UNIVERSIDAD DE EXTREMADURA

TESIS DOCTORAL

EFECTOS DEL EJERCICIO VIBRATORIO CORPORAL EN HIPOXIA NORMOBÁRICA SOBRE LA DENSIDAD MINERAL ÓSEA

Marta Camacho Cardeñosa

Programa de Doctorado en Ciencias del Deporte

2019

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DOCTORAL THESIS

EFFECTS OF WHOLE BODY VIBRATION UNDER NORMOBARIC HYPOXIC CONDITIONS ON BONE MINERAL DENSITY

Marta Camacho Cardeñosa

Sport Sciences Doctorate Program

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CERTIFICA:

Que la Tesis Doctoral realizada por **Dña. Marta Camacho Cardeñosa**, con el título "**Efectos del ejercicio vibratorio corporal en hipoxia normobárica sobre la densidad mineral ósea**", bajo mi co-dirección, reúne los requisitos necesarios de calidad, originalidad y presentación para optar al grado de Doctor, y está en condiciones de ser sometida a valoración de la Comisión encargada de juzgarla.

Y para que conste a los efectos oportunos, firmo la presente en Cáceres, a 5 de julio de 2019.

Fdo: Dr. RAFAEL TIMÓN ANDRADA

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Fdo: Dr. PABLO TOMÁS CARÚS

2019

Enjoy the way…
The best is yet to come!
(Anonymous)

ACKNOWLEDGMENTS

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I would especially like to thank nurses and other workers of the home for the elderly "CIUDAD JARDÍN" and orthopaedic clinic "ALEJO LEAL". All of you have been there to support me when I recruited participants and collected data for my PhD thesis.

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CONTENTS

ABSTRACT

BACKGROUND: Age-related physiological changes, chronic diseases and multimorbidity are commonly known as geriatric syndromes, which result in the particular health states in older age. Physical activity is widely considered as a powerful tool for both, the treatment and prevention. As inactivity is more common in older adults, the benefits of physical exercise in this population are strongest, having also a protective effect. However, very few older adults are meeting the recommended levels of physical activity due to a large number of barriers. In the research of new physical activity therapies, the therapeutic use of hypoxia has been recently suggested for elderly population or chronic diseases patients. Combined with exercise, hypoxic sessions could have a lower stress on the locomotive systems for a similar physiological strain than in normoxia. Currently, interest in hypoxic conditioning has increased and experts around the globe have contributed in promoting its clinical applications in diseases prevention and treatment. However, the influence of hypoxia on bone remodelling is unclear and different physiological mechanisms could mediate in its effects. On the other hand, fewer studies up to date have applied this innovative therapeutic strategy in older adults.

AIMS: the general objectives were: (1) to review the current literature of bone metabolism and hypoxic stimulus; (2) to study the effects of passive hypoxia conditioning on bone mineral density of healthy elderly people; and (3) to study the effects of active hypoxia conditioning on healthy parameters of healthy elderly people.

METHODS: a systematic review was carried out following Preferred Reporting Item for Systematic Reviews and Meta-Analyses. The PubMed and Web of Science databases were searched for experimental studies written in English that investigated the effects of modification of ambient oxygen on bone remodelling parameters of healthy organisms. To

know the effects of passive hypoxia on bone mineral density of elderly people, twenty-four healthy elderly participants were randomly assigned to a hypoxic group (HYP) or control group. During 36 sessions, HYP group performed an intellectual activity while they were exposed to normobaric hypoxic conditions in a hypoxic chamber (16.1% fraction of inspired oxygen; FiO_2). Whole-body and proximal femur bone mineral density ($g \cdot cm^{-2}$) were measured using dual-energy X-ray absorptiometry. To study the effects of active hypoxia conditioning on healthy parameters of healthy elderly people, forty-six healthy elderly were randomly assigned to a hypoxic whole-body vibration group (HWBV), normoxic whole-body vibration group (NWBV) or control group. During 36 sessions, experimental groups received the same vibration treatment in a hypoxia chamber (HWBV: 16.1% FiO_2; NWBV: 21.0% FiO_2). A vibration session included 4 bouts of 30 s (12.6 Hz - 4 mm) with 1 min rest between bouts. Whole-body and proximal femur bone mineral density and body composition were measured using dual-energy X-ray absorptiometry. Isokinetic leg muscle strength was evaluated using a Biodex System-3 isokinetic dynamometer. The "Timed Up and Go Test" evaluated functional mobility.

