to trabecular and cortical bone among young adults and children with low BMD or physical impairments. In a recent randomized controlled trial by Matute-Llorente et al. (218) which involved adolescents with Down syndrome, WBV training improved BMC and BMD of the whole body, lumbar spine, tibia and radius.

These results seem to be in conflict with the ones presented by Rittweger (181), who suggested that WBV training could be more effective in the elderly than in young adults. However, this statement is supported by just two studies in postmenopausal women and one in young adults and therefore does not take into account several other studies that may find different outcomes.

There are three studies including different WBV protocols in order to compare vertical with side-alternating vibration (228), high-frequency with low-frequency vibration (229) and high-intensity with low-intensity WBV (230). No differences between vibration protocols have been found in any of the studies. However, only one of them (229) compared frequencies, maintaining stable the rest of the variables. Therefore, further controlled trials

focusing on one specific variable while controlling the rest of them are needed to clarify the optimal vibration parameters.

Strengths and limitations

To the best of our knowledge, this is the first systematic overview of reviews regarding the effects of WBV training on BMC and BMD. This is a matter of increasing interest as it can be observed by the number of both narrative and systematic reviews related to this topic.

Most included reviews showed medium or high methodological quality and all of them were published in the last ten years. A high degree of overlapping studies within the reviews was identified and taken into account while evaluating the results, referring even to the primary study when discrepancies were found.

One of the main limitations of this overview is the difficulty encountered when trying to assess separately different vibration protocols, since only one review (196) assessed whether the vibration parameters were clearly established in the primary studies. Regarding this topic, the guidelines provided by Rauch et al (231) could prove useful in the standardization of WBV intervention reports. In addition, different protocols are usually pooled together in the framework of the reviews. Moreover, some of the reviews included in this overview did not report the results from all the individual studies they included.

It was not possible to assess the publication bias as it was only considered by one of the reviews (195), even though it may affect this field of research, as suggested by Cardinale and Rittweger (199).

Implications for future research

Most authors agree that there is a necessity of finding the ideal vibration protocol to maximize the benefits of WBV. In order to achieve this goal, randomized controlled trials focused on identifying the specific role of vibration amplitude, frequency and duration in

the bone response to WBV are needed. Additionally, differences between subjects in the response to this training should be evaluated as well, since the optimal vibration parameters may vary within subjects (179).

As it has been mentioned, there is a scarcity of studies aimed at the evaluation of the effectiveness of WBV training in young adults. Future research might help understanding the evolution of the applicability of WBV to increase bone mineral content and density throughout life.

Conclusions

WBV training seems to be more effective on increasing BMC and BMD in children and adolescents with compromised bone mass than in postmenopausal women. Benefits of WBV training in BMC and BMD of postmenopausal women are limited to the lower limbs and are described as having little clinical relevance. Future research should establish the effects of this intervention in young adults as well as the precise vibration parameters required to elicit an optimal response in each population.

Conflicts of Interest

The authors declare that no competing interests exist.

Funding Statement

JMP received a Grant FPU014/04302 from ‘Ministerio de Educación Cultura y Deportes’. This study was funded by ‘Centro Universitario de la Defensa de Zaragoza’ (UZCUD2016-BIO-01) and ‘Ministerio de Economía, Industria y Competitividad’ (DEP2016-78309-R).

4.5 Effects of whole-body vibration training on bone turnover markers in adolescent swimmers

ABSTRACT

Introduction: Whole-body vibration training has recently been proposed as a complementary training modality to improve the bone health of adolescent swimmers. However, there is no longitudinal studies regarding the effects of this training combination on bone metabolism. Therefore, the main goal was to analyze the effects of swimming and vibration training on bone turnover markers during adolescence.

Subjects and methods: The present study included 68 adolescent swimmers, and 41 normoactive controls (CON). Swimmers were randomly selected to either continue with their regular swimming training (SWI) or to participate in an additional vibration protocol (VIB). Anthropometric measurments and serum level determinations of osteocalcin (OC), procollagen type 1 N-terminal propeptide (P1NP) and C-terminal telopeptide crosslaps (CTX) were performed before and after the 6-month intervention.

Results: Statistically significant group by time interactions were found for both bone formation markers. VIB showed a decrease over time in OC (baseline: 101.4 μg/ml, follow-up: 82.8 μg/ml, $p<0.05$) and P1NP (baseline: 528.4 μg/ml, follow-up: 389.0 μg/ml, $p<0.05$) and SWI had analogous reductions in P1NP (baseline: 685.8 μg/ml, follow-up: 542.0 μg/ml, $p<0.05$), whereas CON experienced an increase in OC levels (baseline: 94.4 μg/ml, follow-up: 103.4 μg/ml, $p<0.05$). After stratifying the sample according to the pubertal status, similar interactions were observed.

Discussion: The combination of swimming training and this particular vibration protocol lead to a decrease in bone formation markers, especially during early puberty. Whole-body vibration might not induce an osteogenic stimulus in adolescent swimmers.

Keywords: bone metabolism, bone formation, bone resorption, osteoporosis prevention, exercise, osteocalcin

INTRODUCTION

Osteoporosis, a metabolic disease characterized by low bone mineral density (BMD) and compromised bone microarchitecture (29), is suffered by 22.1% of women and 6.6% of men over 50 years in the European Union (30). Additionally, with the expected demographic variations the number of osteoporotic patients is expected to increase (30). This structural bone fragility found in these subjects results in a lower tolerance to stress and can lead to osteoporotic fractures, which have been linked in turn to a poor quality of life, disabilities or even mortality (35). Around 40% of adult bone mass is created during adolescence and therefore it is a decisive period for osteoporosis prevention (18). This bone gain during adolescence will influence the peak BMD achieved in early adulthood, a critical parameter for the prevention of osteoporosis later in life as an increase of one standard deviation is expected to reduce future fracture risk up to 50% (156).

Weight-bearing physical exercise is considered one of the most effective non-pharmacological interventions in order to increase both the peak BMD and the BMD gain during adolescence (16,99). Non-impact sports, such as swimming or cycling have been traditionally considered as either neutral or detrimental to adolescent bone development (232–234). However, a systematic review suggested that while swimming practice may not confer direct BMD improvements, swimmers show increased bone turnover marker levels when compared to untrained controls, which might result in structural bone benefits (235). This is also supported by a recent cross-sectional study in which bone structural and mechanical properties of female swimmers were compared with soccer and ice hockey players using dual-energy X-ray absorptiometry (DXA) and perimetral quantitative computed tomography. It was shown that in loading regions, weight-bearing athletes showed higher bone densities than swimmers and controls but no differences at the lumbar spine or total hip. Furthermore, no differences among groups were found in any variable at

the radial or tibial mid-shaft. Based on these results, the authors suggest that bone benefits of swimming might be site-specific and therefore they might not be adverted in regular BMD scans (68).

While BMD assessment using DXA is widely used, as it is the method used for osteoporosis diagnosis, there are additional parameters that can give useful information regarding bone health (42). More specifically, bone turnover markers are recommended for short interventions, since changes in bone biomarkers are faster and more pronounced than changes in BMD (11). In patients undergoing pharmacological treatment for osteoporosis, changes in bone turnover markers can be observed as soon as in two weeks, reaching a plateau state in 3-6 months, whereas meaningful changes in BMD can take more than one year (236,237).

There is no clear consensus regarding the specific effects of swimming on bone turnover markers, since there are studies that have shown lower (238), similar (239) and higher (240) concentrations of bone formation markers in swimmers compared to normoactive controls. Similar controversial results can be found for bone resorption markers, with studies showing lower levels in swimmers than runners (241) and others presenting no differences among swimmers, controls and runners (239,242).

One relatively novel training strategy aimed to improve bone health of various populations is whole-body vibration (WBV) training. In a recent overview of systematic reviews we found that one of the populations that could benefit the most from WBV are adolescents with compromised bone health (243). Acute increases in bone formation markers have been found following WBV sessions in prepubertal boys (244) and young adults (245,246). However, to the best of our knowledge, the longitudinal effects of WBV training on bone turnover markers in adolescent swimmers are yet to be described by scientific literature.

Given the above reasons, the main goal of the present study was to determine the longitudinal effects of concurrent swimming and WBV training on bone turnover markers in adolescents.

MATERIAL AND METHODS

Participants and study design

Ninety adolescent swimmers and sixty normoactive controls (CON), all Caucasian and aged 10-18 years at baseline took part in the present 6-month longitudinal study within the framework of the RENACIMIENTO project (75). Adolescents who smoked, were taking medication known to affect bone or suffered from chronic diseases of musculoskeletal disorders were not allowed to take part in the study. Additionally, swimmers had to have a minimum of 3 years of regional swimming competition experience and train at least 6 hours per week in order to participate in the study.

The protocol study was approved by the Ethics Committee of Clinical Research from the Government of Aragón (ref. CP08/2012, CEICA, Spain) and adhered to the Declaration of Helsinki as revised on Seoul 2008. Parents or legal custodians of all participants provided written informed consent and all participants expressed their agreement. The RENACIMIENTO project is registered in the ClincalTrials.gov repository under reference NCT02380664.

After baseline measurements that included body composition measurements, anthropometric assessments and blood sampling, swimmers were randomly allocated into two groups. One of the groups (SWI) continued with their regular swimming training whereas the second group, designated as vibration group (VIB) completed a WBV protocol in addition to their habitual training. All measurements and tests performed at baseline were repeated after the 6-month intervention period.

WBV intervention

The vibration training was performed standing on a synchronous platform (PowerPlate Pro5; PowerPlate, Amsterdam, The Netherlands). Vibration amplitude and frequency as well as exercise duration and number of sets were progressively increased across the six months of intervention as shown in figure 1.

Figure 1 – Whole body vibration training protocol

1.a. Half squat 120º | 1.b. Squat 90º | 1.c. Lunge with right foot in front | 1.d. Lunge with left foot in front

	Sessions	Frequency (Hz)	Peak-to-peak displacement (mm)	Duration (s)	Rest (s)	Repetitions	Vibration total time (min)	Training total time (min)	Peak acceleration (g)
Month 1	12	30	2	45	45	2	7.5	15	3.6
Month 2	12	30	4	45	45	2	7.5	15	7.2
Month 3	12	32	4	45	45	2	7.5	15	8.2
Month 4	12	34	4	60	60	2*	8	16	9.3
Month 5	12	36	4	60	60	2*	8	16	10.4
Month 6	12	38	4	60	60	2*	8	16	11.6

*Squat 120, 90º and dynamic were performed twice, while lunge with each leg was only performed once.

Participants were asked to attend to three weekly sessions wearing sport shoes and in pairs in order to alternate the exercising and resting periods between participants. All sessions were supervised by a sport scientist that controlled attendance and ensured the correct execution of all exercises, caring primarily for the safety of the participants.

Five different exercises were performed while standing on the platform. The following sequence was repeated twice on each session: Half squat (static position, knees bent 120°, figure 1a), squat (static position, knees bent 90°, figure 1b), dynamic flexion and extension

from the previous two positions, lunge with right foot in front (figure 1c) and lunge with left foot in front (figure 1d).

Anthropometric and body composition measurements

Participants wore no shoes and minimal clothing during the anthropometric examination. Height was measured with a stadiometer to the nearest 0.1 cm (SECA 225, SECA, Hamburg, Germany) and weight to the nearest 0.1 kg with an electronic scale (SECA 861, SECA, Hamburg, Germany).

Pubertal maturation was determined following the method established by Tanner (84), by self-assessment of secondary sexual characteristics, using a graphical scale as a reference. This method has been previously utilized to assess sexual maturity among adolescent athletes and it was found that the method was reliable and precise (247). Participants were categorized as early pubertal if they reported Tanner stages 1-3 at baseline and as late pubertal for stages 4 and 5. Even though Tanner stage 1 can be considered as prepubertal, in the present study only two participants were at this stadium. Therefore it was decided to merge them with the Tanner 2 and 3 stages.

Bone mineral status was determined using DXA scans at the subtotal whole body (whole body minus head), lumbar spine and hip (including femoral neck and total hip) evaluated with the pediatric version of the QDR-Explorer software, version 12.4 (Hologic Corp., Bedford, MA, USA). The same trained operator performed all the scans. Coefficients of variation for the DXA measurements in our laboratory were 2.3% for bone mineral content (BMC) and 1.3% for aBMD, as shown elsewhere (83).

Biomarker evaluation

Fasting blood samples were extracted from all participants prior to the baseline and follow-up measurements. In order to minimize the effects of the intraindividual circadian

variations, all samples were taken in the same time frame, between 8:00 and 10:00 in the morning (11). The blood was allowed to clot for 30 min at room temperature before centrifugation at 10.000 rpm for 15 min and storage at -80 ºC. Electrochemiluminescence immunoassays "ECLIA" were performed in an independent laboratory to measure the levels of bone formation markers osteocalcin (OC) and procollagen type 1 N-terminal propeptide (P1NP) and the bone resorption marker beta-C-terminal telopeptide crosslaps (CTX) in serum, using an Elecsys 2010 analyzer (Roche Diagnostics, GmbH, Germany). The intra-assay CV values for serum OC were less than 1% at 0.02 ng/ml and 1.2% at 253.0 ng/ml, for serum P1NP were 1.6% at 20.5 ng/ml and less than 1% at 811 ng/ml and for serum CTX were 2.0% and at 0.06 ng/ml and 1.7% at 1.92 ng/ml.

Statistical analysis

All statistical analyses were performed using SPSS for Windows version 22.0 (SPSS Inc., Chicago, IL, USA) with the significance level set at $p<0.05$. Kolmogorov-Smirnov tests were used to confirm the normality assumption for all variables included in the study. Additionally, no participants were excluded from the analysis after exploration for outliers.

Cross tabulation of sex and pubertal development status across groups was performed and chi-square statistics were used to determine the homogeneity of the group distribution. No sex stratification was used for further analysis, since no differences were found in the distribution among groups. Analyses of covariance adjusting by baseline age, Tanner stage and body weight were conducted to test the differences in bone biomarker levels among groups. Also, t-tests for related samples were used to check the differences within each group over time. Additionally, ANCOVA for repeated measures was used to check the group by time interaction. Partial Pearson's correlation coefficients were calculated to analyze the relationship between the levels of the different biomarkers and bone mineral development.

RESULTS

From the original sample of 150 participants, complete data (baseline and follow-up measurements) were obtained from 109 participants (42 females). Baseline characteristics of the sample including anthropometric measurements, BMC and BMD at the whole body, hip and lumbar spine are shown in table 1. No significant differences between groups were found in any of these parameters, except for the BMD at the femoral neck, which was higher in untrained controls than in swimmers. Additionally, no differences in the sex or pubertal stage distribution were found among the intervention groups.

Table 1 – Descriptive characteristics and bone mineral parameters of the sample at baseline

Descriptive characteristics	Overall (n=109)	Swimmers (n=68)	Controls (n=41)
Chronological age (y)	14.1 ± 2.1	14.2 ± 1.8	14.0 ± 2.6
Height (cm)	162.0 ± 12.7	163.6 ± 12.9	159.2 ± 12.1
Body mass (kg)	54.5 ± 13.2	55.0 ± 12.8	53.7 ± 13.9
BMI (kg/m^2)	20.48 ± 2.93	20.25 ± 2.62	20.87 ± 3.37
Tanner stage (I/II/III/IV/V)	2/20/23/49/15	2/14/14/32/6	0/6/9/17/9
Sex (male/female)	67/42	40/28	27/14
Bone mineral parameters	Overall (n=96)	Swimmers (n=62)	Controls (n=34)
SUBT BMC (g)	1407 ± 450	1414 ± 447	1395 ± 460
SUBT aBMD (g/cm^2)	0.879 ± 0.127	0.870 ± 0.122	0.894 ± 0.135
LS BMC (g)	46.1 ± 14.9	46.2 ± 13.9	45.8 ± 16.9
LS aBMD (g/cm^2)	0.844 ± 0.155	0.836 ± 0.146	0.861 ± 0.171
Hip BMC (g)	30.4 ± 10.1	30.4 ± 10.0	30.6 ± 10.3
Hip aBMD (g/cm^2)	0.912 ± 0.156	0.892 ± 0.147	0.947 ± 0.167
FN BMC (g)	4.06 ± 1.02	4.00 ± 0.94	4.18 ± 1.15
FN aBMD (g/cm^2)	0.838 ± 0.148	0.816 ± 0.129	0.878 ± 0.173*

*Significant differences between swimmers and controls ($p<0.05$)
BMI: Body mass index; SUBT: Subtotal whole body; LS: Lumbar spine
FN: Femoral neck; BMC: Bone mineral content; aBMD: Areal bone mineral density
Values are expressed as mean ± standard deviation (Tanner stage and sex as frequencies)

Correlation coefficients between bone mineral status and serum levels of biomarkers at baseline are displayed in table 2. Baseline values of all biomarkers presented significant and positive correlations with the future change in both BMC and BMD at all sites measured. All significant relationships found while comparing the variation of biomarkers and bone mineral status were negative, with CTX being the marker that consistently showed significant correlations across all sites whereas no significant correlation was found between subtotal bone mineral status and neither OC or P1NP.

Table 2 – Partial correlations between biomarkers and change in bone mineral parameters

Baseline	Δ SUBT BMC	Δ SUBT aBMD	Δ LS BMC	Δ LS aBMD	Δ Hip BMC	Δ Hip aBMD	Δ FN BMC	Δ FN aBMD
OC	**0.597**	**0.480**	**0.638**	**0.652**	**0.532**	**0.561**	**0.455**	**0.351**
P1NP	**0.746**	**0.656**	**0.696**	**0.675**	**0.606**	**0.595**	**0.574**	**0.376**
CTX	**0.627**	**0.478**	**0.601**	**0.582**	**0.506**	**0.477**	**0.417**	**0.247**
Absolute change	Δ SUBT BMC	Δ SUBT aBMD	Δ LS BMC	Δ LS aBMD	Δ Hip BMC	Δ Hip aBMD	Δ FN BMC	Δ FN aBMD
Δ OC	-0.079	-0.073	**-0.254**	**-0.245**	-0.138	**-0.219**	-0.078	-0.155
Δ P1NP	-0.100	-0.127	**-0.211**	-0.162	-0.114	**-0.220**	-0.198	**-0.229**
Δ CTX	**-0.267**	**-0.228**	**-0.332**	**-0.364**	-0.186	**-0.354**	**-0.252**	**-0.289**

Correlation coefficients are adjusted for age, pubertal stage and body weight
Bold characters indicate significant correlations
SUBT: Subtotal whole body; LS: Lumbar spine; FN: Femoral neck
BMC: Bone mineral content; aBMD: Areal bone mineral density

Table 3 presents the baseline and follow-up values for all bone biomarkers analyzed distributed by groups and subdivided into pubertal development categories. There were no differences in any of the bone biomarker levels at baseline among the intervention groups in either early puberty, late puberty or the complete sample. Overall, VIB significantly decreased their OC levels, whereas CON experienced an increase in this variable. For P1NP, a decrease over time for both VIB and SWI was observed, while the levels for CON remained stable. It should be highlighted that, when focusing on early pubertal participants, differences in all biomarkers emerged between VIB and CON, as there was a gain in OC

Table 3 – Differences in biomarkers (after adjustment for age, pubertal stage and body weight) between groups at baseline and follow-up

	Baseline			**Follow-up**			**Group by time interaction**
Overall	Vibration (n=36)	Swimmers (n=32)	Control (n=41)	Vibration (n=36)	Swimmers (n=32)	Control (n=41)	(η_p^2)
OC (µg/ml)	101.4 ± 6.5	123.3 ± 7.1	94.4 ± 6.3†	**82.8 ± 6.4**	118.8 ± 6.8*	**103.4 ± 6.0**	0.149⌟
P1NP (µg/ml)	528.4 ± 45.7	685.8 ± 50.0	544.9 ± 44.0	**389.0 ± 38.6**	**542.0 ± 41.5***	527.4 ± 36.4*	0.061⌟
CTX (µg/ml)	1.606 ± 0.109	1.905 ± 0.119	1.822 ± 0.105	1.509 ± 0.097	1.896 ± 0.104*	1.724 ± 0.092	0.005
Early puberty	Vibration (n=13)	Swimmers (n=17)	Control (n=15)	Vibration (n=13)	Swimmers (n=17)	Control (n=15)	
OC (µg/ml)	111.6 ± 11.1	127.2 ± 10.2	108.4 ± 11.9	102.4 ± 10.1	**145.5 ± 9.7***	118.1 ± 11.2	0.179⌟
P1NP (µg/ml)	696.4 ± 63.3	803.1 ± 58.0	590.0 ± 68.1	**523.0 ± 62.8**	747.4 ± 55.7*	652.7 ± 64.1	0.159⌟
CTX (µg/ml)	1.751 ± 0.140	1.969 ± 0.128	1.873 ± 0.151	1.806 ± 0.130	**2.255 ± 0.105***	1.894 ± 0.133	0.075
Late puberty	Vibration (n=23)	Swimmers (n=15)	Control (n=26)	Vibration (n=23)	Swimmers (n=15)	Control (n=26)	
OC (µg/ml)	89.8 ± 8.3	121.5 ± 10.8	89.9 ± 7.9	**67.4 ± 8.0**	**94.9 ± 10.0**	93.9 ± 7.5	0.167⌟
P1NP (µg/ml)	388.6 ± 61.3	636.4 ± 79.8	510.3 ± 58.4	**280.3 ± 50.2**	**375.2 ± 63.3**	446.3 ± 47.4	0.081
CTX (µg/ml)	1.447 ± 0.155	1.872 ± 0.201	1.837 ± 0.147	1.285 ± 0.135	**1.619 ± 0.170**	**1.600 ± 0.128**	0.015

Values are expressed as mean ± standard error

Bold characters indicate significant intra-group differences with baseline values ($p<0.05$)

* Significant differences compared to vibration group ($p<0.05$)

† Significant differences compared to swimmers group ($p<0.05$)

⌟ Significant group by time interaction ($p<0.05$)

and CTX for SWI and a decline in P1NP levels for VIB. Even though no significant differences in biomarker levels at follow-up were found among groups for the late puberty stage, a trend for lower OC (p=0.058) and P1NP (p=0.059) levels in VIB in contrast with CON appeared. Group by time interaction was found for OC at both early and late puberty stages and for P1NP among the early pubertal participants.