RESULTS: in the systematic review thirty-nine studies analysed the effect of sustained or cyclic hypoxia exposure on genetic and protein expression and mineralisation capacity of different cell models; three studies carried out in animal models implemented sustained or cyclic hypoxia and ten studies examined the effects of sustained, intermittent or cyclic hypoxia on bone health and hormonal responses in humans. After hypoxic exposure, the whole body bone mineral density had a significant increase in HYP compared to the control group, whose values decreased ($p=0.008$). In the within group analysis, HYP group significantly increased theirs whole body bone mineral density ($p=0.004$) and control group significantly decreased this parameters with a large effect size ($p=0.004$; $d=1.18$). Regarding the effects of active hypoxia, compared to NWBV and CON (between-group analysis) the

HWBV group showed an increase whole-body bone mineral density ($p=0.07$), which was significant only in the within-groups analysis ($p<0.05$). Within the HWBV group, also the prevalence of osteopenia/osteoporosis decreased significantly ($p<0.001$). There were no significant differences between groups in either muscle mass or strength parameters. Neither statistically significant within group variations nor statistically significant differences between both groups were detected to "Timed Up and Go Test". The NWBV group showed statistically significant improvements in the maximal strength of knee extensors, with a small effect size ($p = 0.004$; $d = 0.54$).

CONCLUSIONS: Different modes of hypoxic conditioning may have different impacts on bone metabolism both in vivo and in vitro. Additional research is necessary to establish the optimal cyclical dose of oxygen concentration and exposure time. On the other hand, passive hypoxia exposure at 16.1% FiO_2 during 36 sessions is apparently enough to significantly increase total body bone mineral density in older adults, but not in a specific region such as proximal femoral. Combined with WBV training, hypoxic stimuli seemed to generate positive effects on whole body and proximal femur bone mineral density of elderly population. Although changes did not differ significantly between groups, the benefits observed only within the vibration exercise plus hypoxia may be considered promising but they should be confirmed in future large-scale studies. However, active hypoxia does not cause any effect on muscle mass nor strength of older adults. Functional mobility neither changed following an active hypoxia protocol. Our finding suggests that the work/rest ratio and the accumulated volume could be important factors when untrained elderly people are subjected to vibration training in hypoxic conditions. High stress during the session could limit their capacity for improvement.

KEYWORDS: oxygen deprivation; vibration; osteogenesis; bone mineral density; muscle strength dynamometer; physical performance; ageing.

RESUMEN

ANTECEDENTES: Los cambios fisiológicos relacionados con la edad, las enfermedades crónicas y la comorbilidad se conocen comúnmente como síndromes geriátricos y dan como resultado estados de salud característicos del envejecimiento. La actividad física es considerada como una herramienta poderosa tanto para el tratamiento como para la prevención en todas las edades. Como la inactividad es más común en los adultos mayores, los beneficios del ejercicio físico en esta población son más fuertes, poseyendo un importante efecto protector. Sin embargo, muy pocos adultos mayores cumplen con los niveles recomendados de actividad física debido a una gran número de barreras. En la investigación de nuevas terapias de actividad física, el uso terapéutico de la hipoxia se ha sugerido recientemente para pacientes ancianos o con enfermedades crónicas. Combinado con el ejercicio, las sesiones hipóxicas podrían tener un menor estrés en los sistemas locomotores provocando un estrés fisiológico similar a las condiciones de normoxia. Actualmente, el interés en el acondicionamiento hipóxico ha aumentado y los expertos de todo el mundo han contribuido a promover sus aplicaciones clínicas en la prevención y el tratamiento de enfermedades. Sin embargo, la influencia de la hipoxia en la remodelación ósea no está clara y diferentes mecanismos fisiológicos podrían mediar en sus efectos. Por otro lado, pocos estudios hasta la fecha han aplicado esta innovadora estrategia terapéutica en adultos mayores.

OBJETIVOS: los objetivos generales perseguidos en este proyecto fueron: (1) revisar la literatura científica actual relacionada con el metabolismo óseo y el estímulo hipóxico; (2) estudiar los efectos de la hipoxia pasiva sobre la densidad mineral ósea de adultos mayores sanos; y (3) estudiar los efectos de la hipoxia activa sobre parámetros de salud de adultos mayores.