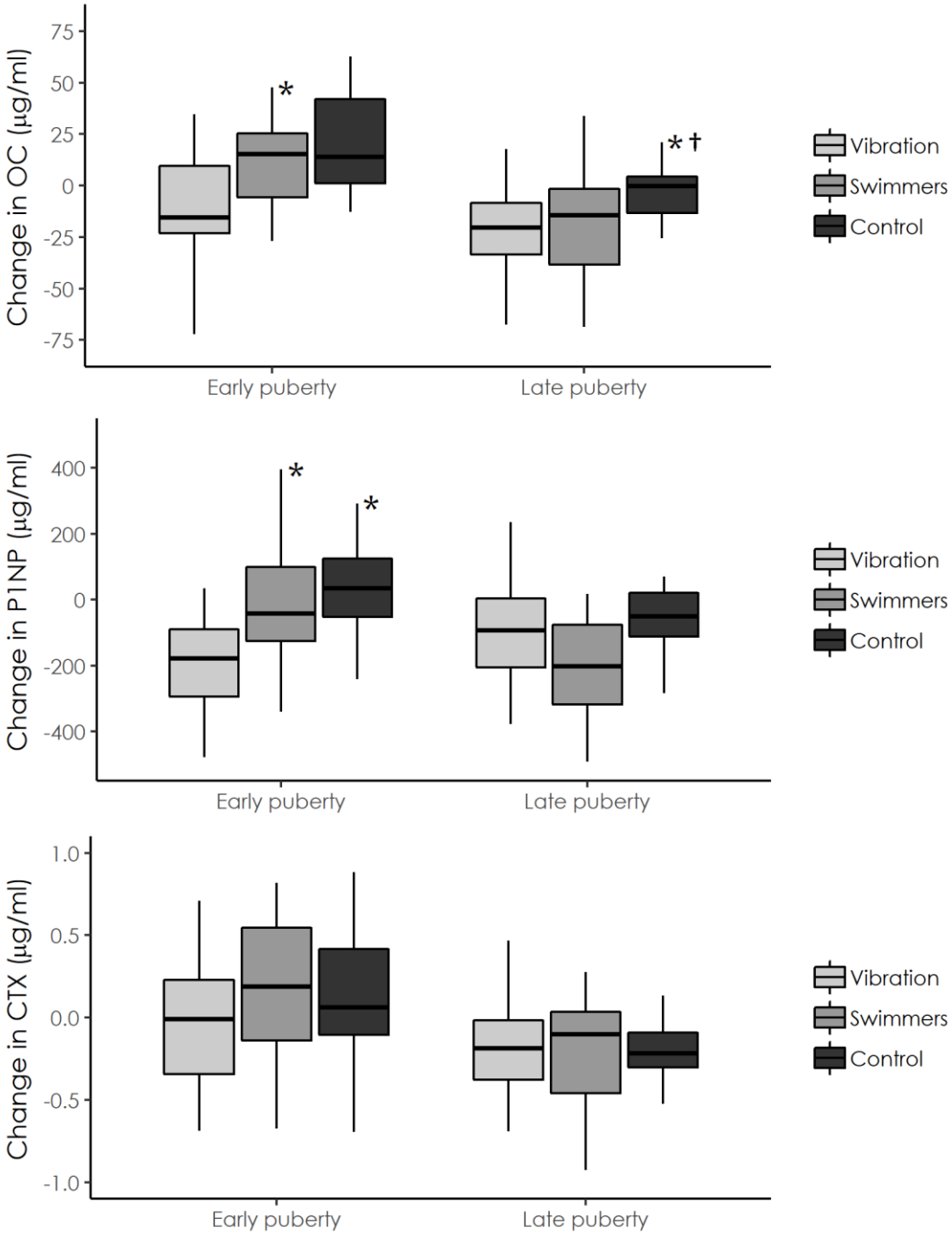
To further analyze the group differences on the evolution of biomarker levels, changes in these parameters were compared and shown in figure 2. As it can be observed, among the early pubertal participants, the group by time interaction found is due to VIB presenting lower changes than SWI for both OC and P1NP and lower than CON for P1NP. Also, a trend emerged for a difference in the change of OC levels between CON and VIB (p=0.062). On the other hand, during late puberty, both VIB and SWI showed a bigger decrease in OC levels in comparison to CON.

DISCUSSION

The main result from the present longitudinal study is that overall, our WBV training protocol seems to decrease global bone turnover rate in adolescent swimmers, since those in the vibration group presented lower levels of both formation and resorption markers after the intervention than those just swimming, while no differences were found between these groups at baseline.

After stratifying the sample according to their pubertal status, further statistical analysis revealed that these differences are found solely within the early pubertal participants. This could be partly due to the high metabolic activity characteristic of early puberty (119). Interpretation should be made with caution in this population since, even though bone markers are sensitive to remodeling (248), they are not able to discriminate between bone modeling and remodeling (27).

Figure 2 – Group comparison of the changes in biomarker levels after the intervention



* Significant differences compared to vibration group ($p<0.05$)

† Significant differences compared to swimmers group ($p<0.05$)

Taking normoactive controls into consideration it can be observed that statistically significant group by time interactions are found in formation markers but not in resorption. Also, no difference in either formation or resorption marker serum levels were found between swimmers and controls at both baseline and follow-up. This may suggest that the group that behaves differently is the vibration group, showing decreases in OC and P1NP levels after the intervention.

These results were surprising, especially taking into account the previously reported acute increases in bone markers following WBV sessions of both low (0.9 g) (244) and high magnitude (6.4 g) (249). However, when vibration training is combined with other forms of physical exercise, this results may vary, as shown by two studies in which combined resistance and vibration training did not alter the formation marker levels of male and female young adults (245,246). Therefore, it is not clear if these discrepancies are caused by differences between vibration protocols, inherent differences between acute and long-term adaptations or by the presence of concurrent training.

The potential interest of WBV training for the improvement of bone health underlies in Frost's mechanostat theorem (45) that establishes that bone reacts to mechanical stimuli. Although the specific mechanisms involved in the osteogenic response from WBV are not completely understood, it has been suggested that vibration might affect a wide array of physiologic systems, including an endocrine response (179). Trabecular bone has been shown to be more responsive to vibration than cortical tissue (70). According to the authors, the higher metabolic activity of trabecular bone compared to cortical bone is among the possible causes for this result. However, in this particular study bone metabolism has not been increased by vibration.

This is not the first study failing to find positive effects of WBV training on bone health. In a previously published paper within the framework of the RENACIMIENTO project, no benefits for aBMD in adolescent swimmers were found following the vibration intervention (82). Controlled trials (228), systematic reviews (186) and overviews (243) have described vibration training as an alternative method, best suited for subjects unable to engage in regular physical training but not particularly useful in healthy participants. Therefore, in order to improve bone status in adolescent swimmers it might be necessary to further investigate the effects of different vibration protocols or entirely new complementary training methods, such as plyometric training which has already shown improvements in bone mineral content, density and structure in children and adolescents (53).

The selected bone turnover markers have already been used in large adolescent samples (119) and are in accordance with scientific literature (11) and the recommendations issued by the International Osteoporosis Foundation and the International federation of Clinical Chemistry (12). Sampling, storing and chemical analysis were also conducted in compliance with the mentioned clinical guidelines. This can be considered one of the strengths of the present study along with the use of gold standard methods to evaluate aBMD.

There are some limitations that should also be acknowledged such as the intrinsic variability of bone turnover markers which could have been further controlled with repeated blood and urine sampling. Also, the wide age range might compromise the applicability of the results. However, it should be pointed out that chi-square tests showed that there were no differences among the study groups regarding the sex and pubertal stage distributions.

In conclusion, this specific vibration training protocol resulted in decreases in bone turnover markers in comparison with control swimmers, especially in bone formation markers in early puberty. Therefore, WBV training might not be an ideal intervention to elicit an osteogenic response in adolescent swimmers and alternative training protocols or modalities should be explored.

Acknowledgements and funding

We would like to thank the participants, their families and the coaches for their collaboration in this project. This work was supported by the Spanish "Ministerio de Economía y Competitividad" "Plan Nacional I+D+i2008–2011 (Project DEP2011-29093)" and by "Ministerio de Educación y Ciencia" (EXERNET - Project DEP2005-00046). This project has been co-financed by "Fondo Europeo de Desarrollo Regional" (MICINN-FEDER). JMP received a Grant FPU014/04302 from "Ministerio de Educación, Cultura y Deportes".

4.6 Design of a computer model for the identification of adolescent swimmers with low BMD

ABSTRACT

Purpose: This paper aims to elaborate a decision tree for the early detection of adolescent swimmers at risk of presenting low bone mineral density (BMD), based on easily measurable fitness and performance variables.

Methods: Bone mineral status of 78 adolescent swimmers was determined using dual-energy X-ray absorptiometry (DXA) scans at the hip and subtotal body. Participants also underwent physical fitness (upper and lower body strength, running speed and cardiovascular endurance) and performance (swimming history, speed and ranking) assessments. A gradient boosting machine regression tree was built in order to predict BMD of the swimmers and to further develop a simpler individual decision tree, using a subtotal BMD height-adjusted Z-score of -1 as threshold value.

Results: The predicted BMD using the gradient boosted model was strongly correlated with the actual BMD values obtained from DXA ($r=0.960$, $p<0.0001$) with a root mean squared error of 0.034 g/cm^2. According to a simple decision tree, that showed a 73.9% of classification accuracy, swimmers with a body mass index (BMI) lower than 17 kg/m^2 or a handgrip strength inferior to 43kg with the sum of both arms could be at higher risk of having low BMD.

Conclusions: Easily measurable fitness variables (BMI and handgrip strength) could be used for the early detection of adolescent swimmers at risk of suffering from low BMD. The presented decision tree could be used in training settings to determine the necessity of further BMD assessments.

Keywords: Osteoporosis prevention; decision tree; physical fitness

INTRODUCTION

Osteoporosis is a metabolic disease that is characterized by a deterioration in skeletal tissue including a clinically low bone mineral density (BMD) and a compromise for the microarchitecture of the bone (29). Osteoporosis affects 22.1% of women and 6.6% of men over 50 years in the European Union and the total number of patients with this condition is expected to increase in the following years due to demographic variations (30). This structural fragility entails a lower tolerance to stress which may play a role in up to a 90% of bone fractures (33). These osteoporotic fractures have been linked to a decrease in the quality of life, to the apparition of disabilities or even mortality (35). Nowadays, osteoporosis treatment and prevention has become one of the primary concerns for healthcare systems in developed countries (34). In fact, a total of 33 different clinical practice guides on osteoporosis screening and management issued by institutions all around the globe have been identified and evaluated in a recent systematic review (250), concluding that collaboration and consensus is needed in the elaboration of these guidelines.

Adolescence stands as a decisive period for osteoporosis prevention, since around 40% of adult bone mass is created during this stage (18) and will influence the peak BMD reached in early adulthood. Achieving the highest peak BMD possible is key for the prevention of osteoporosis later in life (18), given that fracture risk is expected to be halved with an increase of one standard deviation in peak bone mass (156).

Physical activity participation and calcium intake are among the controllable factors that are known to affect bone health during childhood and adolescence (16). Consistent and positive effects on bone development have been found by different reviews focused on sport practice (55) and exercise interventions (53). Additionally, these benefits on bone

status obtained from physical activity have been observed to persist in later stages in life (134). However, not all sport modalities have proven to be beneficial to bone, especially non-weight bearing activities such as swimming may not have a positive effect on bone health (251).

Osteoporosis has been defined as a "silent" disease, given that no pain or other symptoms are perceived by the subject who is affected by this condition (33). For this reason, osteoporosis and low BMD can remain undetected for years and sometimes it is only discovered once the osteoporotic fracture has occurred. There are subjects, thus, who are unknowingly affected by this condition, unaware of their higher risk of fracture and while this condition remains unnoticed no preventive measures could be taken.

Early detection of subjects affected by low BMD is therefore of paramount importance. As such, public health recommendations include an osteoporosis screening for the elderly (42) using the current gold standard method for BMD evaluation, which is the dual-energy X-ray absorptiometry (DXA). However, this clinical evaluation is rarely performed in other population segments, which might also be susceptible from suffering of a decreased BMD such as swimmers.

Correctly identifying the target group for BMD assessments is important regarding the cost-effectiveness of osteoporosis management (252). However, performing a DXA scan to all adolescent swimmers would not be cost-effective, especially taking into account that the younger the target group, the higher the number of scans needed to prevent one fracture (43). For this reason, the assessment of different variables related to BMD, more accessible to researchers or healthcare workers, may provide a tool for determining which subjects might need a deeper evaluation of their bone mineral status.

Therefore, the main goal of the present study is to elaborate a screening method for detecting potential risk of low BMD in adolescent swimmers based on easily measurable variables.

MATERIAL AND METHODS

Participants and study design

Eighty-six adolescent swimmers (41 females, all Caucasian and aged 10-18 years) participated in the present cross-sectional study, which is part of the broader RENACIMIENTO project (75). Participants had to have a minimum of 3 years of regional swimming competition experience and train at least 6 hours per week in order to be part of the study. Exclusion criteria included smoking, taking medication known to alter bone and suffering from chronic diseases or musculoskeletal disorders.

Written informed consent from parents was obtained and all participants expressed their agreement. The protocol study was approved by the Ethics Committee of Clinical Research from the Government of Aragón (ref. CP08/2012, CEICA, Spain) and the ethical guidelines for human research outlined by the Declaration of Helsinki (revision of Seoul 2008) were followed.

Anthropometric and bone measurements

Participants underwent the anthropometric examination wearing no shoes and minimal clothing. Height was measured with a stadiometer to the nearest 0.1 cm (SECA 225, SECA, Hamburg, Germany) and weight to the nearest 0.1 kg with an electronic scale (SECA 861, SECA, Hamburg, Germany). Body mass index (BMI) was calculated as weight (kg) divided by squared height (m^2).

Bone mineral content (g) and areal BMD (g/cm^2) was determined by means of a dual-energy X-ray (DXA) scan at the whole body and the hip, evaluated with the pediatric

version of the QDR-Explorer software, version 12.4 (Hologic Corp., Bedford, MA, USA). All the scans were performed by the same qualified operator who had been trained in the operation of the scanner, the positioning of subjects, and the analysis of scans, according to the manufacturer's guidelines. Coefficients of variation for the DXA measurements in our laboratory have already been published (83) and were 2.3% for BMC and 1.3% for BMD.

Subtotal (whole body less head) BMD height-adjusted Z-scores were calculated according to the reference values provided by Zemel et al. (253). A Z-score of -1 was used as the threshold value for the purpose of categorizing subjects with a low BMD. Subtotal whole body was used as it is one of the regions recommended by the International Society for Clinical Densitometry in pediatric populations (38). Height-adjustment is also advised in this official stand (38).

Evaluation of pubertal stage

Pubertal maturation was determined by self-assessment of secondary sexual characteristics, with the assistance of a graphical scale, following the method established by Tanner (84), which has been demonstrated as a valid and reliable method to assess sexual maturity among adolescent athletes (247).

Fitness assessment

Four different components of physical fitness were assessed using field tests. Strength of the upper limbs was determined by the sum of both arms in a maximum isometric handgrip strength with a dynamometer (TKK 5101, Takei Corp., Tokio, Japan), while strength of the lower limbs was assessed by the standing long jump test. Running speed was calculated from the time to complete a 30-m sprint, and aerobic endurance was assessed by means of a 20-m shuttle run test (78). All tests were performed twice and the best result from both

attempts was recorded with the exception of the aerobic endurance test, which was performed once.

Performance and questionnaires

Swimming history was acquired from a self-reported questionnaire in which participants stated their weekly hours of training and their swimming competition experience (in years). The structured questionnaire also included information of current and past participation in other sports. Additionally, swimming performance was obtained consulting the official timing of swimming competitions, recording the participants' time in 50-m free-style and their FINA ranking points, an official metric used by the International Swimming Federation to track the performance of swimmers. Daily calcium intake (in milligrams) was calculated from a food frequency questionnaire that included daily, weekly and monthly consumption frequency of various calcium-containing aliments such as cheese or bread (254), which has been validated for adolescent swimmers (85).

Statistical analysis

Statistical analyses were performed using SPSS for Windows version 22.0 (SPSS Inc., Chicago, IL, USA) with the significance level set at $p<0.05$. Additionally, the construction of the decision tree was performed with the statistical programming language R (90), including the packages *rpart* (255) and *gbm* (256).

Kolmogorov-Smirnov tests were used to confirm the normality assumption and outlier exploration was performed for all variables included in the study. T-tests for independent samples were used to check the differences between subjects with and without low BMD values on the fitness and performance variables.

Decision tree modelling

70% of the sample was randomly selected to build a decision tree in order to identify the fitness and performance variables that perform a better discrimination between BMD groups, whereas the remaining 30% of the sample was used to test its classification accuracy. Cross tabulation of sex and pubertal development status across groups was performed and chi-square statistics were used to determine the homogeneity of the group distribution in categorical variables between the training and testing subsamples.

Two decision trees were constructed, following different approaches. The first one was a regression tree (treating hip BMD as a continuous variable) fitting all measured variables. Gradient boosting (257,258) was implemented to increase its precision. From this initial computer model, a second model was developed. This decision tree (259) included solely the nine variables that were significant in the previous model and considered only two possible outcomes; being above or below a Z-score of -1 on the total hip BMD. A summary of the variables included in the decision tree modelling is provided in **Supplementary Table 1**. The code used for the construction of both models can be consulted in **Supplementary Material 1**.

RESULTS

Participant characteristics

After removing participants with incomplete or outlier data, 78 participants out of the total sample of 86 swimmers were analyzed. **Table 1** presents their descriptive characteristics, stratified according to their random allocation to the training or testing subsample. No differences between groups were found for any studied variable.

Table 1 – Descriptive characteristics of the participants

General characteristics and anthropometric variables	Overall (n=78)	Training (n=55)	Testing (n=23)
Sex (male/female)	40 / 38	26 / 29	14 / 9
Tanner stage (I/II/III/IV/V)	2 / 18 / 16 / 38 / 7	1 / 10 / 13 / 27 / 4	1 / 6 / 3 / 11 / 2
Age (years)	14.3 ± 1.9	14.3 ± 1.8	14.3 ± 2.2
Height (cm)	163.8 ± 12.0	163.1 ± 11.3	165.4 ± 13.5
Weight (kg)	54.3 ± 12.1	53.3 ± 11.2	56.7 ± 13.9
BMI (kg/m^2)	20.0 ± 2.5	19.8 ± 2.3	20.4 ± 2.8
Bone mineral variables	Overall (n=81)	Training (n=57)	Testing (n=24)
Subtotal BMC (g)	1388 ± 416	1350 ± 392	1481 ± 465
Subtotal BMD (g/cm^2)	0.867 ± 0.113	0.858 ± 0.108	0.889 ± 0.123
Total hip BMC (g)	29.9 ± 9.1	28.9 ± 8.2	32.1 ± 10.6
Total hip BMD (g/cm^2)	0.888 ± 0.133	0.879 ± 0.136	0.907 ± 0.126

Categorical variables expressed as frequencies
Continuous variables expressed as mean ± standard deviation
No significant differences between groups were found

The results from the fitness and performance comparison between participants above and below the threshold in total hip BMD Z-score can be observed in **table 2**. Differences were found between groups for the handgrip strength, long jump, 50-m swim and FINA points (all $p<0.05$).

Table 2 – Fitness and performance comparison between subjects with and without low BMD

Fitness variables	Overall (n=78)	Low BMD (n=24)	Not low BMD (n=54)
Handgrip strength (kg)	53.5 ± 17.3	46.3 ± 16.1	56.7 ± 17.0*
Long jump (cm)	185.6 ± 30.4	174.3 ± 31.8	191.0 ± 28.5*
30-m run (s)	5.21 ± 0.48	5.33 ± 0.42	5.15 ± 0.50
VO_{2max} (mL/kg/min)	50.4 ± 5.3	49.3 ± 5.0	51.0 ± 5.3
Training and performance variables	Overall (n=78)	Low BMD (n=24)	Not low BMD (n=54)
Weekly training (h)	10.0 ± 2.1	9.8 ± 2.1	10.0 ± 2.1
Swimming history (years)	7.9 ± 2.9	7.6 ± 2.7	8.0 ± 3.0
50-m swim (s)	31.2 ± 3.5	32.8 ± 4.4	30.5 ± 2.8*
FINA points	354 ± 84	317 ± 82	370 ± 80*

Values expressed as mean ± standard deviation
*Significant differences compared to the low BMD group

Gradient boosting machine regression tree

After scouting and tuning hyper-parameters for the gradient boosting machine (**Supplementary Material 1**), the optimal robustness was found using a total of 5 iterations of the gradient boosting model. Nine of the variables included in the model had a significant influence. An overview of the significant variables is provided in **figure 1**, where it can be observed that height and weight are the variables that contribute the most to the model prediction (41.9 and 18.5% respectively), followed by the handgrip strength (8.4%).