MÉTODOS: se buscaron estudios en las bases de datos PubMed y Web of Science que fuesen experimentales, escritos en inglés y que investigaran los efectos de la modificación del oxígeno ambiental en parámetros de remodelado óseo de organismos sanos. Para conocer los efectos de la hipoxia pasiva en la densidad mineral ósea de las personas mayores, veinticuatro participantes ancianos sanos fueron aleatoriamente asignados a un grupo de hipoxia (HYP) o a un grupo control. Durante 36 sesiones, el grupo HYP realizó una actividad intelectual a la vez que estaban expuestos a condiciones de hipóxia normobárica en una cámara de hipoxia (16.1% fracción de oxígeno inspirado; FiO2). Se midió la densidad mineral ósea de todo el cuerpo y de la región proximal del fémur (g · cm-2) utilizando la absorciometría de rayos X de energía dual. Para estudiar los efectos de la hipoxia activa en los parámetros de salud de personas mayores sanas, cuarenta y seis participantes fueron aleatoriamente asignados a un grupo de vibración hipóxica de cuerpo completo (HWBV), a un grupo de vibración de cuerpo entero en situación de normoxia (NWBV) o a un grupo control. Durante 36 sesiones, los grupos experimentales recibieron el mismo tratamiento de vibración en una cámara de hipoxia (HWBV: 16.1% FiO_2; NWBV: 21.0% FiO_2). Cada sesión de ejercicio vibratorio incluyó 4 series de 30 s (12,6 Hz - 4 mm) con un descanso de 1 minuto entre series. Se midió la densidad mineral ósea y la composición corporal de todo el cuerpo y de la región proximal del fémur utilizando absorciometría de rayos X de energía dual. La fuerza muscular isocinética del tren inferior se evaluó utilizando un dinamómetro isocinético Biodex System-3. El "Timed Up and Go Test" evaluó la movilidad funcional.

RESULTADOS: treinta y nueve estudios de la revisión sistemática analizaron el efecto de la exposición a hipoxia mantenida o cíclica sobre la expresión genética y de proteínas y la capacidad de mineralización de diferentes modelos celulares. Tres estudios realizados en modelos animales implementaron hipoxia mantenida o cíclica y diez estudios examinaron el efecto de la hipoxia mantenida, intermitente o cíclica sobre la salud ósea y la respuesta

hormonal en humanos. Después de la exposición a la hipoxia, la densidad mineral ósea de todo el cuerpo tuvo un aumento significativo en comparación con el grupo control, cuyos valores disminuyeron (p = 0,008). En el análisis intragrupo, el grupo HYP aumentó significativamente su densidad mineral ósea en todo el cuerpo (p = 0,004) y el grupo control disminuyó significativamente estos parámetros con un tamaño del efecto elevado (p = 0,004; d = 1,18). Con respecto a los efectos de la hipoxia activa, en comparación con NWBV y CON (análisis entregrupos), el grupo HWBV mostró un aumento de la densidad mineral ósea en todo el cuerpo (p = 0.07), que fue significativa solo en el análisis dentro de los grupos (p <0.05). Dentro del grupo HWBV, también la prevalencia de osteopenia / osteoporosis disminuyó significativamente (p <0,001). No hubo diferencias significativas entre los grupos en ninguno de los parámetros de fuerza muscular. Tampoco hubo diferencias significativas en "Timed Up and Go Test" para ninguno de los análisis. El grupo NWBV mostró mejoras estadísticamente significativas en la fuerza máxima de los extensores de rodilla, con un tamaño de efecto pequeño (p = 0,004; d = 0,54).