When this computer regression model was applied to the 30% of the sample intended to test its prediction accuracy, a root mean squared error of 0.034 g/cm^2 was obtained. The BMD values obtained from the model were significantly correlated with the actual BMD

Figure 1 - Relative contribution of the main variables of the ensemble model

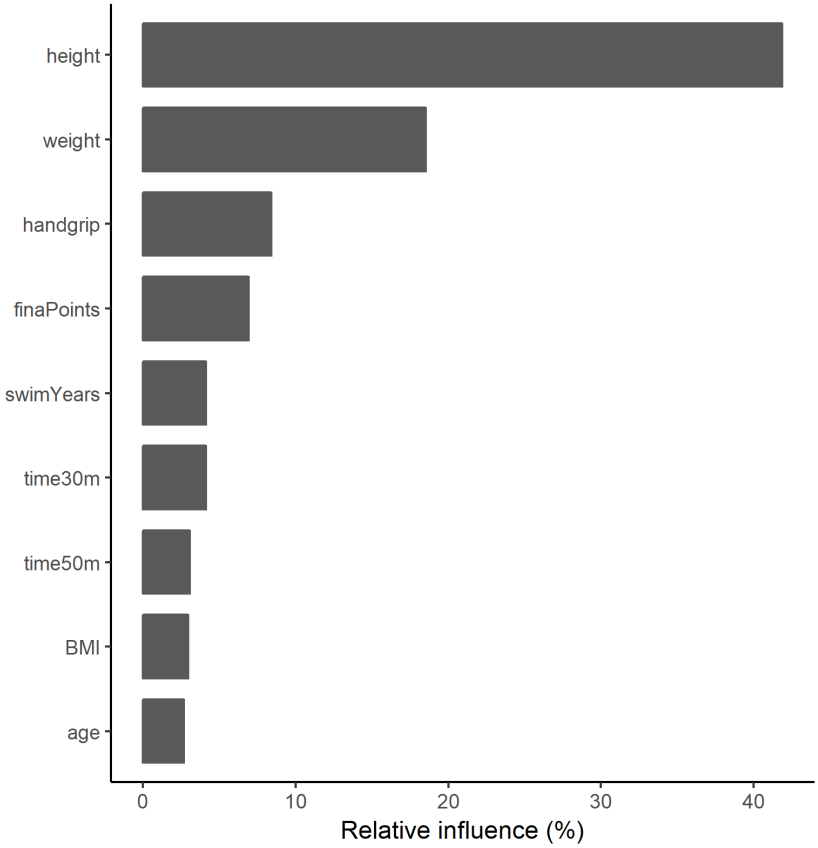
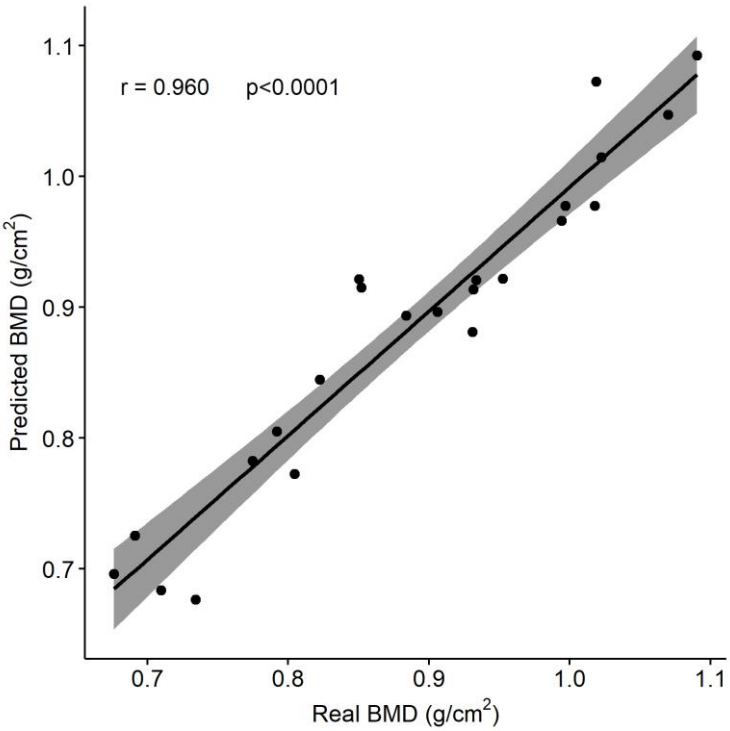


Figure 2 - Comparison between the predicted and actual subtotal BMD



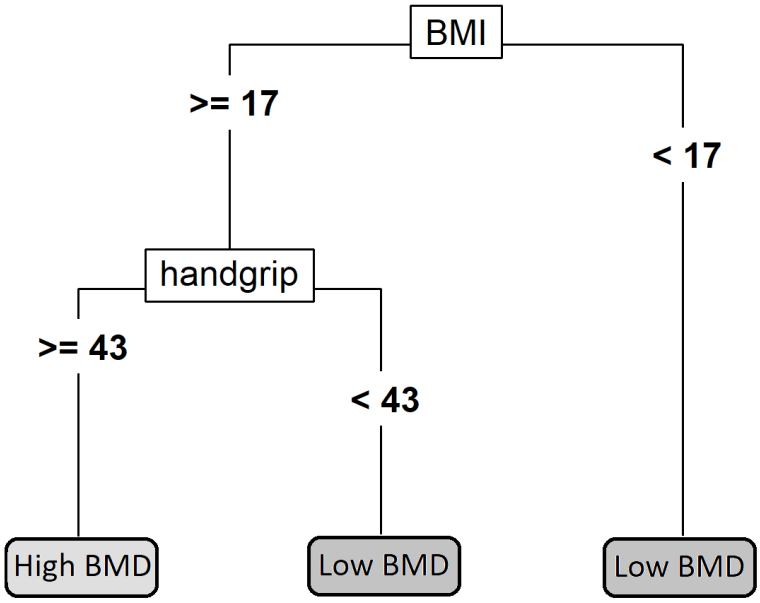
The solid line represents the regression function between the variables, while the grey area shows its 95% confidence interval

values as measured by DXA (r=0.960, p<0.0001, **figure 2**). When converted into the correspondent height-adjusted Z-scores, an 73.9% of classification accuracy was reached when comparing participants above and below the proposed threshold.

Individual decision tree

Figure 3 shows the development of a single classification tree that splits the training sample into four terminal nodes. According to this model, subjects who have either a BMI under 17 kg/m^2 or less than 43 kg of handgrip strength (summing both arms) present the higher risk of having low subtotal BMD for their age and height. This individual decision tree has an overall classification accuracy of 73.9%, a sensitivity of 50% and a specificity of 82.4%.

Figure 3 - Individual decision tree



Handgrip strength is measured in kg, adding the results from both arms
BMI is measured in kg/m^2

DISCUSSION

Main results and relevance

The main results from the present document are that physical fitness and performance variables can be used for the prediction of low BMD in adolescent swimmers and establish the appropriateness of further body composition examinations.

The relevance of the present study is the development of a practical tool for an initial screening of adolescent swimmers at risk of suffering from low BMD. The swimming trainers or healthcare workers can easily measure all the variables included in the model without demanding requirements of material or human resources. Most variables can be measured with the help of a questionnaire or a chronometer, being the main exception the handgrip test, which requires a dynamometer. However, the use of this test is justified for three reasons, since it has been related to other health and performance parameters (260,261), it contributes significantly to both the regression and decision models and it should also be noted that it is a particularly quick, cheap and simple test.

Regarding the specific results of the gradient boosting machine model, height and weight of the participants were the variables that affected the most to their BMD. However, some physical fitness and performance variables accounted for some of the model prediction, especially handgrip strength. The mean squared error of the model did not result in a systematic or proportional bias in the prediction of the total hip BMD. The classification accuracy of 0.739 is not perfect but it is better than chance alone and similar than the areas under the curve (AUC) reported (262) for other screening strategies devised for postmenopausal women such as the FRAX tool (263) (AUC: 0.60), the Simple Calculated Osteoporosis Risk Score (264) (AUC: 0.72) and the Osteoporosis Self-Assessment Tool (265) (AUC: 0.73).

These results are obtained from the model that includes all sixteen variables in a gradient boosting machine and can provide interesting theoretical information. However, this model might not be easy to implement, since it requires a high number of measurements and a computer evaluation of the model. In order to offer a useful tool for trainers that might not have the time and skills needed to perform the complete evaluation, an individual decision tree is provided, which is based solely on the handgrip test and BMI measurement.

In the particular case of our testing sample, three out of the six participants that actually had a subtotal BMD Z-score below -1 would have been recommended to undergo a DXA scan based on the results from our model. Also, only two of the other seventeen participants of the subsample would have been erroneously advised to have their BMD checked.

The accuracy and validity of both the gradient boosting model and the individual decision tree have been assessed with the perspective of trying to simultaneously optimize both the sensitivity and the specificity of the model. However, decision tree analysis allows penalizing differently false positive and false negative cases, by assigning their specific costs. This might be interesting, since it could be argued that in this particular it might be preferable to have a healthy subject scanned rather than failing to identify a participant at risk of having low BMD. A cost-effective analysis of the osteoporosis prevention system could reveal the actual cost of a false negative over a false positive that would in turn provide the optimal balance between specificity and sensitivity that the model should seek. However, doing so would require specific evaluation of the costs of healthcare interventions, which may vary between countries and would therefore limit the geographical applicability of the model.

It is important to note that the purpose of the presented models is not to provide clinical diagnostic or to replace the need DXA scans in any way, but rather is to complement them,

serving as a previous screening filter for detecting those subjects who would benefit the most from a DXA assessment. The cost-effectiveness of the osteoporosis screening protocol for postmenopausal women has been confirmed (252). However, the final implementation of these protocols is not always comprehensive, and sensitization strategies have been implemented (266,267). A previous study that evaluated the effects of the implementation of a temporary clinical case finding strategy for osteoporosis detection in postmenopausal women showed a great improvement of the screening policy using a simple index based solely on the age and weight of the subject (268). Additionally, model-based tools have been successfully implemented to assess fracture risk in the elderly in the framework of clinical osteoporosis management (263). However, to the best of our knowledge, no similar studies have been performed with adolescent swimmers. We consider that this is of primary importance, given the popularity of this sport nowadays in the young populations, and that it will permit the early identification of individuals with low BMD, and it will allow to start taking action early and act preventively in order to avoid future cases of osteoporosis.

Limitations and future research

Some limitations have to be acknowledged as well. The reduced size and the wide age range of the test subsample might require the confirmation of the validity and adjustment of the model in specific populations. However, it should be pointed out that no differences in age or sex distribution were found between the testing and training subgroups or among the low and normal BMD groups. Additionally, even though fitness and nutrition variables were considered, there might be other variables that affect BMD included in the regression algorithm. Future research should confirm the applicability of the model in other samples as well as to investigate if the addition of other easily measurable variables could improve the prediction accuracy.

Conclusion

In conclusion, this study presents a theoretical model and a practical tool for early detection of adolescent swimmers at risk of low BMD that can be quickly applied by swimming trainers or health professionals that work as a first step to seek clinical advice to those subjects that were previously unaware of their condition.

Acknowledgements

We would like to thank the participants, their families and the coaches for their collaboration in this project. This work was supported by the Spanish "Ministerio de Economía y Competitividad" "Plan Nacional I+D+i2008–2011 (Project DEP2011-29093)" and by "Ministerio de Educación y Ciencia" (Project DEP2005-00046). This project has been co-financed by "Fondo Europeo de Desarrollo Regional" (MICINN-FEDER). JMP received a Grant FPU014/04302 from 'Ministerio de Educación, Cultura y Deportes'.

Competing interests

The authors declare no conflicts of interest.

Capítulo 5

Conclusiones y aportaciones principales

Conclusiones

- **Artículo I.** La condición física, considerada de forma holística, está relacionada con parámetros estructurales y mecánicos del hueso ya en la etapa preescolar. Considerando los componentes individuales de la condición física, los valores de fuerza y agilidad son los que mayor correlación muestran con los parámetros óseos. No obstante, esta relación parece ser exclusiva de los parámetros estructurales, puesto que no se encontraron resultados significativos con respecto a la densidad mineral ósea volumétrica.
- **Artículo II.** La intensidad de actividad física que muestra una mayor correlación con el contenido y densidad mineral óseos es la vigorosa. Los adolescentes que participaron en al menos una tanda de cinco minutos consecutivos de actividad física vigorosa presentaron mejores parámetros óseos, especialmente a nivel del cuello femoral, que aquellos que no lo hicieron. La participación en tandas más prolongadas, de al menos 15 minutos, parece relacionarse con valores de densidad mineral ósea aún más elevados.
- **Artículo III.** Se observa un claro efecto osteogénico de la práctica de actividad física vigorosa durante la pubertad temprana. Los adolescentes que eran inactivos en la medición inicial pero incrementaron sus niveles de actividad física en la evaluación final experimentaron mayores incrementos en su densidad mineral ósea que aquellos que permanecieron inactivos en ambas etapas del estudio, llegando a hacer desaparecer las diferencias observadas en la evaluación inicial con respecto a los participantes que ya eran activos al comienzo del estudio.
- **Artículo IV.** El entrenamiento vibratorio de cuerpo completo muestra potencial para la mejora del contenido mineral óseo de niños y adolescentes con valores reducidos de estos parámetros. Por otra parte, con respecto a la preservación de la

masa ósea en mujeres postmenopáusicas los beneficios observados del entrenamiento vibratorio son inexistentes o poco relevantes clínicamente, por lo que su uso principal sería como terapia alternativa para aquellas personas que fueran incapaces de realizar un entrenamiento convencional. Existe una gran heterogeneidad entre los distintos protocolos de entrenamiento vibratorio y todavía se desconocen los parámetros idóneos para maximizar la respuesta osteogénica.

- **Artículo V.** Los nadadores que participaron en la intervención con plataforma vibratoria presentaron valores inferiores de marcadores de metabolismo óseo en comparación con los controles normoactivos y el resto de nadadores. Esta reducción se observa especialmente en los marcadores de formación ósea de aquellos participantes en las etapas tempranas de la pubertad. Por lo tanto, no se recomienda el uso de este protocolo en nadadores adolescentes, siendo necesaria la exploración de otras alternativas, como distintos protocolos de vibración o modalidades de ejercicio completamente distintos.
- **Artículo VI.** Un sencillo algoritmo de clasificación basado en el índice de masa corporal y la fuerza de prensión manual puede ayudar a identificar a los nadadores en riesgo de presentar una densidad mineral ósea inferior a la media. Estas variables se pueden evaluar de manera rápida y sencilla por lo que se podría implementar su valoración en el entorno de los clubes deportivos. Además, la utilización de una batería mayor de pruebas junto con un modelo de clasificación más complejo permitió la estimación del contenido mineral óseo, presentando una elevada correlación con los resultados obtenidos mediante DXA.

Conclusions

- **Manuscript I.** Physical fitness as a whole is related to structural and mechanical properties of bone, already at preschool age. Regarding the individual components of physical fitness, strength and agility are the ones that present the highest correlation with bone parameters. This relationship, however, seems to be exclusive of structural variables, since no significant correlations were found with volumetric bone density.
- **Manuscript II.** Vigorous physical activity is the activity intensity that shows the highest correlation with bone mineral content and density. Adolescents that engaged in at least one 5-minute bout of vigorous physical activity presented higher bone mineral parameters, especially at the femoral neck, than those who didn't. Participation in longer bouts, of 15 minutes or more, seems to be related with an even better bone mineral status.
- **Manuscript III.** A clear osteogenic effect of vigorous physical activity was observed during early puberty. Boys who were inactive initially but increased their physical activity participation during the follow-up measurements presented higher increments of bone mineral density than those who remained inactive. Moreover, these participants were able to reach a similar bone mineral status than those who were already active at the beginning of the study.
- **Manuscript IV.** Whole-body vibration training might be useful to improve bone mineral status in children and adolescents with compromised bone mass. On the other hand, the effects of this type of training on postmenopausal women are small and clinically irrelevant, and therefore its main application would be as an alternative training for those patients unable to take part in regular exercise

interventions. There is a great heterogeneity among the different vibration protocols and the optimal parameters for eliciting an osteogenic response are still unknown.

- **Manuscript V.** Swimmers that took part in the whole-body vibration training showed lower levels of bone turnover markers when compared to normoactive controls and other swimmers, especially regarding the formation markers in early pubertal participants. Therefore, adolescent swimmers should not participate in this vibration protocol and different training modalities should be explored.
- **Manuscript VI.** A simple classification algorithm based on the body mass index and the handgrip strength might help identifying those swimmers at risk of having low bone mineral density. These variables can be measured in a quick an easy manner and therefore, this assessment could be included in training settings. Additionally, the use of a more extensive set of tests alongside with a more complex classification algorithm allowed to estimate the bone mineral density of the swimmers, showing a high correlation with the values obtained by DXA.

Aportaciones principales de la Tesis Doctoral

- Gracias a los resultados obtenidos en el estudio del proyecto PREFIT (artículo I), se demuestra que la condición física juega un papel fundamental en la estructura ósea, ya en las primeras etapas de la vida. Además, se recalca la importancia de considerar la condición física en su conjunto, ya que puede proporcionar resultados que van más allá de la mera suma de sus componentes individuales.
- A partir del estudio transversal realizado en Estonia (artículo II) se han definido los patrones de práctica de actividad física vigorosa que se relacionan con una mejor salud ósea, atendiendo no sólo a la intensidad y duración total sino también a la frecuencia y duración de cada periodo de práctica de actividad vigorosa. Asimismo, al ampliarse estos datos en el estudio longitudinal (artículo III) se dan claros argumentos en favor de la participación en actividad física vigorosa, al demostrarse que esta es beneficiosa para la salud ósea de los adolescentes que comienzan a realizarla, así como para la de aquellos que perseveran en su práctica.
- La revisión de revisiones sistemáticas (artículo IV) cumple una doble finalidad, al recopilar en un solo documento la información existente actualmente con respecto al entrenamiento vibratorio de cuerpo completo como herramienta enfocada hacia la mejora de la salud ósea y permitiendo a su vez la identificación de los grupos poblacionales que más se pueden beneficiar de este tipo de entrenamiento.
- Aunque los resultados encontrados en el estudio de intervención del proyecto RENACIMIENTO (artículo V) fueron inesperados, esto no impide la extracción de conclusiones y aplicaciones a partir de ellos. Se ha identificado un protocolo de entrenamiento que no es efectivo de cara a la estimulación osteogénica de los

nadadores adolescentes, lo que a su vez puede servir para redirigir los esfuerzos de posibles intervenciones o líneas de investigación futuras.

- Por último, el desarrollo del algoritmo de decisión del estudio transversal del proyecto RENACIMIENTO (artículo VI) se realizó teniendo presente la importancia de su transferibilidad práctica. De esta manera, se ha conseguido proporcionar a los entrenadores y monitores una herramienta rápida y sencilla que permite identificar a aquellos nadadores que pueden ser más vulnerables a un problema de deficiente mineralización ósea.

Main contributions of the Thesis

- Thanks to the results obtained in the study that was part of the PREFIT project (manuscript I), the crucial role that physical fitness plays on bone structure already on the first stages of life is highlighted. Moreover, it is shown that physical fitness considered as a whole can provide results that go beyond the mere addition of its individual components.
- From the Estonian cross-sectional study (manuscript II), the patterns of vigorous physical activity participation that are related to an improved bone health were defined, taking into consideration not only the overall duration and intensity but also the frequency and duration of the vigorous activity bouts. When these data are completed with the longitudinal results (manuscript III) a clear point towards the encouragement of vigorous physical participation for the improvement of bone health is provided. It is proven that both engaging and maintaining high levels of vigorous physical activity participation are beneficial for bone development during puberty.
- The overview of systematic reviews (manuscript IV) served a dual purpose, summarizing the scientific literature currently available on the topic of whole-body vibration training as a tool for the improvement of bone health as well as identifying the target populations that might benefit the most from this type of training.
- Even though the results found in the intervention study that was part of the RENACIMIENTO project (manuscript V) were unexpected, this does not mean that these results are not useful or that conclusions cannot be drawn from them. The training protocol used has been identified as ineffective in the promotion of

osteogenesis, which could, in turn, help redirect the focus of future interventions or research.

- Lastly, during the development of the decision algorithm from the RENACIMIENTO project (manuscript VI) its applicability on real practice was considered of paramount importance. As a result, swimming coaches can now use this quick and simple tool that might help them identify those swimmers that are more likely to be suffering from low bone mineral density.

Referencias [References]

1. Calbet JAL. Osteoporosis y ejercicio. Ediciones Díaz de Santos; 2013.

2. Wolff J. The law of bone formation. Hirschwald, Berlin. 1892;

3. Reith EJ, Ross MH. Atlas of Descriptive Histology: With 281 Illustr. Harper & Row; 1968.

4. Seibel MJ, Robins SP, Bilezikian JP. Dynamics of bone and cartilage metabolism: principles and clinical applications. Elsevier; 2006.

5. Applegate EJ. The anatomy and physiology learning system. Saunders/Elsevier; 2011. 518 p.

6. Seeman E. Structural basis of growth-related gain and age-related loss of bone strength. Rheumatology (Oxford). 2008 Jul;47 Suppl 4:iv2-8.

7. Currey JD. Princeton University Press; Princeton, NJ: 2002. Bones Struct Mech.

8. Burtis CA, Ashwood ER, Bruns DE. Tietz textbook of clinical chemistry and molecular diagnostics-e-book. Elsevier Health Sciences; 2012.

9. Bernardi D, Zaninotto M, Plebani M. Requirements for improving quality in the measurement of bone markers. Clin Chim acta. 2004;346(1):79–86.

10. Hill PA. Bone remodelling. Br J Orthod. 1998;25(2):101–7.

11. Hlaing TT, Compston JE. Biochemical markers of bone turnover - uses and limitations. Ann Clin Biochem. 2014;51(2):189–202.

12. Vasikaran S, Cooper C, Eastell R, Griesmacher A, Morris HA, Trenti T, et al.

International Osteoporosis Foundation and International Federation of Clinical Chemistry and Laboratory Medicine position on bone marker standards in osteoporosis. Clin Chem Lab Med. 2011;49(8):1271–4.