CONCLUSIONES: Diferentes protocolos de administración de la hipoxia pueden tener efectos diferentes sobre el metabolismo óseo. Son necesarias más investigaciones para establecer la dosis óptima de concentración de oxígeno y tiempo de exposición. La exposición a la hipoxia pasiva al 16,1% de FiO2 durante 36 sesiones parece suficiente para aumentar significativamente la densidad mineral ósea de todo el cuerpo en adultos mayores, pero no en una región específica como la femoral. Combinado con el entrenamiento vibratorio, los estímulos hipóxicos podrían generar efectos positivos en la densidad mineral ósea de todo el cuerpo y del fémur de población anciana. Aunque los cambios no fueron diferentes entre los grupos de intervención, los beneficios observados solo dentro del grupo sometido a hipoxia activa pueden considerarse prometedores, aunque se necesitan estudios a gran escala para poder confirmar dichos resultados. Sin embargo, la hipoxia activa no causa

efecto sobre la masa muscular o la fuerza de adultos mayores, ni sobre la movilidad funcional. Nuestros resultados sugieren que la relación trabajo / descanso y el volumen acumulado podrían ser factores importantes cuando las personas mayores se someten a entrenamiento vibratorio en condiciones de hipoxia. El alto estrés durante la sesión podría limitar su capacidad de mejora.

PALABRAS CLAVE: falta de oxígeno; vibración; osteogénesis; densidad mineral ósea; dinamómetro de fuerza muscular; rendimiento físico; envejecimiento.

RESULTS DIVULGATION

During the development of this thesis project, different activities have been carried out to the scientific results divulgation. Three scientific articles have already been published in Journal Citation Reports (JCR) indexed journals. Below, references and the journal data are showed. Furthermore, other manuscript has been submitted to *Frontriers in Physiology* Journal, being currently under review to various experts in the field. Finally, we have completed a last manuscript, which will be submitted soon.

	**EVALUATION OF 18-WEEK WHOLE-BODY VIBRATION TRAINING IN NORMOBARIC HYPOXIA ON LOWER-EXTREMITY MUSCLE STRENGTH IN ELDERLY POPULATION** *Camacho-Cardenosa, M., Camacho-Cardenosa, A., Brazo-Sayavera, J., Olcina, G.; Tomas-Carus, P.; Timon, R.* ***High Altitude Medicine & Biology*** JCR (WoS) - SPORT SCIENCES – SCI Impact Factor: 3.854 Position: Q3
	**CAN HYPOXIC CONDITIONING IMPROVE BONE METABOLISM? A SYSTEMATIC REVIEW** *Camacho-Cardenosa, M., Camacho-Cardenosa, A., Timon, R., Olcina, G.; Tomas-Carus, P.; Brazo-Sayavera, J.* ***International Journal of Environmental Research and Public Health*** JCR (WoS) - PUBLIC, ENVIR & OCCUP HEALTH – SCIE Impact Factor: 2.468 Position: Q2
	**EFFECTS OF WHOLE-BODY VIBRATION UNDER HYPOXIC EXPOSURE ON MUSCLE MASS AND FUNCTIONAL MOBILITY IN OLDER ADULTS** *Camacho-Cardenosa, M., Camacho-Cardenosa, A., Tomas-Carus, P.; Olcina, G.; Timon, R., Brazo-Sayavera, J.* ***Aging Clinical and Experimental Research*** JCR (WoS) - GERIATRICS AND GERONTOLOGY – SCI Impact Factor: 2.331 Position: Q3
(under review)	**EFFECTS OF WHOLE-BODY VIBRATION TRAINING COMBINED WITH CYCLIC HYPOXIA ON BONE MINERAL DENSITY IN ELDERLY PEOPLE** *Camacho-Cardenosa, M., Camacho-Cardenosa, A., Burtscher, M., Brazo-Sayavera, J., Tomas-Carus, P.; Olcina, G.; Timon, R.* ***Frontiers in Physiology*** JCR (WoS) - PHYSIOLOGY – SCI Impact Factor: 3.201 Position: Q2

On the other hand, during this period, some data are been presented in different Conferences.

Camacho-Cardenosa, M. Título: EFECTOS DEL EJERCICIO VIBRATORIO CORPORAL EN HIPOXIA NORMOBÁRICA SOBRE LA DENSIDAD MINERAL ÓSEA Conference: II Congreso Multidisciplinar de jóvenes investigadores extremeños National Conference City/Country: Cáceres/Spain YEAR: 2016
Camacho-Cardenosa, M.; Camacho-Cardenosa, A.; Brazo-Sayavera, J. ENTRENAMIENTO VIBRATORIO CORPORAL EN HIPOXIA NORMOBÁRICA PARA LA MEJORA DE LA MOVILIDAD DE PERSONAS MAYORES Conference: I Congreso Internacional de "Salud y Calidad de Vida" International Conference City/Country: Almería/Spain YEAR: 2018
Timón, R.; Camacho-Cardenosa, A.; Martínez-Guardado, I.; Olcina, G.; Leal, A.; Camacho-Cardenosa, M. EFFECT OF HYPOXIC TRAINING AND WHOLE-BODY VIBRATION ON MUSCLE STRENGTH IN ELDERLY Conference: IX Simposio Internacional en Entrenamiento de la Fuerza International Conference City/Country: Madrid/Spain YEAR: 2018

Finally, four research stays in international and national universities and research centres have been done.