13. Sadler TW, Langman J. Langman: Embriología médica con orientación clínica. Médica Panamericana,; 2007.

14. Rigutti A. Atlas ilustrado de anatomía. Susaeta Ediciones; 2002.

15. Bailey DA, McKay HA, Mirwald RL, Crocker PRE, Faulkner RA. A six-year longitudinal study of the relationship of physical activity to bone mineral accrual in growing children: the university of Saskatchewan bone mineral accrual study. J bone Miner Res. 1999;14(10):1672–9.

16. Weaver CM, Gordon CM, Janz KF, Kalkwarf HJ, Lappe JM, Lewis R, et al. The National Osteoporosis Foundation's position statement on peak bone mass development and lifestyle factors: a systematic review and implementation recommendations. Osteoporos Int. 2016/02/10. 2016;27(4):1281–386.

17. BONJOUR J-P, THEINTZ G, BUCHS B, SLOSMAN D, RIZZOLI R. Critical years and stages of puberty for spinal and femoral bone mass accumulation during adolescence. J Clin Endocrinol Metab. 1991;73(3):555–63.

18. Baxter-Jones ADG, Faulkner RA, Forwood MR, Mirwald RL, Bailey DA. Bone mineral accrual from 8 to 30 years of age: An estimation of peak bone mass. J Bone Miner Res. 2011;26(8):1729–39.

19. Robling AG, Castillo AB, Turner CH. Biomechanical and molecular regulation of bone remodeling. Annu Rev Biomed Eng. 2006;8:455–98.

20. Riggs B, Khosia S, Melton L. Sex steroids and the conservation of the adult skeleton. Endocrinol Rev. 2002;23(3):279–302.

21. Seeman E. Bone quality: the material and structural basis of bone strength. J Bone Miner Metab. 2008;26(1):1–8.

22. Duan Y, Wang X-F, Evans A, Seeman E. Structural and biomechanical basis of racial and sex differences in vertebral fragility in Chinese and Caucasians. Bone. 2005;36(6):987–98.

23. Parfitt AM, Travers R, Rauch F, Glorieux FH. Structural and cellular changes during bone growth in healthy children. Bone. 2000;27(4):487–94.

24. Van der Linden JC, Homminga J, Verhaar JAN, Weinans H. Mechanical consequences of bone loss in cancellous bone. J Bone Miner Res. 2001;16(3):457–65.

25. Rauchenzauner M, Schmid A, Heinz-Erian P, Kapelari K, Falkensammer G, Griesmacher A, et al. Sex- and age-specific reference curves for serum markers of bone turnover in healthy children from 2 months to 18 years. J Clin Endocrinol Metab. 2007;92(2):443–9.

26. van Coeverden SCCM, Netelenbos JC, de Ridder CM, Roos JC, Popp-Snijders C, Delemarre-van de Waal HA. Bone metabolism markers and bone mass in healthy pubertal boys and girls. Clin Endocrinol (Oxf). 2002 Jul 1;57(1):107–16.

27. Jürimäe J. Interpretation and application of bone turnover markers in children and adolescents. Curr Opin Pediatr. 2010;22(4):494–500.

28. Jurimae J, Maestu J, Jurimae T. Bone turnover markers during pubertal

development: relationships with growth factors and adipocytokines. Med Sport Sci. 2010;55:114–27.

29. Peck WA, Burckhardt P, Christiansen C, Fleisch HA, Genant HK, Gennari C, et al. Consensus development conference: diagnosis, prophylaxis, and treatment of osteoporosis. In: American Journal of Medicine. 1993. p. 646–50.

30. Hernlund E, Svedbom A, Ivergård M, Compston J, Cooper C, Stenmark J, et al. Osteoporosis in the European Union: Medical management, epidemiology and economic burden: A report prepared in collaboration with the International Osteoporosis Foundation (IOF) and the European Federation of Pharmaceutical Industry Associations (EFPIA). Arch Osteoporos. 2013;8(1–2):136.

31. Encuesta Europea de Salud. Estado de salud y enfermedades crónicas. [Internet]. Instituto Nacional de Estadística. 2014 [cited 2019 Sep 8]. Available from: http://www.ine.es/jaxi/Tabla.htm?path=/t15/p419/p01/a2003/l0/&file=01011.px&L=0

32. Wright NC, Saag KG, Dawson-Hughes B, Khosla S, Siris ES. The impact of the new National Bone Health Alliance (NBHA) diagnostic criteria on the prevalence of osteoporosis in the USA. Osteoporos Int. 2017 Apr 13;28(4):1225–32.

33. Daly RM, Petit MA. Optimizing bone mass and strength. Vol. 53, Journal of Chemical Information and Modeling. Basel: Karger; 2007. 1689-1699 p.

34. Cooper C, Cole ZA, Holroyd CR, Earl SC, Harvey NC, Dennison EM, et al. Secular trends in the incidence of hip and other osteoporotic fractures. Osteoporos Int. 2011;22(5):1277–88.

35. Bliuc D, Nguyen ND, Milch VE, Nguyen T V, Eisman JA, Center JR. Mortality risk associated with low-trauma osteoporotic fracture and subsequent fracture in men and women. JAMA. 2009;301(5):513–21.

36. Simón L, Thuissard I, Gogorcena M La. Atención a la Fractura de Cadera en los Hospitales del SNS. Madrid Minist Sanid y Política Soc. 2010;

37. Writing Group for the ISCD Position Development Conference. Diagnosis of osteoporosis in men, premenopausal women, and children. Vol. 7, J Clin Densitom. . 2004. p. 17–26.

38. Crabtree NJ, Arabi A, Bachrach LK, Fewtrell M, Fuleihan GE-H, Kecskemethy HH, et al. Dual-energy X-ray absorptiometry interpretation and reporting in children and adolescents: the revised 2013 ISCD Pediatric Official Positions. J Clin Densitom. 2014;17(2):225–42.

39. Carter DR, Bouxsein ML, Marcus R. New approaches for interpreting projected bone densitometry data. J Bone Miner Res. 2009 Dec 3;7(2):137–45.

40. Silva BC, Leslie WD, Resch H, Lamy O, Lesnyak O, Binkley N, et al. Trabecular Bone Score: A Noninvasive Analytical Method Based Upon the DXA Image. J Bone Miner Res. 2014 Mar 1;29(3):518–30.

41. Beck TJ. Extending DXA beyond bone mineral density: understanding hip structure analysis. Curr Osteoporos Rep. 2007;5(2):49–55.

42. Cosman F, de Beur SJ, LeBoff MS, Lewiecki EM, Tanner B, Randall S, et al. Clinician's Guide to Prevention and Treatment of Osteoporosis. Osteoporos Int. 2014 Oct 15;25(10):2359–81.

43. Nelson HD, Haney EM, Dana T, Bougatsos C, Chou R. Screening for Osteoporosis: An Update for the U.S. Preventive Services Task Force. Ann Intern Med. 2010;153(2):99–111.

44. Rizzoli R, Bianchi ML, Garabédian M, McKay HA, Moreno LA. Maximizing bone mineral mass gain during growth for the prevention of fractures in the adolescents and the elderly. Bone. 2010;46(2):294–305.

45. Frost HM. Bone's mechanostat: a 2003 update. Anat Rec Part A Discov Mol Cell Evol Biol. 2003;275(2):1081–101.

46. Frost HM. Bone "mass" and the "mechanostat": a proposal. Anat Rec. 1987;219(1):1–9.

47. Pivonka P, Park A, Forwood MR. Functional Adaptation of Bone: The Mechanostat and Beyond. In: Multiscale Mechanobiology of Bone Remodeling and Adaptation. Springer; 2018. p. 1–60.

48. Forwood MR, Turner CH. Skeletal adaptations to mechanical usage: results from tibial loading studies in rats. Bone. 1995;17(4 SUPPL.):197–205.

49. Calbet JA, Dorado C, Diaz-Herrera P, Rodriguez-Rodriguez LP. High femoral bone mineral content and density in male football (soccer) players. Med Sci Sports Exerc. 2001;33(10):1682–7.

50. Gomez-Cabello A, Ara I, Gonzalez-Aguero A, Casajus JA, Vicente-Rodriguez G. Effects of training on bone mass in older adults: a systematic review. Sport Med. 2012/03/02. 2012;42(4):301–25.

51. French SA, Fulkerson JA, Story M. Increasing Weight-Bearing Physical Activity

and Calcium Intake for Bone Mass Growth in Children and Adolescents: A Review of Intervention Trials. Prev Med (Baltim). 2000;31(6):722–31.

52. Poitras VJ, Gray CE, Borghese MM, Carson V, Chaput J, Janssen I, et al. Systematic review of the relationships between objectively measured physical activity and health indicators in school-aged children and youth. Appl Physiol Nutr Metab. 2016;6(Suppl 3):S197-239.

53. Gómez-Bruton A, Matute-Llorente Á, González-Agüero A, Casajús JA, Vicente-Rodríguez G. Plyometric exercise and bone health in children and adolescents: a systematic review. World J Pediatr. 2017 Apr 15;13(2):112–21.

54. Gutin B, Owens S, Okuyama T, Riggs S, Ferguson M, Litaker M. Effect of physical training and its cessation on percent fat and bone density of children with obesity. Obes Res. 1999;7(2):208–14.

55. Gruodytė R, Jürimäe J, Saar M, Jürimäe T. The relationships among bone health, insulin-like growth factor-1 and sex hormones in adolescent female athletes. J Bone Miner Metab. 2010;28(3):306–13.

56. MacKelvie KJ, Khan KM, McKay HA. Is there a critical period for bone response to weight-bearing exercise in children and adolescents? A systematic review. Br J Sports Med. 2002;36(4):250–7.

57. Mitchell JA, Chesi A, Elci O, McCormack SE, Roy SM, Kalkwarf HJ, et al. Physical Activity Benefits the Skeleton of Children Genetically Predisposed to Lower Bone Density in Adulthood. J Bone Miner Res. 2016;31(8):1504–12.

58. Khan K, McKay HA, Haapasalo H, Bennell KL, Forwood MR, Kannus P, et al.

Does childhood and adolescence provide a unique opportunity for exercise to strengthen the skeleton? J Sci Med Sport. 2000;3(2):150–64.

59. Warden SJ, Mantila Roosa SM, Kersh ME, Hurd AL, Fleisig GS, Pandy MG, et al. Physical activity when young provides lifelong benefits to cortical bone size and strength in men. Proc Natl Acad Sci. 2014 Apr 8;111(14):5337–42.

60. Cardadeiro G, Baptista F, Ornelas R, Janz KF, Sardinha LB. Sex Specific Association of Physical Activity on Proximal Femur BMD in 9 to 10 Year-Old Children. PLoS One. 2012;7(11):e50657.

61. Ivuskans A, Latt E, Maestu J, Saar M, Purge P, Maasalu K, et al. Bone mineral density in 11-13-year-old boys: relative importance of the weight status and body composition factors. Rheumatol Int. 2013 Jul;33(7):1681–7.

62. Gracia-Marco L, Rey-López JP, Santaliestra-Pasías AM, Jiménez-Pavón D, Díaz LE, Moreno LA, et al. Sedentary behaviours and its association with bone mass in adolescents: the HELENA cross-sectional study. BMC Public Health. 2012;12(1):971.

63. Vicente-Rodríguez G, Ortega FB, Rey-López JP, España-Romero V, Blay VA, Blay G, et al. Extracurricular physical activity participation modifies the association between high TV watching and low bone mass. Bone. 2009;45(5):925–30.

64. Gracia-Marco L, Moreno LA, Ortega FB, Len F, Sioen I, Kafatos A, et al. Levels of physical activity that predict optimal bone mass in adolescents: The HELENA study. Am J Prev Med. 2011;40(6):599–607.

65. Vicente-Rodríguez G. How does exercise affect bone development during growth? Sport Med. 2006;36(7):561–9.

66. Gabel L, Macdonald HM, Nettlefold L, McKay HA. Bouts of Vigorous Physical Activity and Bone Strength Accrual During Adolescence. Pediatr Exerc Sci. 2017;29(4):465–75.

67. Gómez-Bruton A, Gónzalez-Agüero A, Gómez-Cabello A, Casajus JA, Vicente-Rodriguez G. Is bone tissue really affected by swimming? A systematic review. PLoS One. 2013;8(8):e70119.

68. Bruininks BD, Korak JA, Scibora LM, Sage SK, Mellick PF. Swimming and Bone Density: A DXA and pQCT Study Examining Swimming as an Osteogenic Activity. J Exerc Physiol Online. 2019;22(1):75–97.

69. Rubin CT, Sommerfeldt DW, Judex S, Qin Y-X. Inhibition of osteopenia by low magnitude, high-frequency mechanical stimuli. Drug Discov Today. 2001;6(16):848–58.

70. Rubin C, Turner AS, Mallinckrodt C, Jerome C, McLeod K, Bain S. Mechanical strain, induced noninvasively in the high-frequency domain, is anabolic to cancellous bone, but not cortical bone. Bone. 2002;30(3):445–52.

71. Cardinale M, Wakeling J. Whole body vibration exercise: are vibrations good for you? Br J Sports Med. 2005/08/25. 2005;39(9):585–9; discussion 589.

72. Abercromby AFJ, Amonette WE, Layne CS, McFarlin BK, Hinman MR, Paloski WH. Vibration exposure and biodynamic responses during whole-body vibration training. Med Sci Sports Exerc. 2007;39(10):1794.

73. WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI-Seoul revision: Ethical Principles for Medical Research Involving Human Subjects. J Korean Med Assoc. 2010 Sep 1;53(9):739.

74. World Medical Association. World Medical Association Declaration of Helsinki. JAMA. 2013 Nov 27;310(20):2191.

75. Gómez-Bruton A, Gonzalez-Agüero A, Casajus JA, Vicente-Rodriguez G. Swimming training repercussion on metabolic and structural bone development; benefits of the incorporation of whole body vibration or pilometric training; the RENACIMIENTO project. Nutr Hosp. 2014 Aug 1;30(2):399–409.

76. Binkley TL, Specker BL. pQCT measurement of bone parameters in young children: validation of technique. J Clin Densitom. 2000;3(1):9–14.

77. Cadenas-Sanchez C, Martinez-Tellez B, Sanchez-Delgado G, Mora-Gonzalez J, Castro-Piñero J, Löf M, et al. Assessing physical fitness in preschool children: Feasibility, reliability and practical recommendations for the PREFIT battery. J Sci Med Sport. 2015;

78. Leger L, Lambert J. A maximal multistage 20-m shuttle run test to predict. Eur J Appl Physiol. 1982;49:1–12.

79. Greulich WW, Pyle SI. Radiographic atlas of skeletal development of the hand and wrist. Am J Med Sci. 1959;238(3):393.

80. Groell R, Lindbichler F, Riepl T, Gherra L, Roposch A, Fotter R. The reliability of bone age determination in central European children using the Greulich and Pyle method. Br J Radiol. 1999;72(MAY):461–4.

81. Evenson KR, Catellier DJ, Gill K, Ondrak KS, McMurray RG. Calibration of two objective measures of physical activity for children. J Sports Sci. 2008;26(14):1557–65.

82. Gómez-Bruton A, González-Agüero A, Matute-Llorente A, Julián C, Lozano-Berges G, Gómez-Cabello A, et al. Do 6 months of whole-body vibration training improve lean mass and bone mass acquisition of adolescent swimmers? Arch Osteoporos. 2017 Dec 21;12(1):69.

83. Gracia-Marco L, Ortega FB, Jiménez-Pavón D, Rodríguez G, Castillo MJ, Vicente-Rodríguez G, et al. Adiposity and bone health in Spanish adolescents. the HELENA study. Osteoporos Int. 2012;23(3):937–47.

84. Tanner J, Whitehouse R, Takaishi M. Standards from birth to maturity for height, weight, height velocity, and weight velocity: British children, 1965. II. Arch Dis Child. 1966;41:613–35.

85. Julian Almarcegui C, Huybrechts I, Gomez Bruton A, Matute Llorente A, Gonzalez Aguero A, Gomez Cabello A, et al. Validity of a food-frequency questionnaire for estimating calcium intake in adolescent swimmers. Nutr Hosp. 2015 Oct;32(4):1773–9.

86. Smith V, Devane D, Begley CM, Clarke M. Methodology in conducting a systematic review of systematic reviews of healthcare interventions. BMC Med Res Methodol. 2011/02/05. 2011;11(1):15.

87. Becker LA, Oxman AD. Overviews of reviews. Cochrane Handbook for Systematic Reviews of Interventions. Chichester: John Wiley & Sons.; 2008. 607-631 p.

88. Shea BJ, Grimshaw JM, Wells GA, Boers M, Andersson N, Hamel C, et al. Development of AMSTAR: a measurement tool to assess the methodological quality of systematic reviews. BMC Med Res Methodol. 2007;7(1):10.

89. Pieper D, Antoine SL, Mathes T, Neugebauer EA, Eikermann M. Systematic review finds overlapping reviews were not mentioned in every other overview. J Clin Epidemiol. 2014/03/04. 2014;67(4):368–75.

90. R Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2019.

91. Basterfield L, Reilly JK, Pearce MS, Parkinson KN, Adamson AJ, Reilly JJ, et al. Longitudinal associations between sports participation, body composition and physical activity from childhood to adolescence. J Sci Med Sport. 2015 Mar;18(2):178–82.

92. Esteban-Cornejo I, Tejero-Gonzalez CM, Sallis JF, Veiga OL. Physical activity and cognition in adolescents: A systematic review. J Sci Med Sport. 2015 Sep;18(5):534–9.

93. Herrmann D, Buck C, Sioen I, Kouride Y, Marild S, Molnár D, et al. Impact of physical activity, sedentary behaviour and muscle strength on bone stiffness in 2–10-year-old children-cross-sectional results from the IDEFICS study. Int J Behav Nutr Phys Act. 2015 Dec;12(1):112.

94. Tan VP, Macdonald HM, Kim S, Nettlefold L, Gabel L, Ashe MC, et al. Influence of physical activity on bone strength in children and adolescents: a systematic review and narrative synthesis. J Bone Min Res. 2014;29(10):2161–81.

95. Gu X, Chang M, Solmon MA. Physical Activity, Physical Fitness, and Health-Related Quality of Life in School-Aged Children. J Teach Phys Educ. 2016 Apr;35(2):117–26.

96. Ortega FB, Ruiz JR, Hurtig-Wennlöf A, Vicente-Rodríguez G, Rizzo NS, Castillo MJ, et al. Cardiovascular fitness modifies the associations between physical activity and abdominal adiposity in children and adolescents: the European Youth Heart Study. Br J Sports Med. 2010 Mar;44(4):256–62.

97. Ortega FB, Ruiz JR, Castillo MJ, Sjöström M. Physical fitness in childhood and adolescence: a powerful marker of health. Int J Obes. 2008;32(1):1–11.

98. Smith JJ, Eather N, Morgan PJ, Plotnikoff RC, Faigenbaum AD, Lubans DR. The Health Benefits of Muscular Fitness for Children and Adolescents: A Systematic Review and Meta-Analysis. Sport Med. 2014 Sep;44(9):1209–23.

99. Gracia-Marco L, Vicente-Rodríguez G, Casajús JA, Molnar D, Castillo MJ, Moreno LA. Effect of fitness and physical activity on bone mass in adolescents: The HELENA Study. Eur J Appl Physiol. 2011;111(11):2671–80.

100. Gordon CM, Bachrach LK, Carpenter TO, Crabtree N, El-Hajj Fuleihan G, Kutilek S, et al. Dual energy X-ray absorptiometry interpretation and reporting in children and adolescents: the 2007 ISCD Pediatric Official Positions. J Clin Densitom. 2008;11(1):43–58.

101. Sievänen H. A physical model for dual-energy X-ray absorptiometry--derived bone mineral density. Invest Radiol. 2000 May;35(5):325–30.

102. Janz KF, Letuchy EM, Burns TL, Francis SL, Levy SM. Muscle Power Predicts

Adolescent Bone Strength: Iowa Bone Development Study. Med Sci Sport Exerc. 2015;47(10):2201–6.

103. Duckham RL, Rantalainen T, Ducher G, Hill B, Telford RD, Telford RM, et al. Effects of Habitual Physical Activity and Fitness on Tibial Cortical Bone Mass, Structure and Mass Distribution in Pre-pubertal Boys and Girls: The Look Study. Calcif Tissue Int. 2016;99(1):56–65.

104. Clark EM, Ness AR, Tobias JH. Bone Fragility Contributes to the Risk of Fracture in Children, Even After Moderate and Severe Trauma. J Bone Miner Res. 2007 Oct;23(2):173–9.

105. Kalkwarf HJ, Laor T, Bean JA. Fracture risk in children with a forearm injury is associated with volumetric bone density and cortical area (by peripheral QCT) and areal bone density (by DXA). Osteoporos Int. 2011;22(2):607–16.

106. Rizzoli R, Bianchi ML, Garabedian M, McKay HA, Moreno LA. Maximizing bone mineral mass gain during growth for the prevention of fractures in the adolescents and the elderly. Bone. 2010;46(2):294–305.

107. Ortega FB, Cadenas-Sánchez C, Sánchez-Delgado G, Mora-González J, Martínez-Téllez B, Artero EG, et al. Systematic review and proposal of a field-based physical fitness-test battery in preschool children: The PREFIT battery. Sport Med. 2015 Apr;45(4):533–55.

108. Cadenas-Sanchez C, Martinez-Tellez B, Sanchez-Delgado G, Mora-Gonzalez J, Castro-Piñero J, Löf M, et al. Assessing physical fitness in preschool children: Feasibility, reliability and practical recommendations for the PREFIT battery. J Sci Med Sport. 2016 Nov;19(11):910–5.