Centre/Institute: **University of Central Lancashire**			
City: Preston	Country: United Kingdom	Year: 2015	Duration (months): 3
Centre/Institute: **University of Innsbruck**			
City: Innsbruck	Country: Austria	Year: 2017	Duration (months): 3
Centre/Institute: **Instituto Maimónides de Investigación Médica de Córdoba (IMIBIC)**			
City: Córdoba	Country: Spain	Year: 2018	Duration (months): 5
Centre/Institute: **University of Évora**			
City: Évora	Country: Portugal	Year: 2019	Duration (months): 3

Conferences proceedings and scientific stays inform are available in the Appendix (Appendix A) section.

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

1,25 (OH) D	*25-Hydroxyvitamin D*
25 (OH) D	*1,25 Dihydroxyvitamin D*
25-Vit D	*25 Hidroxi Vitamine D*
ALP	*Alkaline phosphatase*
ANOVA	*Analysis of variance*
ASCs	*Adipose-derived stromal cells*
ATP	*Adenosine triphosphate*
B-ALP	*Bone specific alkaline phosphatase*
BMC	*Bone Mineral Content*
BMD	*Bone Mineral Density*
BMI	*Body Mass Index*
BMSCs	*Bone Marrow Stromal Cells*
BPAQ	*Bone Physical Activity Questionnaire*
Ca^{2+}	*Calcium*
CESCs	*Cartilage Endplate Stem Cells*
CICP	*C-terminal Propeptide of type I Collagen*
CIS	*Commonwealth of Independent States*
COL1A1	*Collagen Type 1 Alpha 1*
CON	*Control*
CTX	*C-terminal Telopeptide*
DPD	*Desoxipiridinoline*
DPD/Cr	*Desoxipiridinoline/Creatine ratio*
DXA	*Dual-energy X-ray Absorptiometry*
EPO	*Erythropoietin*
EU	*European Union*
Ext	*Extension*
FiO_2	*Inspired Oxygen Fraction*
Flex	*Flexion*
GDP	*Gross Domestic Product*
GLUTs	*Glucose Transporters*
H/Q	*Hamstring/Quadriceps*
HAT	*High Altitude Training*
HC	*Hypoxic Conditioning*
HIF	*Hypoxia Inducible Factor*
HR	*Heart Rate*
HRE	*Hypoxia Response Element*
HWBV	*Hypoxia Whole Body Vibration*
HYP	*Hypoxia group*
PTH	*Parathyroid Hormone*
IH	*Intermittent Hypoxia*
IHT	*Intermittent Hypoxia Training*
LDHA	*Lactate Dehydrogenase A*
MDC	*Minimum Detectable Change*
MSCs	*Mesenchymal Stem Cells*
NO	*Nitric Oxide*
NTX	*N-telopeptide of Type I Collagen*
NWBV	*Normoxia Whole Body Vibration*
OC	*Osteocalcin*
ODD	*Oxygen-Dependent Degradation*
OHAT	*Office of Health Assessment and*
OPG	*Osteoprogeterin*
OPN	*Osteopontin*
OSAS	*Obstructive Sleep Apnoea Syndrome*
P	*Phosphorous*
PDK1	*Pyruvate Dehydrogenase Kinase 1*
PHD	*Prolyl Hydroxylase*
PiO_2	*Inspired Partial Pressure of Oxygen*
PMSCs	*Placental Mesenchymal Stem Cells*
POST	*Assessed after intervention*
PRE	*Assessed in baseline*
PRISMA	*Preferred Reporting Item for Systematic Reviews and Meta-Analyses*
PT	*Peak Torque*
pVHL	*Von Hippel–Lindau protein*
RANK	*Receptor Activator of Nuclear Factor κB*
RANKL	*Receptor Activator of Nuclear Factor κB Ligand*
ROS	*Reactive Oxygen Species*
RUNX2	*Runt-related Transcription Factor 2*
SaO_2	*Oxygen Saturation Arterial*
SD	*Standard Deviations*
SPPB	*Short Physical Performance Battery*
TDSCs	*Tendon-derived Stem Cells*
TUGT	*Timed Up and Go Test*
UCPVCs	*Umbilical Cord Perivascular cells*
VEGF	*Vascular endothelial Growth Factor*
W	*Work*
WBV	*Whole-Body Vibration*

Chapter 1. BACKGROUND

Chapter 1. BACKGROUND

1.1 Healthy ageing

Based on data of (Nations United, 2017), the world's population is ageing: in 1980 there were 382 million older persons worldwide; in 2017 the global population aged 60 years or over numbered 962 million and is project that, in 2050, the number of older person will reach nearly 2.1 billion. The increase of number of older persons is also accompanied by a lower number of people in all younger age groups (Figure 1). These projections indicate that in 2050 there will be more old persons that youth at ages 10-24 years and the number of advanced ages people could be to triple compared with 2017 (137 million to 425 million).

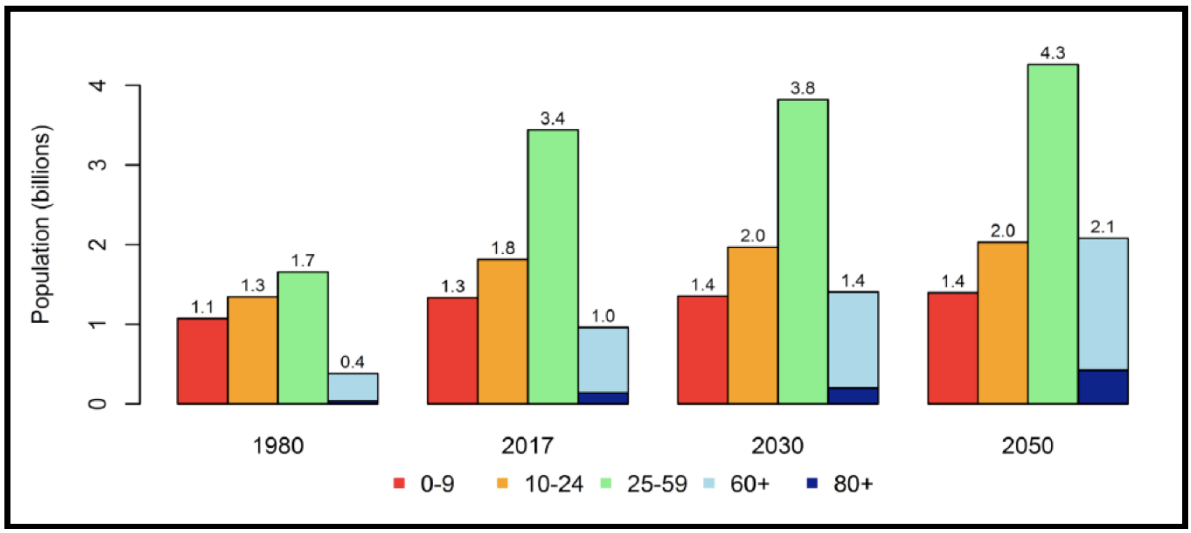

Figure 1 Global Population by broad age groups since 1980 until 2050
Data source: (Nations United, 2017)

Moreover, in spite of this increase is being much faster in developing regions, it is expected that every country in the world will experience a substantial increase in the size of population aged 60 years or over. In Europe, percentage change between 2017 and 2050 will be 35 per cent (Figure 2).

	Number of persons aged 60 years or older in 2017 (millions)	Number of persons aged 60 years or over in 2050 (millions)	Percentage change between 2017 and 2050	Distribution of older persons in 2017 (percentage)	Distribution of older persons in 2050 (percentage)
World	962.3	2080.5	116.2	100.0	100.0
Africa	68.7	225.8	228.5	7.1	10.9
Asia	549.2	1273.2	131.8	57.1	61.2
Europe	183.0	247.2	35.1	19.0	11.9
Northern America	78.4	122.8	56.7	8.1	5.9
Latin America and the Caribbean	76.0	198.2	160.7	7.9	9.5
Oceania	6.9	13.3	92.6	0.7	0.6

Figure 2 Number and distribution of person aged 60 years or over in 2017 and 2050
Data source: (Nations United, 2017)

Since 1980, countries located in Europe have composed the list of the world's ten most aged population, but the percentage of this population had not yet reached 25 per cent in any county (Figure 3). In 2050, it is expected that older person will comprise more than 39 per cent of the population in these ten most aged countries.