109. Binkley TL, Specker BL. pQCT measurement of bone parameters in young children: validation of technique. J Clin Densitom. 2000;3(1):9–14.

110. Gómez-Bruton A, González-Agüero A, Gómez-Cabello A, Matute-Llorente A, Zemel BS, Moreno LA, et al. Bone structure of adolescent swimmers; a peripheral quantitative computed tomography (pQCT) study. J Sci Med Sport. 2016;19(9).

111. Lozano-Berges G, Matute-Llorente Á, Gómez-Bruton A, González-Agüero A, Vicente-Rodríguez G, Casajús JA. Bone geometry in young male and female football players: a peripheral quantitative computed tomography (pQCT) study. Arch Osteoporos. 2018;13(1).

112. Gómez-Bruton A, Gonzalez-Agüero A, Casajús JA, Rodríguez GV. Swimming training repercussion on metabolic and structural bone development; benefits of the incorporation of whole body vibration or pilometric training; the RENACIMIENTO project | Repercusión del entrenamiento y la práctica de la natación sobre el desar. Nutr Hosp. 2014;30(2).

113. Adams JE, Engelke K, Zemel BS, Ward KA. Quantitative Computer Tomography in Children and Adolescents: The 2013 ISCD Pediatric Official Positions. 2013;

114. Cadenas-Sanchez C, Intemann T, Labayen I, Peinado AB, Vidal-Conti J, Sanchis-Moysi J, et al. Physical fitness reference standards for preschool children: The PREFIT project. J Sci Med Sport. 2018 Sep;

115. Vicente-Rodríguez G, Urzanqui A, Mesana MI, Ortega FB, Ruiz JR, Ezquerra J, et al. Physical fitness effect on bone mass is mediated by the independent association between lean mass and bone mass through adolescence: A cross-sectional study. J Bone Miner Metab. 2008;26(3):288–94.

116. Prokasky A, Rudasill K, Molfese VJ, Putnam S, Gartstein M, Rothbart M. Identifying child temperament types using cluster analysis in three samples. J Res Pers. 2017;67:190–201.

117. Sanson A, Letcher P, Smart D, Prior M, Toumbourou JW, Oberklaid F. Associations between early childhood temperament clusters and later psychosocial adjustment. 2009;55(1):26–54.

118. Eshghi A, Haughton D, Legrand P, Skaletsky M, Woolford S. Identifying groups: A comparison of methodology. J Data Sci. 2011;9:271–91.

119. Gracia-Marco L, Vicente-Rodriguez G, Valtuena J, Rey-Lopez JP, Martinez AED, Mesana MI, et al. Bone mass and bone metabolism markers during adolescence: The HELENA Study. Horm Res Paediatr. 2010;74(5):339–50.

120. Nguyen T V, Maynard LM, Towne B, Roche AF, Wisemandle W, Li J, et al. Sex differences in bone mass acquisition during growth: the Fels Longitudinal Study. J Clin Densitom. 2001;4(2):147–57.

121. Moon RJ, Cole ZA, Crozier SR, Curtis EM, Davies JH, Gregson CL, et al. Longitudinal changes in lean mass predict pQCT measures of tibial geometry and mineralisation at 6-7 years. Bone. 2015 Jun;75:105–10.

122. Schoenau E. The Development of the Skeletal System in Children and the Influence of Muscular Strength. Horm Res Paediatr. 1998;49(1):27–31.

123. Haapasalo H, Kontulainen S, Sievanen H, Kannus P, Jarvinen M, Vuori I. Exercise-induced bone gain is due to enlargement in bone size without a change in volumetric bone density: a peripheral quantitative computed tomography study of

the upper arms of male tennis players. Bone. 2000;27(3):351–7.

124. Nogueira R, Weeks B, Beck B. Exercise to improve pediatric bone and fat: a systematic review and meta-analysis. Med Sci Sport Exerc. 2014;46(3):610–21.

125. Ginty F, Rennie KL, Mills L, Stear S, Jones S, Prentice A. Positive, site-specific associations between bone mineral status, fitness, and time spent at high-impact activities in 16- to 18-year-old boys. Bone. 2005 Jan;36(1):101–10.

126. VLACHOPOULOS D, UBAGO-GUISADO E, BARKER AR, METCALF BS, FATOUROS IG, AVLONITI A, et al. Determinants of Bone Outcomes in Adolescent Athletes at Baseline. Med Sci Sport Exerc. 2017 Jul;49(7):1389–96.

127. Vicente-Rodriguez G, Ara I, Perez-Gomez J, Serrano-Sanchez JA, Dorado C, Calbet JA. High femoral bone mineral density accretion in prepubertal soccer players. Med Sci Sport Exerc. 2004;36(10):1789–95.

128. Kemper HC, Twisk JW, van Mechelen W, Post GB, Roos JC, Lips P. A fifteen-year longitudinal study in young adults on the relation of physical activity and fitness with the development of the bone mass: The Amsterdam Growth And Health Longitudinal Study. Bone. 2000;27(6):847–53.

129. Barnekow-Bergkvist M, Hedberg G, Pettersson U, Lorentzon R. Relationships between physical activity and physical capacity in adolescent females and bone mass in adulthood. Scand J Med Sci Sport. 2006 Dec;16(6):447–55.

130. Specker B, Binkley T. Randomized trial of physical activity and calcium supplementation on bone mineral content in 3- to 5-year-old children. J Bone Min Res. 2003;18(5):885–92.

131. Gómez-Bruton A, González-Agüero A, Matute-Llorente A, Julián C, Lozano-Berges G, Gómez-Cabello A, et al. Effects of Whole Body Vibration on Tibia Strength and Structure of Competitive Adolescent Swimmers: A Randomized Controlled Trial. PM&R. 2018 Sep;10(9):889–97.

132. Cole ZA, Harvey NC, Kim M, Ntani G, Robinson SM, Inskip HM, et al. Increased fat mass is associated with increased bone size but reduced volumetric density in pre pubertal children. Bone. 2012 Feb;50(2):562–7.

133. Kannus P, Parkkari J, Niemi S. Age-adjusted incidence of hip fractures. Lancet. 1995;346(8966):50–1.

134. Holroyd C, Harvey N, Dennison E, Cooper C. Epigenetic influences in the developmental origins of osteoporosis. Osteoporos Int. 2012;23(2):401–10.

135. Hernandez CJ, Beaupré GS, Carter DR. A theoretical analysis of the relative influences of peak BMD, age-related bone loss and menopause on the development of osteoporosis. Osteoporos Int. 2003;14(10):843–7.

136. Kemper HCG. Skeletal Development During Childhood and Adolescence and the Effects of Physical Activity. Pediatr Exerc Sci. 2000;12(47):198–216.

137. Karlsson MK, Linden C, Karlsson C, Johnell O, Obrant K, Seeman E. Exercise during growth and bone mineral density and fractures in old age. Lancet (London, England). 2000 Feb 5;355(9202):469–70.

138. Sardinha LB, Baptista F, Ekelund U. Objectively Measured Physical Activity and Bone Strength in 9-Year-Old Boys and Girls. Pediatrics. 2008;122(3):e728–36.

139. Robling AG, Hinant FM, Burr DB, Turner CH. Improved bone structure and

strength after long-term mechanical loading is greatest if loading is separated into short bouts. J Bone Miner Res. 2002;17(8):1545–54.

140. Ivuškāns A, Jurimae T, Latt E, Jurimae J, Purge P, Saar M, et al. Role of physical activity in bone health in peripubertal boys. Pediatr Int. 2014;56(5):763–7.

141. Ivuškāns A, Mäestu J, Jürimäe T, Lätt E, Purge P, Saar M, et al. Sedentary time has a negative influence on bone mineral parameters in peripubertal boys: a 1-year prospective study. J Bone Miner Metab. 2015;33(1):85–92.

142. Vaitkeviciute D, Lätt E, Mäestu J, Jürimäe T, Saar M, Purge P, et al. Physical activity and bone mineral accrual in boys with different body mass parameters during puberty: A longitudinal study. PLoS One. 2014;9(10).

143. Ottevaere C, Huybrechts I, de Meester F, de Bourdeaudhuij I, Cuenca-Garcia M, de Henauw S. The use of accelerometry in adolescents and its implementation with non-wear time activity diaries in free-living conditions. J Sports Sci. 2011;29(1):103–13.

144. Trost SG, Mciver KL, Pate RR. Conducting accelerometer-based activity assessments in field-based research. Med Sci Sports Exerc. 2005;37(11 SUPPL.):531–43.

145. Trost SG, Loprinzi PD, Moore R, Pfeiffer KA. Comparison of accelerometer cut points for predicting activity intensity in youth. Med Sci Sports Exerc. 2011;43(7):1360–8.

146. Janz KF, Letuchy EM, Francis SL, Metcalf KM, Burns TL, Levy SM. Objectively measured physical activity predicts hip and spine bone mineral content in children

and adolescents ages 5-15 years: Iowa bone development study. Front Endocrinol (Lausanne). 2014;5(JUL):1–7.

147. Sayers A, Mattocks C, Deere K, Ness A, Riddoch C, Tobias JH. Habitual levels of vigorous, but not moderate or light, physical activity is positively related to cortical bone mass in adolescents. J Clin Endocrinol Metab. 2011;96(5):793–802.

148. Janz KF, Burns TL, Torner JC, Levy SM, Paulos R, Willing MC, et al. Physical activity and bone measures in young children: the Iowa Bone Development Study. Pediatrics. 2001;107(6):1387–93.

149. Kanis JA, McCloskey E V., Johansson H, Oden A, Melton LJ, Khaltaev N. A reference standard for the description of osteoporosis. Bone. 2008;42(3):467–75.

150. Bakker I, Twisk JWR, Van Mechelen W, Kemper HCG. Fat-free body mass is the most important body composition determinant of 10-yr longitudinal development of lumbar bone in adult men and women. J Clin Endocrinol Metab. 2003;88(6):2607–13.

151. Kriemler S, Zahner L, Puder JJ, Braun-Fahrländer C, Schindler C, Farpour-Lambert NJ, et al. Weight-bearing bones are more sensitive to physical exercise in boys than in girls during pre- and early puberty: A cross-sectional study. Osteoporos Int. 2008;19(12):1749–58.

152. Heidemann M, Mølgaard C, Husby S, Schou AJ, Klakk H, Møller NC, et al. The intensity of physical activity influences bone mineral accrual in childhood: the childhood health, activity and motor performance school (the CHAMPS) study, Denmark. BMC Pediatr. 2013;13(1):32.

153. Vainionpää A, Korpelainen R, Sievänen H, Vihriälä E, Leppäluoto J, Jämsa T. Effect of impact exercise and its intensity on bone geometry at weight-bearing tibia and femur. Bone. 2007;40(3):604–11.

154. Wright NC, Looker AC, Saag KG, Curtis JR, Delzell ES, Randall S, et al. The Recent Prevalence of Osteoporosis and Low Bone Mass in the United States Based on Bone Mineral Density at the Femoral Neck or Lumbar Spine. J Bone Miner Res. 2014 Nov;29(11):2520–6.

155. Pocock NA, Eisman JA, Hopper JL, Yeates MG, Sambrook PN, Eberl S. Genetic determinants of bone mass in adults: A twin study. J Clin Invest. 1987;80(3):706–10.

156. Bonjour JP, Chevalley T, Ferrari S, Rizzoli R. The importance and relevance of peak bone mass in the prevalence of osteoporosis. Salud Publica Mex. 2009;51(SUPPL.1):5–17.

157. Lappe JM, Watson P, Gilsanz V, Hangartner T, Kalkwarf HJ, Oberfield S, et al. The longitudinal effects of physical activity and dietary calcium on bone mass accrual across stages of pubertal development. J Bone Miner Res. 2015;30(1):156–64.

158. Gunter KB, Almstedt HC, Janz KF. Physical activity in childhood may be the key to optimizing lifespan skeletal health. Exerc Sport Sci Rev. 2012;40(1):13–21.

159. Robling AG. Is bone's response to mechanical signals dominated by muscle forces? Med Sci Sports Exerc. 2009;41(11):2044–9.

160. Borer KT. Physical activity in the prevention and amelioration of osteoporosis in

women: interaction of mechanical, hormonal and dietary factors. Sports Med. 2005;35(9):779–830.

161. Daly RM. The effect of exercise on bone mass and structural geometry during growth. Med Sport Sci. 2007;51:33–49.

162. Gunter K, Baxter-Jones AD, Mirwald RL, Almstedt H, Fuchs RK, Durski S, et al. Impact Exercise Increases BMC During Growth: An 8-Year Longitudinal Study. J Bone Miner Res. 2007;23(7):986–93.

163. Janz KF, Letuchy EM, Burns TL, Eichenberger Gilmore JM, Torner JC, Levy SM. Objectively measured physical activity trajectories predict adolescent bone strength: Iowa Bone Development Study. Br J Sports Med. 2014;48(13):1032–6.

164. Hasselstrøm H, Karlsson KM, Hansen SE, Grønfeldt V, Froberg K, Andersen LB. Peripheral bone mineral density and different intensities of physical activity in children 6-8 years old: The Copenhagen School Child Intervention Study. Calcif Tissue Int. 2007;80(1):31–8.

165. KATZMAN DK, BACHRACH LK, CARTER DR, MARCUS R. Clinical and Anthropometric Correlates of Bone Mineral Acquisition in Healthy Adolescent Girls*. J Clin Endocrinol Metab. 1991 Dec 1;73(6):1332–9.

166. Holman RM, Carson V, Janssen I. Does the fractionalization of daily physical activity (sporadic vs. bouts) impact cardiometabolic risk factors in children and youth? PLoS One. 2011;6(10):1–7.

167. Gore PA. Cluster analysis. In: Tinsley HEA, Brown SD, editors. Handbook of Applied Multivariate Statistics and Mathematical Modeling. San Diego, CA:

Academic Press; 2000. p. 297–321.

168. Francis SL, Letuchy EM, Levy SM, Janz KF. Sustained effects of physical activity on bone health: Iowa Bone Development Study. Bone. 2014;63:95–100.

169. Christoffersen T, Winther A, Nilsen OA, Ahmed LA, Furberg A-S, Grimnes G, et al. Does the frequency and intensity of physical activity in adolescence have an impact on bone? The Tromsø Study, Fit Futures. BMC Sports Sci Med Rehabil. 2015;7(1):26.

170. Deere K, Sayers A, Rittweger J, Tobias JH. Habitual levels of high, but not moderate or low, impact activity are positively related to hip BMD and geometry: Results from a population-based study of adolescents. J Bone Miner Res. 2012;27(9):1887–95.

171. Marcus R. Mechanisms of exercise effects on bone. Princ bone Biol. 1996;151:1135–46.

172. Klein-Nulend J, Bacabac RG, Bakker AD. Mechanical loading and how it affects bone cells: the role of the osteocyte cytoskeleton in maintaining our skeleton. Eur Cell Mater. 2012 Sep 24;24:278–91.

173. Consensus development conference: Diagnosis, prophylaxis, and treatment of osteoporosis. In: The American Journal of Medicine. Elsevier; 1993. p. 646–50.

174. Center JR, Nguyen T V, Schneider D, Sambrook PN, Eisman JA. Mortality after all major types of osteoporotic fracture in men and women: an observational study. Lancet. 1999;353(9156):878–82.

175. Hopkins RB, Tarride JE, Leslie WD, Metge C, Lix LM, Morin S, et al. Estimating

the excess costs for patients with incident fractures, prevalent fractures, and nonfracture osteoporosis. Osteoporos Int. 2013 Feb;24(2):581–93.

176. Loro ML, Sayre J, Roe TF, Goran MI, Kaufman FR, Gilsanz V. Early Identification of Children Predisposed to Low Peak Bone Mass and Osteoporosis Later in Life. J Clin Endocrinol Metab. 2000;85(10):3908–18.

177. Gomez-Bruton A, Matute-Llorente A, Gonzalez-Agüero A, Casajus JA, Vicente-Rodríguez G. Plyometric exercise and bone health in children and adolescents: a systematic review. World J Pediatr. (In Press).

178. Gómez-Cabello A, Ara I, González-Agüero A, Casajús JA, Vicente-Rodríguez G. "Ossa sanus in corpore sano" A sound bone in a sound body: the importance of nutrition, physical activity, and nonpharmacologicalmanagement in osteoporosis. Medicographia. 2014;36:170–5.

179. Prisby RD, Lafage-Proust MH, Malaval L, Belli A, Vico L. Effects of whole body vibration on the skeleton and other organ systems in man and animal models: what we know and what we need to know. Ageing Res Rev. 2008/09/03. 2008;7(4):319–29.

180. Rauch F. Vibration therapy. Dev Med Child Neurol. 2009/09/18. 2009;51 Suppl 4:166–8.

181. Rittweger J. Vibration as an exercise modality: how it may work, and what its potential might be. Eur J Appl Physiol Occup Physiol. 2009/12/17. 2010;108(5):877–904.

182. Totosy de Zepetnek JO, Giangregorio LM, Craven BC. Whole-body vibration as

potential intervention for people with low bone mineral density and osteoporosis: a review. J Rehabil Res Dev. 2009/11/03. 2009;46(4):529–42.

183. Amatya B, Khan F, La Mantia L, Demetrios M, Wade DT. Non pharmacological interventions for spasticity in multiple sclerosis. Cochrane Database Syst Rev. 2013/03/02. 2013;2:CD009974.

184. Chanou K, Gerodimos V, Karatrantou K, Jamurtas A. Whole-body vibration and rehabilitation of chronic diseases: a review of the literature. J Sport Sci Med. 2012/01/01. 2012;11(2):187–200.

185. Lam FM, Lau RW, Chung RC, Pang MY. The effect of whole body vibration on balance, mobility and falls in older adults: a systematic review and meta-analysis. Maturitas. 2012/05/23. 2012;72(3):206–13.

186. Lau RW, Liao LR, Yu F, Teo T, Chung RC, Pang MY. The effects of whole body vibration therapy on bone mineral density and leg muscle strength in older adults: a systematic review and meta-analysis. Clin Rehabil. 2011/08/19. 2011;25(11):975–88.

187. Lau RW, Teo T, Yu F, Chung RC, Pang MY. Effects of whole-body vibration on sensorimotor performance in people with Parkinson disease: a systematic review. Phys Ther. 2011/01/08. 2011;91(2):198–209.

188. Madou KH, Cronin JB. The effects of whole body vibration on physical and physiological capability in special populations. Hong Kong Physiother J. 2008;26(1):24–38.

189. Matute-Llorente A, Gonzalez-Aguero A, Gomez-Cabello A, Vicente-Rodriguez G,

Casajus Mallen JA. Effect of whole-body vibration therapy on health-related physical fitness in children and adolescents with disabilities: a systematic review. J Adolesc Heal. 2014/01/07. 2014;54(4):385–96.

190. Merriman H, Jackson K. The effects of whole-body vibration training in aging adults: a systematic review. J Geriatr Phys Ther. 2010/02/05. 2009;32(3):134–45.

191. Mikhael M, Orr R, Fiatarone Singh MA. The effect of whole body vibration exposure on muscle or bone morphology and function in older adults: a systematic review of the literature. Maturitas. 2010/02/23. 2010;66(2):150–7.

192. del Pozo-Cruz B, Adsuar JC, Parraca JA, Pozo-Cruz J del, Olivares PR, Gusi N. Using whole-body vibration training in patients affected with common neurological diseases: a systematic literature review. J Altern Complement Med. 2012;18(1):29–41.

193. Rehn B, Nilsson P, Norgren M. Effects of whole-body vibration exercise on human bone density–systematic review. Phys Ther Rev. 2008;13(6):427–33.

194. Sitja-Rabert M, Rigau D, Fort Vanmeerghaeghe A, Romero-Rodriguez D, Bonastre Subirana M, Bonfill X. Efficacy of whole body vibration exercise in older people: a systematic review. Disabil Rehabil. 2012/01/10. 2012;34(11):883–93.

195. Slatkovska L, Alibhai SM, Beyene J, Cheung AM. Effect of whole-body vibration on BMD: a systematic review and meta-analysis. Osteoporos Int. 2010/04/22. 2010;21(12):1969–80.

196. Pollock RD, Martin FC, Newham DJ. The effect of whole body vibration on older people: a systematic review. Phys Ther Rev. 2012;17(2):110–23.

197. Wysocki A, Butler M, Shamliyan T, Kane RL. Whole-body vibration therapy for osteoporosis: state of the science. Ann Intern Med. 2011/11/16. 2011;155(10):680–6, w206-13.

198. Verschueren SMP, Roelants M, Delecluse C, Swinnen S, Vanderschueren D, Boonen S. Effect of 6-Month Whole Body Vibration Training on Hip Density, Muscle Strength, and Postural Control in Postmenopausal Women: A Randomized Controlled Pilot Study. J Bone Miner Res. 2004;19(3):352–9.

199. Cardinale M, Rittweger J. Vibration exercise makes your muscles and bones stronger: fact or fiction? J Br Menopause Soc. 2006/03/04. 2006;12(1):12–8.

200. Bell Jennifer M, Blackwood B, Shields Michael D, Watters J, Hamilton A, Beringer T, et al. Interventions to prevent steroid-induced osteoporosis and osteoporotic fractures in Duchenne muscular dystrophy. Cochrane Database Syst Rev. 2014;(3).

201. Ma C, Liu A, Sun M, Zhu H, Wu H. Effect of whole-body vibration on reduction of bone loss and fall prevention in postmenopausal women: a meta-analysis and systematic review. J Orthop Surg Res. 2016/02/19. 2016;11(1):24.

202. Oliveira LC, Oliveira RG. Effects of whole body vibration on bone mineral density in postmenopausal women : a systematic review and meta-analysis. Osteoporos Int. 2016;16(3):193–203.

203. Luo X, Zhang J, Zhang C, He C, Wang P. The effect of whole-body vibration therapy on bone metabolism, motor function, and anthropometric parameters in women with postmenopausal osteoporosis. Disabil Rehabil. 2017;39(22):2315–23.

204. Jepsen DB, Thomsen K, Hansen S, Jørgensen NR, Masud T, Ryg J. Effect of whole-body vibration exercise in preventing falls and fractures: a systematic review and meta-analysis. BMJ Open. 2017;7(12):e018342.

205. Dionello CF, Sá-Caputo D, Pereira H, Sousa-Gonçalves CR, Maiworm AI, Morel DS, et al. Effects of whole body vibration exercises on bone mineral density of women with postmenopausal osteoporosis without medications: novel findings and literature review. J Musculoskelet Neuronal Interact. 2016;16(3):193.

206. Saquetto MB, Pereira FF, Queiroz RS, da Silva CM, Conceição CS, Gomes Neto M. Effects of whole-body vibration on muscle strength, bone mineral content and density, and balance and body composition of children and adolescents with Down syndrome: a systematic review. Osteoporos Int. 2018;29(3):527–33.

207. Mikton C, Butchart A. Child maltreatment prevention: a systematic review of reviews. Bull World Health Organ. 2009;87(5):353–61.

208. Seo H-J, Kim KU. Quality assessment of systematic reviews or meta-analyses of nursing interventions conducted by Korean reviewers. BMC Med Res Methodol. 2012;12(1):1.

209. Bemben DA, Palmer IJ, Bemben MG, Knehans AW. Effects of combined whole-body vibration and resistance training on muscular strength and bone metabolism in postmenopausal women. Bone. 2010;47(3):650–6.

210. Russo CR, Lauretani F, Bandinelli S, Bartali B, Cavazzini C, Guralnik JM, et al. High-frequency vibration training increases muscle power in postmenopausal women1,2. Arch Phys Med Rehabil. 2003;84(12):1854–7.

211. Fjeldstad C, Palmer IJ, Bemben MG, Bemben DA. Whole-body vibration augments resistance training effects on body composition in postmenopausal women. Maturitas. 2009;63(1):79–83.

212. Iwamoto J, Takeda T, Sato Y, Uzawa M. Effect of whole-body vibration exercise on lumbar bone mineral density, bone turnover, and chronic back pain in post-menopausal osteoporotic women treated with alendronate. Aging Clin Exp Res. 2005;17(2):157–63.

213. Turner S, Torode M, Climstein M, Naughton G, Greene D, Baker MK, et al. A randomized controlled trial of whole body vibration exposure on markers of bone turnover in postmenopausal women. J Osteoporos. 2011;2011:710387.

214. Kiel DP, Hannan MT, Barton BA, Bouxsein ML, Sisson E, Lang T, et al. Low-Magnitude Mechanical Stimulation to Improve Bone Density in Persons of Advanced Age: A Randomized, Placebo-Controlled Trial. J Bone Miner Res. 2015;30(7):1319–28.

215. Corrie H, Brooke-Wavell K, Mansfield NJ, Cowley A, Morris R, Masud T. Effects of vertical and side-alternating vibration training on fall risk factors and bone turnover in older people at risk of falls. Age Ageing. 2015;44(1):115–22.

216. Slatkovska L, Beyene J, Alibhai SMH, Wong Q, Sohail QZ, Cheung AM. Effect of Whole-Body Vibration on Calcaneal Quantitative Ultrasound Measurements in Postmenopausal Women: A Randomized Controlled Trial. Calcif Tissue Int. 2014;95(6):547–56.

217. Torvinen S, Kannus P, Sievänen H, Järvinen TAH, Pasanen M, Kontulainen S, et al. Effect of 8-Month Vertical Whole Body Vibration on Bone, Muscle

Performance, and Body Balance: A Randomized Controlled Study. J Bone Miner Res. 2003;18(5):876–84.

218. Matute-Llorente A, Gonzalez-Aguero A, Gomez-Cabello A, Olmedillas H, Vicente-Rodriguez G, Casajus JA. Effect of whole body vibration training on bone mineral density and bone quality in adolescents with Down syndrome: a randomized controlled trial. Osteoporos Int. 2015/05/23. 2015;26(10):2449–59.

219. Gilsanz V, Wren TAL, Sanchez M, Dorey F, Judex S, Rubin C. Low-Level, High-Frequency Mechanical Signals Enhance Musculoskeletal Development of Young Women With Low BMD. J Bone Miner Res. 2006;21(9):1464–74.

220. Ward K, Alsop C, Caulton J, Rubin C, Adams J, Mughal Z. Low Magnitude Mechanical Loading Is Osteogenic in Children With Disabling Conditions. J Bone Miner Res. 2004;19(3):360–9.

221. Söderpalm A-C, Kroksmark A-K, Magnusson P, Karlsson J, Tulinius M, Swolin-Eide D. Whole body vibration therapy in patients with Duchenne muscular dystrophy–a prospective observational study. J Musculoskelet Neuronal Interact. 2013;13(1):13–8.

222. von Stengel S, Kemmler W, Engelke K, Kalender WA. Effects of whole body vibration on bone mineral density and falls: results of the randomized controlled ELVIS study with postmenopausal women. Osteoporos Int. 2010/03/23. 2011;22(1):317–25.

223. Gusi N, Raimundo A, Leal A. Low-frequency vibratory exercise reduces the risk of bone fracture more than walking: a randomized controlled trial. BMC Musculoskelet Disord. 2006;7(1):92.

224. Raimundo AM, Gusi N, Tomas-Carus P. Fitness efficacy of vibratory exercise compared to walking in postmenopausal women. Eur J Appl Physiol. 2009;106(5):741–8.

225. Xu J, Lombardi G. Effects of Exercise on Bone Status in Female Subjects, from Young Girls to Postmenopausal Women: An Overview of Systematic Reviews and Meta-Analyses. Sport Med. 2016;1–18.

226. Gómez-Cabello A, González-Agüero A, Morales S, Ara I, Casajús JA, Vicente-Rodríguez G. Effects of a short-term whole body vibration intervention on bone mass and structure in elderly people. J Sci Med Sport. 2014;17(2):160–4.

227. Cheung AM, Giangregorio L. Mechanical stimuli and bone health: what is the evidence? Curr Opin Rheumatol. 2012/07/27. 2012;24(5):561–6.

228. Von Stengel S, Kemmler W, Bebenek M, Engelke K, Kalender WA. Effects of whole-body vibration training on different devices on bone mineral density. Med Sci Sports Exerc. 2011;43(6):1071–9.

229. Slatkovska L, Alibhai SM, Beyene J, Hu H, Demaras A, Cheung AM. Effect of 12 months of whole-body vibration therapy on bone density and structure in postmenopausal women: a randomized trial. Ann Intern Med. 2011;155(10):668–79, W205.

230. Beck B, Norling TL. The effect of 8 mos of twice-weekly low- or higher intensity whole body vibration on risk factors for postmenopausal hip fracture. Am J Phys Med Rehabil. 2010;89(12):997–1009.

231. Rauch F, Sievanen H, Boonen S, Cardinale M, Degens H, Felsenberg D, et al.

Reporting whole-body vibration intervention studies: recommendations of the International Society of Musculoskeletal and Neuronal Interactions. J Musculoskelet Neuronal Interact. 2010;10(3):193–8.

232. Taaffe DR, Snow-Harter C, Connolly DA, Robinson TL, Brown MD, Marcus R. Differential effects of swimming versus weight-bearing activity on bone mineral status of eumenorrheic athletes. J Bone Miner Res. 1995;10(4):586–93.

233. Nikander R, Sievänen H, Uusi-Rasi K, Heinonen A, Kannus P. Loading modalities and bone structures at nonweight-bearing upper extremity and weight-bearing lower extremity: a pQCT study of adult female athletes. Bone. 2006;39(4):886–94.

234. Rector RS, Rogers R, Ruebel M, Hinton PS. Participation in road cycling vs running is associated with lower bone mineral density in men. Metabolism. 2008;57(2):226–32.

235. Gomez-Bruton A, Montero-Marín J, González-Agüero A, García-Campayo J, Moreno LA, Casajús JA, et al. The Effect of Swimming During Childhood and Adolescence on Bone Mineral Density: A Systematic Review and Meta-Analysis. Sports Med. 2016 Mar 26;46(3):365–79.

236. Song L. Calcium and Bone Metabolism Indices. 1st ed. Vol. 82, Advances in Clinical Chemistry. Elsevier Inc.; 2017. 1-46 p.

237. Wheater G, Elshahaly M, Tuck SP, Datta HK, van Laar JM. The clinical utility of bone marker measurements in osteoporosis. J Transl Med. 2013;11(1):201.

238. Vlachopoulos D, Barker AR, Ubago-Guisado E, Fatouros IG, Knapp KM, Williams CA, et al. Longitudinal Adaptations of Bone Mass, Geometry, and

Metabolism in Adolescent Male Athletes: The PRO-BONE Study. J Bone Miner Res. 2017;32(11):2269–77.

239. Maïmoun L, Coste O, Mura T, Philibert P, Galtier F, Mariano-Goulart D, et al. Specific bone mass acquisition in elite female athletes. J Clin Endocrinol Metab. 2013 Jul;98(7):2844–53.

240. Maïmoun L, Mariano-Goulart D, Couret I, Manetta J, Peruchon E, Micallef JP, et al. Effects of physical activities that induce moderate external loading on bone metabolism in male athletes. J Sports Sci. 2004 Sep;22(9):875–83.

241. O'Kane JW, Hutchinson E, Atley LM, Eyre DR. Sport-related differences in biomarkers of bone resorption and cartilage degradation in endurance athletes. Osteoarthr Cartil. 2006 Jan;14(1):71–6.

242. Derman O, Cinemre A, Kanbur N, Doğan M, Kiliç M, Karaduman E. Effect of swimming on bone metabolism in adolescents. Turk J Pediatr. 50(2):149–54.

243. Marin-Puyalto J, Gomez-Cabello A, Gonzalez-Agüero A, Gomez-Bruton A, Matute-Llorente A, Casajús JA, et al. Is Vibration Training Good for Your Bones? An Overview of Systematic Reviews. Biomed Res Int. 2018 Nov 4;2018:1–16.

244. Harrison R, Ward K, Lee E, Razaghi H, Horne C, Bishop NJ. Acute bone response to whole body vibration in healthy pre-pubertal boys. J Musculoskelet Neuronal Interact. 2015;15(2):112–22.

245. Bemben DA, Sharma-Ghimire P, Chen Z, Kim E, Kim D, Bemben MG. Effects of whole-body vibration on acute bone turnover marker responses to resistance exercise in young men. J Musculoskelet Neuronal Interact. 2015 Mar;15(1):23–31.

246. Sherk VD, Chrisman C, Smith J, Young KC, Singh H, Bemben MG, et al. Acute bone marker responses to whole-body vibration and resistance exercise in young women. J Clin Densitom. 2013;16(1):104–9.

247. Leone M, Comtois A. Validity and reliability of self-assessment of sexual maturity in elite adolescent athletes. J Sports Med Phys Fitness. 2007;47:361–5.

248. Rapún-López M, Olmedillas H, Pradas-de la Fuente F, Gómez-Cabello A, González-Agüero A, Casajús JA, et al. Metabolismo óseo en niños y adolescentes deportistas: revisión sistemática. Nutr Hosp. 2017;34(6):1469–81.

249. Bowtell JL, Jackman SR, Scott S, Connolly LJ, Mohr M, Ermidis G, et al. Short duration small sided football and to a lesser extent whole body vibration exercise induce acute changes in markers of bone turnover. Biomed Res Int. 2016;2016.

250. Hayawi LM, Graham ID, Tugwell P, Abdelrazeq SY. Screening for osteoporosis : A systematic assessment of the quality and content of clinical practice guidelines , using the AGREE II instrument and the IOM Standards for Trustworthy Guidelines. PLoS One. 2018;13(12).

251. Gomez-Bruton A, Montero-Marín J, González-Agüero A, Gómez-Cabello A, García-Campayo J, Moreno LA, et al. Swimming and peak bone mineral density: A systematic review and meta-analysis. J Sports Sci. 2018 Feb 10;36(4):365–77.

252. Dell R, Greene D. Is Osteoporosis Disease Management Cost Effective ? 2010;(February):49–55.

253. Zemel BS, Kalkwarf HJ, Gilsanz V, Lappe JM, Oberfield S, Shepherd JA, et al. Revised Reference Curves for Bone Mineral Content and Areal Bone Mineral

Density According to Age and Sex for Black and Non-Black Children : Results of the Bone Mineral Density in Childhood Study. 2015;96(September):3160–9.

254. Barr SI. Associations of social and demographic variables with calcium intakes of high school students. J Am Diet Assoc. 1994 Mar 1;94(3):260–9.

255. Therneau T, Atkinson B. rpart: Recursive Partitioning and Regression Trees. 2019.

256. Greenwell B, Boehmke B, Cunningham J, Developers G. gbm: Generalized Boosted Regression Models. 2019.

257. Friedman J, Hastie T, Tibshirani R. Additive logistic regression: a statistical view of boosting. Ann Stat. 2000;28(2):337–407.

258. Natekin A, Knoll A. Gradient boosting machines, a tutorial. Front Neurorobot. 2013;7:21.

259. Myles AJ, Feudale RN, Liu Y, Woody NA, Brown SD. An introduction to decision tree modeling. J Chemom. 2004;18(6):275–85.

260. Ortega FB, Silventoinen K, Tynelius P, Rasmussen F. Muscular strength in male adolescents and premature death: cohort study of one million participants. BMJ. 2012;345(19):e7279.

261. Gomez-Bruton A, Matute-Llorente A, Pardos-Mainer E, Gonzalez-Aguero A, Gomez-Cabello A, Casajus JA, et al. Factors affecting children and adolescents 50 meter performance in freestyle swimming. J Sports Med Phys Fitness. 2016 Dec;56(12):1439–47.

262. Crandall CJ, Larson J, Gourlay ML, Donaldson MG, Lacroix A, Cauley JA, et al.

Osteoporosis Screening in Postmenopausal Women 50 to 64 Years Old : Comparison of US Preventive Services Task Force Strategy and Two Traditional Strategies in the Women ' s Health Initiative. J Bone Miner Res. 2014;29(7).

263. Kanis JA, McCloskey E V, Johansson H, Oden A, Ström O, Borgström F. Development and use of FRAX® in osteoporosis. Osteoporos Int. 2010 Jun;21(2):407–13.

264. Lydick E, Cook K, Turpin J, Melton M, Stine R, Byrnes C. Development and validation of a simple questionnaire to facilitate identification of women likely to have low bone density. Am J Manag Care. 1998;4(1):37–48.

265. Koh LKH, Sedrine W Ben, Torralba TP, Kung A, Fujiwara S, Chan SP, et al. A simple tool to identify Asian women at increased risk of osteoporosis. Osteoporos Int. 2001;12(8):699–705.

266. O'Donnell S. Screening, prevention and management of osteoporosis among Canadian adults. Heal Promot chronic Dis Prev Canada Res policy Pract. 2018;38(12):445–54.

267. Ayoub WT, Newman ED, Blosky MA, Stewart WF, Wood GC. Improving detection and treatment of osteoporosis: redesigning care using the electronic medical record and shared medical appointments. Osteoporos Int. 2009;20(1):37–42.

268. Geusens P, Dumitrescu B, Geel T Van, Helden S Van, Vanhoof J, Dinant GJ. Impact of Systematic Implementation of a Clinical Case Finding Strategy on Diagnosis and Therapy of Postmenopausal Osteoporosis. 2008;23(6):812–8.

Agradecimientos [Acknowledgements]

Este apartado probablemente sea diferente al resto del documento, al ser más informal y personal, pero no por ello es menos importante puesto que las personas que han hecho posible que este trabajo salga adelante merecen sin duda alguna su reconocimiento. El trabajo en equipo es indispensable para el avance científico completo y eficiente, pero en este caso he tenido la suerte de poder contar con algo que va más allá del trabajo en equipo; un gran grupo de personas que han aportado a esta Tesis no sólo su conocimiento sino también su apoyo, experiencia y comprensión. Quizá hasta que no me he detenido a pensarlo no he sido consciente de la gran red de personas que me amparan, y probablemente debería reconocer más a menudo lo importantes que sois para mi, así que quiero aprovechar estas páginas para mostrar mi agradecimiento.

En primer lugar quiero agradecer la labor de mis directores de tesis, comenzando con **Germán**, la persona que me introdujo en el mundo de la investigación. Cuando todavía era un estudiante de Grado me diste la oportunidad de conocer vuestro trabajo, instalaciones y equipo, del que un día formaría parte. Dos periodos formativos de estancia lejos de nuestra facultad demoraron ese momento, pero cuando estuve preparado para iniciar mis estudios doctorales volviste a recibirme con los brazos abiertos. Desde entonces no sólo has dirigido mi tesis, sino también el Programa de Doctorado, la Facultad, un Máster Propio y distintas sesiones de cocinado de paella. Con tantas obligaciones y responsabilidades es normal que no siempre sea fácil llegar hasta ti, pero siempre ofreces propuestas innovadoras, soluciones prácticas y un trato amigable que confirman el acierto de mi decisión de realizar la tesis contigo.

José Antonio, creo que no me equivocaría afirmando que representas las cualidades que todos los miembros de GENUD aspiramos a alcanzar algún día. No sólo capitaneas el equipo, siendo el primero que se preocupa por el rigor en nuestro trabajo, sino que además

te aseguras de que este repercuta en una contribución a nuestra sociedad, mientras también atiendes al bienestar de cuantos te rodean. En lo que a mi persona respecta debo agradecer tu cuidado de mi formación, dándome distintas oportunidades tanto en el campo docente como en el investigador, así como tu preocupación para conseguir financiación, desde la impresión de un póster hasta las becas predoctorales.

Alba, fuiste la última en incorporarte como directora de esta Tesis, pero no por ello tu contribución ha sido menos importante. Como tú misma has dicho, en ocasiones puede parecer que no necesite ayuda ya que soy relativamente independiente y autónomo a la hora de sacar adelante el trabajo. Sin embargo, también eres consciente de que tiendo a perder el rumbo de mis esfuerzos si se me deja a la deriva y tú siempre has sido capaz de enderezar el timón, sabiendo tanto animarme como ponerme los puntos sobre las íes cuando era necesario en cada caso. Muchas gracias por esto y por todo.

En el grupo de investigación GENUD hay muchos espejos en los que mirarse y soñar en llegar a ser algún día como ellos. Todos los ya mencionados estarían incluidos en esta lista, pero **Agüero** también es uno de ellos. Cuando llegué por aquí, tan sólo eras una historia que tus directores y compañeros contaban orgullosos: la del compañero que leyó la tesis y cogió las maletas de camino a Gales para luchar por su sueño de dedicarse a la docencia y la investigación. Al poco tiempo regresaste con estos objetivos cumplidos y me demostraste el motivo de tu éxito, ya que eres capaz de trabajar sin descanso y compartir algunas de mis tendencias más “frikis” mientras mantienes un talante que hace que todo el mundo alrededor tuyo quiera ser tu amigo.

Hablando de espejos, aquellos en los que más a menudo he intentado verme reflejado han sido sin duda **Matute** y **Brutón**. Cuando llegué, eran los estudiantes que estaban cerca de defender su tesis, si bien ya habían demostrado suficientemente su excelencia investigadora. Aunque a mí, lo que más me llamó la atención fue la tremenda intensidad

con la que vivían todos los aspectos de su vida, desde la organización de las pruebas de campo hasta las discusiones por cualquier tontería que surgiera en el momento como la distribución de la presión de las patas de una mesa. Con el tiempo, sin embargo, he podido apreciar y admirar cómo esa intensidad se traduce en dedicación al trabajo que finalmente os ha convertido en referencias indiscutibles y casi míticas para los estudiantes que hemos ido detrás (aquellos doctorandos que defendieron sus tesis con ¡13 artículos!). No puedo más que daros la enhorabuena por vuestros logros, desearos que sigan llegando buenas noticias y agradeceros toda la incalculable ayuda e inolvidables recuerdos que habéis supuesto para mí.

Hay dos personas dentro del grupo que empezaron las andanzas del doctorado prácticamente al mismo tiempo que yo, **Gabri** y **Borja**. Al primero tuve la fortuna de conocerlo durante nuestros estudios de Grado, donde ya empezó a dar muestras de la capacidad de trabajo y el compañerismo que le caracterizan, con un corazón bondadoso y una gran preocupación por la calidad de su trabajo. Borja, por su parte, se encontraba en una situación probablemente más complicada al encontrarse sin posibilidad de financiación y centrarse en la faceta de rendimiento, menos habitual dentro de este grupo de investigación. Sin embargo supo acomodar perfectamente estas circunstancias dentro del equipo de trabajo y de los estudios de doctorado, lo cual considero que demuestra una gran valía. Con estas dos personas ha sido probablemente con las que he compartido más horas de despacho, de lamentos, de ensoñaciones y de reflexiones, de las cuales he disfrutado todas y cada una de ellas.

Antes de continuar con el resto de agradecimientos, me gustaría resaltar que, si bien he ido apelando principalmente a las cualidades profesionales de mis compañeros y directores sería tramendamente injusto no mencionar también el extraordinario ambiente de trabajo y trato personal que hace que más que compañeros os llame amigos. Cada viaje

o congreso son fuente de momentos divertidos y anécdotas que se recordarán mucho tiempo, como haré sin duda con la despedida de soltero que os tomásteis la molestia de prepararme y en la que estuvo presente todo el grupo. Os lo agradecí en su momento, pero quiero volver a hacerlo aquí y dejarlo plasmado, porque creo que también es un gran indicador de la calidad humana de todos los miembros de este equipo. Muchas gracias de corazón.

Ese ambiente dentro del grupo no sería el mismo sin todas las personas que voy a mencionar a continuación. Me vais a perdonar que no os dedique un apartado a cada uno, pero con la cantidad de personas que formamos el equipo y la velocidad de las nuevas incorporaciones, este apartado de agradecimientos terminaría extendiéndose más allá de la propia Tesis. Al político **Navarrete** y su "secretaria" **Ana** quiero enviarles ánimos para que continúen con su buen trabajo dentro de sus estudios de doctorado y agradecerles los momentos compartidos en el proyecto de mayores, el cual creo que ha tenido un gran refuerzo con **Ángel**, de quien espero seguir recibiendo correctivos tenísticos. **Adrián** y **José Luis**, indistinguibles para algunos, también están comenzando su trayectoria investigadora, la cual auguro un brillante recorrido vista su avidez por el conocimiento científico, demostrada por los estudios simultáneos del primero y del segundo, la voluntariedad para formar parte de trabajos de investigación, ya desde su primer año como estudiante de Grado, lo cual recuerdo y agradezco. Con **Cristina** puede que llevemos un poco menos de tiempo trabajando juntos, aunque ha sido intenso, caótico y por encima de todo, divertido. A su gemela exergaming **Lorena** lo único que puedo decirle de momento es que aproveche todo el potencial que demuestra y que prometo no repetir ninguna "cobra" estadística. Al igual que con el resto del grupo, espero poder pasar mucho más tiempo trabajando junto a vosotras. A **Jorge**, tocayo y compañero oscense, me gustaría desearle mucho éxito en sus todos proyectos (otro más que se atreve a compaginar los estudios de doctorado y Medicina)

y también muchas tardes de disfrute en el Alcoraz. Por último, al recién llegado **Marcos**, asegurarle que, como probablemente ya haya podido empezar a comprobar, dentro de este grupo podrá desarrollar todo su potencial y que los esfuerzos se ven recompensados.

*Dear **Jaak** and **Jarek**, this PhD Thesis would have not been possible without you. I am deeply thankful not only for your clever advice and scientific insight but also for the kind hospitality that you displayed welcoming me. This is also applicable to the rest of your team; **Liina**, **Evelin**, **Priit** and **Rasmus**, thank you. I am proud to say that I was your first international student and that the memories from Tartu will always bring a smile to my face.*

Antes de cerrar el apartado más académico de estos agradecimientos, me gustaría mencionar la importancia de quienes nos permiten subirnos a los hombros de los gigantes y de quienes eliminan los obstáculos en el camino de la ciencia. También querría dedicar unas líneas a todas aquellas personas que han estado presentes en etapas anteriores de mi formación. Del Grado en Ciencias del Deporte en Huesca, dejar patente el grato recuerdo de todos mis compañeros, especialmente del año de Erasmus vivdo junto a **Paula** y agradecer la gran labor de todos los profesores, y particularmente el altruismo de **Eva Gómez**, quien no dudó un instante en recomendarme al grupo GENUD cuando le comenté mis planes de comenzar el doctorado. Del máster realizado en la UPM, querría agradecer por un lado la formación recibida de todos los profesores y el apoyo de los compañeros, particularmente de **Carlos López** y **Víctor Jiménez**, convertidos ya en brillantes profesionales, y por otro trasladar un profundo agradecimiento al grupo de investigación ImFINE con **Marcela** a la cabeza y transmitir un recuerdo especialmente cariñoso para **Raquel Aparicio** con quien compartí los inolvidables comienzos de mi etapa investigadora. Han ganado mucho en Miami con tu llegada y espero que seas muy feliz allí, especialmente ahora que tienes al recién llegado Leo.

Como he dicho al principio de esta sección, tengo que agradecer a un gran número de personas a mi alrededor su compañía a lo largo de este camino, que en ocasiones puede tornarse arduo. Está claro que los amigos son las personas que más llevadero nos pueden hacer este camino, de manera que quiero agradecer a **Fri, Geppe** y **Bundy** (ya veis que ni en la tesis utilizo vuestros nombres reales) por llenar de carcajadas mi vida desde hace más de 15 años. Ya no nos podemos ver tan a menudo como anteriormente y he de reconocer que no soy precisamente el más ágil y concienzudo a la hora de atender el Whatsapp, pero cuando nos juntamos queda demostrado que nuestra amistad sigue tan fuerte como siempre. He tenido la suerte de poder establecer lazos de amistad con personas de diferentes entornos, ya sea durante la carrera, las estancias en el extranjero o sencillamente en distintos grupos como los amigos de mi hermano o de mi novia. Todos ellos han compartido conmigo una de mis grandes aficiones en alguna noche de juegos de mesa; algunos más bien aguantándolas y otros disfrutándolas tan intensamente como yo (o incluso más). En este último grupo incluiríamos sin lugar a dudas a **Víctor** y **Noelia** con quienes espero pasar muchas más veladas entre tableros.

Como he intentado plasmar, he tenido mucha suerte con mi grupo de amigos, también denominada "la familia que se escoge", pero es que tampoco habría podido tener mejor suerte con la familia que no escogí, la mía.

Ya desde pequeño crecí rodeado por el amor incondicional de mis abuelas **Mari** y **Lola**, que todavía sigo recibiendo y mis abuelos **Antonio** y **Ramiro**, a quienes llevo en mi memoria. Las reuniones familiares siempre han sido y son una fuente constante de alegría, tanto en casa de mi padre con la comida abundante y los juegos cargados de risas y despistes (que a su vez generan más risas) junto a mis tías **Rosa** y **Patricia** y mi prima **Sara**, como en la finqueta, donde también se come mucho y se complemeta con la piscina, el fútbol o simplemente dejando pasar el tiempo jugando a las cartas con mis tíos **Mariajo** e **Iñaki** y

mis primos **Diego** y **Laura**. Otro miembro imprescindible de esas tardes en la finca es mi tío **Rami**, a quien quiero dedicar un apartado especial. Has sido un pilar fundamental a lo largo de toda mi vida, un apoyo constante en el día a día, en el ámbito académico y científico y también en el deportivo. No puedo hacer otra cosa más que agradecértelo profundamente.

Hablando de pilares, está claro que son mis padres, **Toño** y **Mariló** las personas a las que debo todo; en definitiva, las personas que me han hecho ser como soy.

Mamá, qué no decir de ti. Has dado absolutamente todo por mi hermano y por mí, entregándonos todo tu amor, compartiendo los momentos de felicidad y soportando por dentro los momentos duros para que a nosotros no nos faltara de nada. Has sido un apoyo constante, tanto desde el cariño diario como desde la comprensión absoluta en los periodos más difíciles. Papá, a lo largo de las líneas anteriores he ido describiendo la gran capacidad de trabajo de mis compañeros, sin embargo tengo clarísimo que mi referente indiscutible con respecto al trabajo duro eres tú y también sé que el motivo por el que te has esforzado tantísimo es de nuevo el bienestar de mi hermano y el mío. Espero poder ser algún día al menos la mitad de buen padre de lo que vosotros lo habéis sido para mi. Os quiero mucho a los dos.

Ya he mencionado a mi hermano **Daniel** varias veces a lo largo de este apartado, tanto entre los amigos como entre la familia y es que eso eres para mi; no sólo eres mi hermano sino que también eres uno de mis mejores amigos. Se supone que el hermano mayor debe servir como ejemplo al pequeño me encantaría pensar que haya podido ser así ya que estaría tremendamente orgulloso de haber podido contribuir aunque sólo sea en una pequeña fracción en formar la increíble persona en la que te has convertido y que no ha hecho sino mejorar en compañía de tu futura esposa, **Zaza**, a quien también quiero agradecer el tiempo pasado juntos y reconocer su granito de arena dentro de este trabajo como parte de nuestra familia. Espero que sigáis siendo así de felices juntos durante muchísimo tiempo.

Y he dejado para el final a la persona más importante para mí, **Elena.** Llegaste a mi vida aproximadamente al mismo tiempo que comencé los estudios de doctorado y nuestra relación ha ido conviviendo con éstos, ajustándose de tal manera que este cambio de ciclo a nivel académico coincide con un punto muy especial para nosotros, el de nuestro matrimonio. Intentaré no extenderme mucho en la parte sentimental, ya que tengo que dejarme algo para decirte precisamente en la boda. Sin embargo, creo que este es el lugar idóneo para expresarte mi gratitud imperecedera por el apoyo fundamental que me has supuesto durante este período y por aguantar a mi lado pese a todo aquello con lo que has tenido que convivir, incluyendo la incertidumbre de mi disponibilidad, a mi ordenador como compañero inseparable de viaje o mis erráticos horarios de trabajo personal. Y no sólo es que hayas "aguantado" sino que lo has hecho con una sonrisa y sabiendo mantenerme a flote en los momentos más tristes. Tus ánimos y tu convicción en mi capacidad de sacar el trabajo adelante han sido los motores más importantes que me han permitido conseguirlo efectivamente.

Esta tesis está dedicada a todas las personas que he ido mencionando, porque todas ellas son partes importantes tanto de esta Tesis como de mi vida, pero especialmente a ti, puesto que si no estuvieras a mi lado no creo que hubiera podido completar la tesis y no soy capaz de imaginarme mi vida. Te quiero.

Material suplementario

I. Capítulo 4.2 – Tabla suplementaria 221

II. Capítulo 4.3 – Tabla suplementaria 222

III. Capítulo 4.3 – Figura suplementaria 223

IV. Capítulo 4.6 – Tabla suplementaria 224

V. Capítulo 4.6 – Material suplementario 225

Supplementary material

I. Chapter 4.2 – Supplementary table 221

II. Chapter 4.3 – Supplementary table 222

III. Chapter 4.3 – Supplementary figure 223

IV. Chapter 4.6 – Supplementary table 224

V. Chapter 4.6 – Supplementary material 225

Material suplementario I

Supplementary table 1 – Summary of group formation

Number of 5-min VPA bouts	Light or moderate PA: <230 min/day	Light or moderate PA: ≥230 min day
0	*Low volume* *No VPA bout* (n=41)	*High volume* *No VPA bout* (n=42)
≥1	*Low volume* *VPA bout* (n=49)	*High volume* *VPA bout* (n=48)

Material suplementario II

Table A1 – Partial correlation coefficients between physical activity evolution and bone growth, adjusted by body mass and skeletal age change (n=140)

PA mean	Δ WB BMC (g)	Δ WB BMD (g/cm^2)	Δ FN BMC (g)	Δ FN BMD (g/cm^2)	Δ LS BMC (g)	Δ LS BMD (g/cm^2)
Sedentary (min/day)	-0.020	0.039	-0.079	-0.105	0.030	0.068
Light PA (min/day)	-0.001	0.003	-0.015	0.007	-0.094	-0.089
Moderate PA (min/day)	-0.039	-0.004	0.049	0.035	-0.072	-0.103
Vigorous PA (min/day)	**0.182**	**0.214**	**0.208**	**0.245**	0.098	0.070
Overall PA (counts/min)	0.101	0.123	0.149	**0.168**	0.004	-0.032
*PA difference**	Δ WB BMC (g)	Δ WB BMD (g/cm^2)	Δ FN BMC (g)	Δ FN BMD (g/cm^2)	Δ LS BMC (g)	Δ LS BMD (g/cm^2)
Sedentary (min/day)	-0.016	-0.118	-0.135	**-0.216**	-0.094	-0.044
Light PA (min/day)	-0.023	0.044	0.028	0.012	0.009	0.015
Moderate PA (min/day)	-0.080	0.064	0.074	0.105	0.074	0.027
Vigorous PA (min/day)	0.074	**0.198**	**0.197**	**0.254**	0.143	0.081
Overall PA (counts/min)	0.089	**0.214**	**0.215**	**0.279**	**0.192**	0.139

* Calculated as follow up – baseline values

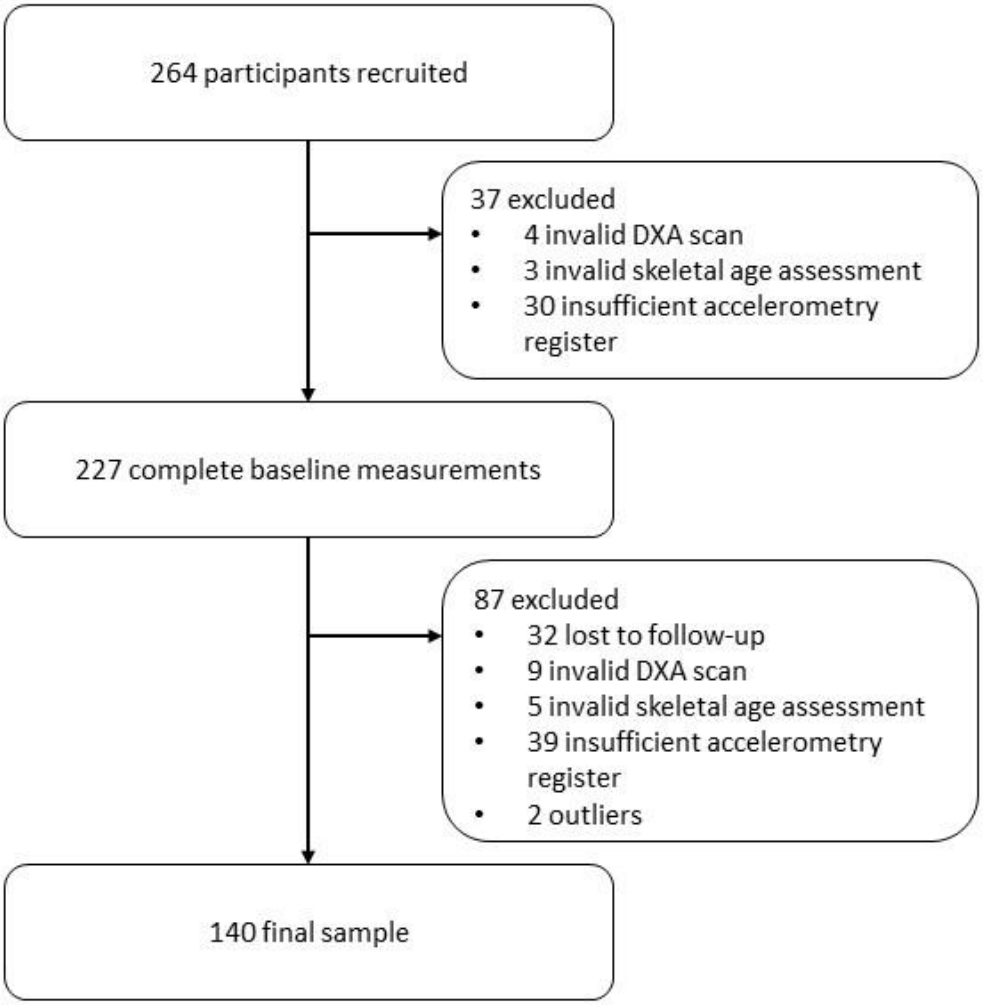
Bold characters indicate significant correlations ($p<0.05$)

PA: Physical activity; WB: Whole body; FN: Femoral neck; LS: Lumbar spine

BMC: Bone mineral content; BMD: Bone mineral density

Material suplementario III

Figure A1 – Flowchart of the study sample



Material suplementario IV

Supplementary Table 1- Summary of the variables included in the regression and decision trees

Variable type	*Variable names*
Predicted outcome:	Subtotal (whole body less head) bone mineral density (BMD)
Physical fitness:	**Speed**: Time to complete a 30-m sprint run
	Handgrip strength: Sum of best attempts with both arms
	Lower-limb strength: Distance in a standing long jump
	Endurance: VO_{2max} estimated from 20-m shuttle-run test
Swimming training and performance:	Weekly training hours, **years of swimming history**, **time in 50-m swim**, **FINA points**
Potential confounders:	**Age**, sex, Tanner stage, **height, weight, BMI**, calcium intake, participation in other sports

The variables highlighted in bold were statistically significant within the ensemble model and were therefore used to construct the individual decision tree

Material suplementario V

```
#loading necessary packages
library(dplyr)
library(rpart)
library(rpart.plot)
library(gbm)
library(ggplot2)
library(vip)
library(pdp)
library(boot)
library(ggpubr)

#loading data and isolating the variables included in model construction
load("RENACIMIENTO.RData")
treeDB<-renacimiento[,c(12,2:9,30:37)]

#for reproducibility
set.seed(1)

#randomly selecting 70% of the sample
random_index<-sample(1:nrow(treeDB),nrow(treeDB))
treeDB<-treeDB[random_index,]
train<-sample(1:78,round(78*0.7,0))

#creating a matrix to test different hyper-parameter values
hyper_grid <- expand.grid(
  shrinkage = c(.01, .1, .3),
  interaction.depth = c(1, 3, 5),
  n.minobsinnode = c(2, 5, 8),
  bag.fraction = c(.5, .75, 1),
  optimal_trees = 0,
  min_RMSE = 0
)

# grid search
for(i in 1:nrow(hyper_grid)) {
  set.seed(123)

  # training model
  gbm.tune <- gbm(
    formula = totalBMD ~ .,
    distribution = "gaussian",
    data = treeDB[train,],
    n.trees = 100,
    interaction.depth = hyper_grid$interaction.depth[i],
    shrinkage = hyper_grid$shrinkage[i],
    n.minobsinnode = hyper_grid$n.minobsinnode[i],
    bag.fraction = hyper_grid$bag.fraction[i],
    train.fraction = .75
  )
```

```
  # adding min training error and trees to grid
  hyper_grid$optimal_trees[i] <- which.min(gbm.tune$valid.error)
  hyper_grid$min_RMSE[i] <- sqrt(min(gbm.tune$valid.error))
}

#checking best fits
hyper_grid %>%
  dplyr::arrange(min_RMSE) %>%
  head(10)

#fine-tuning hyper-parameter based on previous results
hyper_grid_fine <- expand.grid(
  shrinkage = c(.1, .2, .3),
  interaction.depth = c(3,5,7),
  n.minobsinnode = c(2,3,4,5),
  bag.fraction = c(.5,.625,.75),
  optimal_trees = 0,              # a place to dump results
  min_RMSE = 0                    # a place to dump results
)

# new grid search
for(i in 1:nrow(hyper_grid_fine)) {
  set.seed(123)

  # training model
  gbm.tune <- gbm(
    formula = totalBMD ~ .,
    distribution = "gaussian",
    data = treeDB[train,],
    n.trees = 100,
    interaction.depth = hyper_grid_fine$interaction.depth[i],
    shrinkage = hyper_grid_fine$shrinkage[i],
    n.minobsinnode = hyper_grid_fine$n.minobsinnode[i],
    bag.fraction = hyper_grid_fine$bag.fraction[i],
    train.fraction = 0.75
  )

  # adding min training error and trees to grid
  hyper_grid_fine$optimal_trees[i] <- which.min(gbm.tune$valid.error)
  hyper_grid_fine$min_RMSE[i] <- sqrt(min(gbm.tune$valid.error))
}

#checking new best fits
hyper_grid_fine %>%
  dplyr::arrange(min_RMSE) %>%
  head(10)

#building single model with the identified parameters
set.seed(123)
boost.fit<-gbm(totalBMD~.,
               distribution="gaussian",
```

```
        data=treeDB[train,],
        n.trees=50,
        interaction.depth = 3,
        n.minobsinnode = 3,
        bag.fraction=0.75,
        shrinkage=0.3,
        cv.folds=5)
boost.fit

#plotting model performance
png(filename = "cvError.png",width=1600,height=1600,units="px",res=300)
gbm.perf(boost.fit, method = "cv")
dev.off()

#plotting relative importance of variables
png(filename = "relativeImportance.png",width=1600,height=1600,units="px",res=300)
par(mar = c(5, 8, 1, 1))
vip::vip(boost.fit,n.trees=50,num_features=9)+theme_classic()+ylab("Relative influence (%)")
dev.off()

#predicting risk in test data to include them in the database
pred<-predict(boost.fit,n.trees=50,newdata=treeDB[-train,])

#loading database with the predicted and actual BMD values and its correspondent Z-score
load("predMat.RData")

#calculating root mean squared error
sqrt(mean((predMat$realBMD-pred)^2))

#showing the accuracy of the prediction
table(real=!predMat$realAdjZ>-1,pred=!predMat$predAdjZ>-1)

#building single tree for practical application
indTreeDB<-renacimiento[,c(38,2:9,30:37)]
indTreeDB<-indTreeDB[random_index,]
indTree<-
rpart(risk~height+weight+finaPoints+handgrip+swimYears+time30m+time50m+BMI+age,
        data=indTreeDB[train,],method="class",minsplit=20,
        parms=list(loss=matrix(c(0,1,1,0),byrow=TRUE,nrow=2)))

#plotting contents of the individual tree
png(filename = "individualTree.png",width=1600,height=1600,units="px",res=300)
rpart.plot(indTree,box.palette=gray(seq(.6, 1, length.out=9)),type=5,extra=0)
dev.off()

#calculating the prediction on the remaining 30% of the sampling
indPred<-predict(indTree,newdata=indTreeDB[-train,])[,2]>1/2

#showing the accuracy of the prediction
table(real=!predMat$realAdjZ>-1,pred=indPred)
```

Anexos

I. Certificación del comité de ética del proyecto PREFIT 279

II. Consentimiento informado del proyecto PREFIT 280

III. Información para padres de participantes en el proyecto PREFIT 281

IV. Certificación del comité de ética del proyecto RENACIMIENTO 283

V. Consentimiento informado del proyecto RENACIMIENTO 285

VI. Información para padres de participantes en el proyecto RENACIMIENTO 286

VII. Cuestionario utilizado en el proyecto RENACIMIENTO 289

Annexes

I. Ethics committee clearance for the PREFIT project.....279

II. Informed consent for the PREFIT project 280

III. Information for the guardians of participants in the PREFIT project.....281

IV. Ethics committee clearance for the RENACIMIENTO project 283

V. Informed consent for the RENACIMIENTO project 285

VI. Information for the guardians of participants in the RENACIMIENTO project.286

VII. Questionnaire used in the RENACIMIENTO project 289

Anexo I

GOBIERNO DE ARAGON
Departamento de Sanidad, Bienestar Social y Familia

Informe Dictamen Favorable
Proyecto Investigación Biomédica
C.P. - C.I. PI14/00111
19 de noviembre de 2014

Dña. María González Hinjos, Secretaria del CEIC Aragón (CEICA)

CERTIFICA

1º. Que el CEIC Aragón (CEICA) en su reunión del día 19/11/2014, Acta Nº CP18/2014 ha evaluado la propuesta del investigador referida al estudio:

Título: Evaluación de la condición física y estructura ósea en preescolares

Investigador Principal: Germán Vicente Rodríguez. Universidad de Zaragoza

Versión protocolo: noviembre/ 2014
Versión hoja de información para los padres y consentimiento informado: noviembre/ 2014

2º. Considera que

- El proyecto se plantea siguiendo los requisitos de la Ley 14/2007, de 3 de julio, de Investigación Biomédica y su realización es pertinente.
- Se cumplen los requisitos necesarios de idoneidad del protocolo en relación con los objetivos del estudio y están justificados los riesgos y molestias previsibles para el sujeto.
- Son adecuados tanto el procedimiento para obtener el consentimiento informado como la compensación prevista para los sujetos por daños que pudieran derivarse de su participación en el estudio.
- El alcance de las compensaciones económicas previstas no interfiere con el respeto a los postulados éticos.
- La capacidad de los Investigadores y los medios disponibles son apropiados para llevar a cabo el estudio.

3º. Por lo que este CEIC emite **DICTAMEN FAVORABLE a la realización del proyecto.**

Lo que firmo en Zaragoza, a 19 de noviembre de 2014

Fdo.

GOBIERNO DE ARAGÓN DEPARTAMENTO DE SANIDAD, BIENESTAR SOCIAL Y FAMILIA COMITÉ ÉTICO DE INVESTIGACIÓN CLÍNICA

Dña. María González Hinjos
Secretaria del CEIC Aragón (CEICA)

Tel. 976 71 48 57 Fax. 976 71 55 54 Correo electrónico mgonzalezh.ceic@aragon.es

Anexo II

HOJA DE CONSENTIMIENTO PARA PADRES/MADRES: "EVALUACIÓN DE LA CONDICIÓN FÍSICA Y ESTRUCTURA ÓSEA EN PREESCOLARES."

D. / Dña. ..... con D.N.I. nº…… como madre/padre/tutor de …….. con fecha de nacimiento ……. del curso ……de preescolar declaro que:

He leído y comprendo la información que se me ha entregado.

Comprendo que la participación es voluntaria.

Comprendo que mi hijo/a se puede retirar del estudio:

1. Cuando quiera.
2. Sin tener que dar explicaciones.
3. Sin que esto repercuta en los cuidados médicos caso de enfermedad o lesión derivadas del estudio.

Presto libremente mi conformidad para que mi hijo/a pueda participar en el estudio durante el horario escolar en su centro educativo.

Firma del padre/madre o tutor Fecha y lugar

Anexo III

HOJA DE INFORMACIÓN PARA PADRES/MADRES: "EVALUACIÓN DE LA CONDICIÓN FÍSICA Y ESTRUCTURA ÓSEA EN PREESCOLARES."

El centro educativo en el que se encuentra su hijo/a ha sido seleccionado para la participación en un proyecto que pretende estudiar en niños y niñas de 3-5 años el nivel de forma física utilizando un conjunto de test que evalúan: fuerza de prensión manual, salto a pies juntos, velocidad-agilidad 4x10, el test de ida y vuelta de 20 metros, y test de equilibrio con una pierna, así como peso, talla y perímetro de cintura. Todos estos test se usan de forma frecuente en centros educativos de primaria y secundaria en España y resto del mundo. Estos test son sencillos y divertidos, y sólo requieren que la persona no tenga ninguna limitación para realizar Educación Física. Esta nueva batería adaptada a niños/as de infantil recibiría el nombre de Batería de condición física PRE-FIT. Además se estudiará la composición corporal y específicamente la fortaleza del hueso de estos niños.

Información de utilidad:

1. **Beneficios derivados del estudio**.

En la actualidad existen numerosos estudios que han determinado la fiabilidad y validez una batería de evaluación de la condición física en personas de 6 años y mayores, pero es necesario estandarizar la medida de la condición física también en preescolar, es decir, de 3 a 5 años y contar con valores de referencia en este grupo de edad.

El/la niño/a recibirá completa información de su estado de condición física, composición corporal y estructura ósea y sus puntuaciones en las pruebas realizadas.

2. **Posibles acontecimientos adversos**. No se ha descrito ningún efecto adverso grave derivado de estas pruebas.

3. **Voluntariedad**. El participante lo hace de forma voluntaria, pudiéndose retirar del estudio en cualquier momento, habiendo sido informado explícitamente de la finalidad del mismo. Esto no conllevará ningún tipo de discriminación ni consecuencias.

4. **Los datos** obtenidos en el estudio pertenecen tan solo a la persona voluntaria y al entorno investigador, manteniéndose siempre la más estricta confidencialidad. El participante decidirá si quiere conocer o no los datos de la investigación y será informado, si así lo desea, de los resultados durante el proceso. Al finalizar el mismo, obtendrá un informe detallado por especialistas del área.

A continuación se detallan brevemente las pruebas que se le van a realizar a su hijo/a:

1. La batería PRE-FIT consta de las siguientes pruebas:

a. Peso, altura y perímetro de cintura.
b. Fuerza de presión manual: se utilizará un dinamómetro manual para calcular la fuerza de presión manual del alumno/a (dos repeticiones con cada una de las manos). El agarre del dinamómetro se ajusta al tamaño de la mano. Este test pretende medir la fuerza de la mano.
c. Salto de longitud a pies juntos: el alumno/a tendrá que realizar con los pies juntos un salto horizontal (dos repeticiones). Este test pretende medir la fuerza del tren inferior.
d. Test de agilidad 4x10 metros: el alumno/a realiza un recorrido de ida y vuelta de 10m lo más rápido posible (dos repeticiones). Este test pretende medir velocidad/agilidad.
e. Test de ida y vuelta de 20 metros: el alumno/a se desplazará de una línea a otra situadas a 20 metros de distancia según el ritmo indicado por una grabación en CD. El ritmo irá aumentando hasta que el alumno no pueda seguirlo (una repetición). Este test pretende medir la capacidad aeróbica (de resistencia).
f. Test de equilibrio con una pierna: el alumno/a se situará de forma estática sobre el suelo y con una pierna flexionada. El test se basa en contabilizar el tiempo (duración máxima de 60 segundos) que mantiene el equilibrio (una repetición con cada pierna). Este test pretende medir equilibrio estático.

2. Realización de una prueba de tomografía cuantitativa computarizada periférica (pQCT) para obtener información del estado, geometría y arquitectura del hueso y estimar así factores determinantes de la fortaleza y resistencia del esqueleto. Esta prueba genera una irradiación de 1 μSv que es 40 veces menor a una radiografía de pecho y que se considera inocuo (inofensivo) para la salud.

3. Valoración del nivel y la cantidad de actividad física semanal realizada por los participantes mediante los acelerómetros ligeros, triaxiales y resistentes al agua creados por GENEActive. Tienen la apariencia de un reloj, no son manipulables y no se quitan ni para el baño ni para dormir por lo que resultan muy cómodos.

Previamente a la realización de la batería se realizará un breve calentamiento de 3-5 minutos.

Para más información acerca de las pruebas descritas anteriormente pueden visitar la página web:

www.ugr.es/~cts262/ES/documents/MANUALALPHA-Fitness.pdf

O contactando directamente con el investigador principal:

Germán Vicente Rodríguez
Prof. Titular Universidad de Zaragoza
Facultad de Ciencias de la Salud y del Deporte
Universidad de Zaragoza
gervicen@unizar.es

Anexo IV

GOBIERNO DE ARAGON
Departamento de Sanidad, Bienestar Social y Familia

Informe Dictamen Favorable
Proyecto Investigación Biomédica
C.P. - C.I. PI11/0034
18 de abril de 2012

/CEIC Aragón (CEICA)

Dña. María González Hinjos, Secretaria del CEIC Aragón (CEICA)

CERTIFICA

1º. Que el CEIC Aragón (CEICA) en su reunión del día 18/04/2012, Acta Nº CP08/2012 ha evaluado la propuesta del investigador referida al estudio:

Título: Repercusión del entrenamiento y la práctica de la natación sobre el desarrollo metabólico y estructural del hueso en crecimiento. Beneficios de la incorporación de entrenamiento pliométrico o vibratorio. (RENACIMIENTO).

Versión Protocolo: abril 2012
Versión hoja de información al paciente y consentimiento informado abril/2012

1º. Considera que

- El proyecto se plantea siguiendo los requisitos de la Ley 14/2007, de 3 de julio, de Investigación Biomédica y su realización es pertinente.
- Se cumplen los requisitos necesarios de idoneidad del protocolo en relación con los objetivos del estudio y están justificados los riesgos y molestias previsibles para el sujeto.
- Son adecuados tanto el procedimiento para obtener el consentimiento informado como la compensación prevista para los sujetos por daños que pudieran derivarse de su participación en el estudio.
- El alcance de las compensaciones económicas previstas no interfiere con el respeto a los postulados éticos.
- La capacidad de los Investigadores y los medios disponibles son apropiados para llevar a cabo el estudio.

2º. Por lo que este CEIC emite un **DICTAMEN FAVORABLE**.

3º. Este CEIC acepta que dicho estudio sea realizado en los siguientes Centros por los Investigadores:

Dr. Germán Vicente Rodríguez, Universidad de Zaragoza.

Lo que firmo en Zaragoza, a 18 de abril de 2012

Fdo:

GOBIERNO DE ARAGÓN DEPARTAMENTO DE SANIDAD, BIENESTAR SOCIAL Y FAMILIA COMITÉ DE ÉTICA DE LA INVESTIGACIÓN CLÍNICA

Dña. María González Hinjos
Secretaria del CEIC Aragón (CEICA)

Departamento de Salud y Consumo del Gobierno de Aragón
Avda Gómez Laguna 25 Zaragoza 50009 Zaragoza España
Tel. 976 71 48 57 Fax. 976 71 55 54 Correo electrónico mgonzalezh.ceic@aragob.es

GOBIERNO DE ARAGON
Departamento de Salud y Consumo

COMITÉ ÉTICO DE INVESTIGACIÓN
CLÍNICA DE ARAGÓN (CEICA)
Avda. Gómez Laguna, 25 planta 11
50009 Zaragoza

COMPOSICIÓN DEL COMITÉ ÉTICO DE INVESTIGACIÓN CLÍNICA DE ARAGÓN

Dra. María González Hinjos, Secretaria del Comité Ético de Investigación Clínica de Aragón,

CERTIFICA

1º En la reunión celebrada el día 18 de abril de 2012, correspondiente al Acta nº **CP08/2012**, se cumplieron los requisitos establecidos en la legislación vigente -Real Decreto 223/2004 y Decreto 26/2003 del Gobierno de Aragón, modificado por el Decreto 292/2005– para que la decisión del citado CEIC sea válida.

3º El CEIC de Aragón, tanto en su composición, como en sus PNT, cumple con las normas de BPC.

4º La composición del CEIC de Aragón en la citada fecha, era la siguiente:

- **Presidente:** Cesar Loris Pablo; Médico. Servicio de Pediatría. Hospital Universitario Miguel Servet. Representante de Comisión de Investigación.
- **Vicepresidente:** Carlos Aibar Remón; Médico. Servicio de Medicina Preventiva y Salud Pública. Hospital Clínico Universitario Lozano Blesa. Profesional Sanitario experto en epidemiología clínica.
- **Secretaria:** María González Hinjos; Farmacéutica.
- Pilar Comet Cortés; Enfermera. Unidad Mixta de Investigación. Hospital Clínico Universitario Lozano Blesa.
- Marina Heredia Ríos; Representante de las Organizaciones de Consumidores y Usuarios.
- Gabriel Hernández Delgado; Médico. Servicio de Radiología. Hospital Universitario Miguel Servet. Representante de Comisión de Investigación.
- Angela Idoipe Tomás; Farmacéutica. Servicio de Farmacia. Hospital Universitario Miguel Servet. Farmacéutica de Hospital.
- María Jesús Lallana Álvarez. Farmacéutica de Atención Primaria de Zaragoza Sector III.
- Jesús Magdalena Bello; Médico. Centro de Salud de Azuara. Médico con labor asistencial y representante del Comité de Ética Asistencial del Área de Atención Primaria II y V.
- Mariano Mateo Arrizabalaga; Médico. Servicio de Farmacología Clínica. Hospital Clínico Universitario Lozano Blesa.
- Elisa Moreu Carbonell; Jurista. Profesora de la Facultad de Derecho, Universidad de Zaragoza.
- Javier Perfecto Ejarque; Médico. Centro de Salud Arrabal. Médico con labor asistencial.
- Alexandra Prados Torres; Médico. Instituto Aragonés de Ciencias de la Salud. Representante de Comisión de Investigación.
- José Puzo Foncillas; Médico. Servicio de Bioquímica. Hospital General San Jorge. Representante de Comisión de Investigación.
- Mónica Torrijos Tejada; Médico. Instituto Aragonés de Ciencias de la Salud.

Para que conste donde proceda, y a petición del promotor,

Zaragoza, a 18 de abril de 2012

GOBIERNO DE ARAGÓN DEPARTAMENTO DE SANIDAD, BIENESTAR SOCIAL Y FAMILIA COMITÉ ÉTICO DE INVESTIGACIÓN CLÍNICA

Firmado: María González Hinjos

Anexo V

Departamento de Fisiatría y Enfermería
Universidad Zaragoza

CONSENTIMIENTO INFORMADO CORRESPONDIENTE A:

Datos del participante

D./Dª_____

DNI: _____ Edad_____

Que presta su consentimiento:

- ❑ Por si mismo
- ❑ Por medio del tutor legal:

D./Dª_____

DNI: _____

Teléfeno 1:_____ Teléfono 2:_____

En Zaragoza, a ..... de ..... de 20....

D.....

manifiesta que ha recibido información suficiente y en términos comprensibles para tomar la decisión de acuerdo con su propia y libre voluntad y **presta su consentimiento y autorización** a la práctica de la intervención reseñada en el proyecto:

"*Re* *percusión del entrenamiento y la práctica de la* ***na*** *tacion sobre el desarrollo metabólico y estructural del hueso en cre* ***cimiento.*** *Beneficios de la incorporación de entrenemiento pliométrico o vibratorio. (acrónimo: RENACIMIENTO)"* financiado por el MICIN, en el que participa el Departamento de Fisiatría y Enfermería de la Universidad de Zaragoza, del que es investigador principal el profesor Dr. Germán Vicente-Rodríguez, Profesor Contratado Doctor de la Universidad de Zaragoza (Departamento de Fisiatría y Enfermería C/ Domingo Miral s/n 50008 Zaragoza, teléfono 974238422 (ext 853258), e-mail: gervicen@unizar.es).

Facultativos que intervienen:
Profesor Dr. Germán Vicente-Rodriguez, y personal autorizado.

PARTICIPANTE	DOCTOR
Padre, madre o tutor	Dr. G. Vicente-Rodríguez

Anexo VI

GOBIERNO DE ARAGON

Departamento de Fisiatría y Enfermería
Universidad Zaragoza

INFORMACIÓN PARA PADRES PARA EL CONSENTIMIENTO INFORMADO CORRESPONDIENTE A LA PARTICIPACIÓN EN EL PROYECTO "RENACIMIENTO"

Proyecto de investigación para el que se solicita el consentimiento informado

La participación que se solicita es para la realización de un proyecto de investigación sobre "***Re**percusión del entrenamiento y la práctica de la **na**tacion sobre el desarrollo metabólico y estructural del hueso en cre**cimiento.** Beneficios de la incorporación de entrenamiento pliométrico o vibratorio. (acrónimo: RENACIMIENTO)*" financiado por el MICIN, en el que participa el Departamento de Fisiatría y Enfermería de la Universidad de Zaragoza, del que es investigador principal el profesor Dr. Germán Vicente-Rodríguez, Profesor Contratado Doctor de la Universidad de Zaragoza (Departamento de Fisiatría y Enfermería C/ Domingo Miral s/n 50008 Zaragoza, teléfono 974238422 (ext 853258), e-mail: gervicen@unizar.es).

Facultativos que intervienen:
Profesor Dr. Germán Vicente-Rodriguez, y personal autorizado.

Intervención para la que se solicita el consentimiento informado

Realización de una valoración médico-deportiva.

Finalidad de la intervención:
Observar el efecto de la práctica y entrenamiento de natación sobre el metabolismo, la geometría y arquitectura del hueso y sus posibles relaciones.

Confidencialidad de los resultados

Se garantiza la absoluta confidencialidad de los resultados, de forma que en ningún caso, sin consentimiento previo del participante, se dará a conocer ningún dato personal ni de los resultados de su colaboración en este proyecto.

Naturaleza del estudio

Realización de una prueba de tomografía cuantitativa computerizada periférica (pQCT) y otra de densitometría dual de rayos X (DXA) para obtener información del estado, geometría y arquitectura del hueso y estimar así factores determinantes de la fortaleza y resistencia del esqueleto. Estas técnicas conllevan una dosis de radiación total de entre 5 y 10 mrem, que es una dosis 20 veces más baja que la de una radiografía de tórax y similar a la radiación solar que conlleva un día de playa.

Se obtendrán muestras de sangre (punción cubital) para determinaciones de marcadores bioquímicos del metabolismo del hueso.

Además se realizarán pruebas de condición física:

Cardiovascular

El consumo máximo de oxígeno (VO2max) se estimará mediante una prueba de campo incluido en la batería Eurofit para escolares (test de 20 m de ida y vuelta).

Test de velocidad de carrera

El tiempo invertido en correr 30 m (T30) se medirá utilizando células fotoeléctricas (Byomedics, Barcelona).

La fuerza isométrica máxima (FIM)

De pierna

Test de máxima contracción voluntaria isométrica (MCVI)

Se registrará la fuerza ejercida por el sujeto durante 10 segundos con un ángulo de flexión de rodilla de 110º.

De brazo

La dinamometría manual se realizará con un dinamómetro y el adolescente de pie, brazos extendidos a lo largo del cuerpo hará la mayor fuerza posible de prensión manual sin apoyar el brazo en el cuerpo.

Fuerza dinámica de las piernas.

Las fuerzas generadas durante el salto vertical se medirán mediante el cálculo de la altura de vuelo durante el salto con una plataforma que registra la fuerza realizada durante el salto.

Potencia muscular de tren inferior al 10%, 20% y al 30% de la MCVI.

El objetivo de dicha evaluación será cuantificar la potencia máxima que será capaz de imprimir el adolescente durante la fase concéntrica del movimiento de extensión de piernas, utilizando una carga equivalente al 10, 20 y 30% de la *MCVI* que hemos medido anteriormente.

Valoración de la dieta

La dieta se valorará a partir de tres recuerdos de 24 h no consecutivos y en días distintos de la semana, realizados con un software informático.

La duración total de la sesión de laboratorio será de 2 horas (una tarde).

Para la extracción sanguínea se citara al participante un sábado por la mañana a elección del participante

Posibles beneficios

- Detección precoz de mineralización débil u otros problemas de microarquitectura ósea.
- Mejora de la mineralización y desarrollo óseo durante el crecimiento.

Riesgos

No se ha descrito ningún efecto adverso grave derivado de estas pruebas o de la participación en un programa de ejercicio físico de estas características.

Contraindicaciones

Patologías graves que contraindiquen la práctica deportiva escolar.

Advertencias

1. El participante es advertido sobre la posibilidad de utilizar los resultados del diagnóstico en un proceso de investigación, que en ningún caso podrá comportar riesgo adicional para su salud y que no tendrá carácter comercial.
2. El participante es advertido de que puede revocar libremente por escrito su consentimiento en cualquier momento.
3. El participante es advertido de su derecho a que se le dé una copia del documento firmado.
4. El participante contará con la cobertura de un seguro para la realización de las pruebas.
5. El participante puede obtener la información complementaria del investigador principal del proyecto, cuya dirección figura en este escrito.
6. El participante puede solicitar por escrito dirigido al investigador principal del proyecto los resultados concretos obtenidos en su muestra donada.

Un saludo

Germán-Vicente Rodríguez

Anexo VII

FECHA | | **CODIGO:** | |

NOMBRE:

APELLIDOS:

EDAD: FECHA NACIMIENTO: / /

TELÉFONO MÓVIL:

PESO: _____ Kg TALLA: _____ cm

1) Años entrenados exclusivamente de natación (si también haces waterpolo incluirlo en el apartado de "otros deportes practicados"): _____

2) Horas entrenadas/semana (este año): _____

3) ¿Has entrenado hoy?_____

4) Otros deportes practicados en el pasado (años practicados/horas semana) (Solo contar deporte federados, gimnasio o actividades extraescolares. No contar los deportes que haces en educación física o en el recreo del colegio)

Deporte	Horas/semana que entrenabas	Competías Si/No	Años de practica (ejemplo: Desde los 11 hasta los 13)

5) ¿Actualmente realizas actividades deportivas complementarias a la natación?

Deporte	Horas/semana que entrenabas	Competías Si/No	Años de practica (ejemplo: Desde los 11 hasta la actualidad)

6) En caso de competir en que pruebas compites _____

Medicación habitual (En caso de no saber como se llama, marca si en la casilla "nombre", y te llamaremos al número de teléfono indicado)

Nombre	Dosis	Frecuencia

Longitud antebrazo	
Longitud pierna	

¿Padeces alguna enfermedad?