	1980		2017		2050	
Rank	Country or area	Percentage aged 60 years or over	Country or area	Percentage aged 60 years or over	Country or area	Percentage aged 60 years or over
1	Sweden	22.0	Japan	33.4	Japan	42.4
2	Norway	20.2	Italy	29.4	Spain	41.9
3	Channel Islands	20.1	Germany	28.0	Portugal	41.7
4	United Kingdom	20.0	Portugal	27.9	Greece	41.6
5	Denmark	19.5	Finland	27.8	Republic of Korea	41.6
6	Germany	19.3	Bulgaria	27.7	China, Taiwan Province of China	41.3
7	Austria	19.0	Croatia	26.8	China, Hong Kong SAR	40.6
8	Belgium	18.4	Greece	26.5	Italy	40.3
9	Switzerland	18.2	Slovenia	26.3	Singapore	40.1
10	Luxembourg	17.8	Latvia	26.2	Poland	39.5

Figure 3 Ten countries or areas with the largest share or persons aged 60 years or over
Data source: United Nations (2017). *World Population Prospects: the 2017 Revision*

Longer life is an incredibly valuable resource. It provides the opportunity to reconsider what older age might be: many people are looking to spend these extra years in innovative ways, such as a new career, continuing education, or pursuing a neglected passion (Age Wave, 2011). Yet, the extent of the opportunities that arise from these extra years of life

will be very heavily dependent on one key factor: health (Beard et al., 2016). Although increasing longevity is often assumed to be accompanied by an extended period of good health, little evidence exists that older people today are experiencing better health than their parents did at the same age (Crimmins & Beltran-Sanchez, 2011). A good health at these ages could permit to maintain the ability to do the things that matter to them will be as a younger person (Beard et al., 2016).

During the past 50 years, the socioeconomic development has caused important changes in cause of death (Omran, 2005), which occur majority in people older than 70 years old (Beard et al., 2016). Based on World Health Organization (WHO) Global Health Estimates data (World Health Organization, 2013), between the main causes of death in older age, the non-communicable diseases are dominant, independently of the level of socioeconomic development. Sensory impairments, back and neck pain, chronic obstructive respiratory disease, depressive disorders, falls, diabetes, dementia, and osteoarthritis are identified as the greatest causes of years living with disability in old people (World Health Organization, 2013). More than half of older people are likely to experience multimorbidity, which is characterized by the presence of more than one disorder at the same time.

Among others, old age is associated with a decline in bone mass and strength that can culminate with osteoporosis (Beck & Norling, 2010). Two hundred million individuals are affected by osteoporosis worldwide (Reginster & Burlet, 2006), causing 8.9 million fractures yearly (1,000 fractures per hour) with the hip region being the most debilitating (Johnell & Kanis, 2006) Ageing is also accompanied by changes in body composition, which include a loss of muscle mass and function or strength (sarcopoenia; (Gomez-Cabello, Gonzalez-Aguero, Ara, Casajus, & Vicente-Rodriguez, 2013; Walston, 2012). Muscular strength is reduced by 15% per decade after the age of 50, and by 30% after the age of 70 ((Rolland et al., 2008; Verdijk et al., 2007)). Among other risk factors, the loss of muscle strength in the

lower-limbs has been considered responsible for a higher risk of falls (Goudarzian, Ghavi, Shariat, Shirvani, & Rahimi, 2017). The majority of falls among the elderly take place in situations that demand reactive postural control, such as the recovery from external perturbations (Niino, Tsuzuku, Ando, & Shimokata, 2000). Muscle fatigue has been shown to alter the peripheral proprioceptive system and the central processing of sensory inputs, both of which are essential for this reactive postural control (Taylor, Butler, & Gandevia, 2000). This reductions in skeletal muscle mass are also associated with decrease of functional capacity, autonomy and higher dependence (Gomez-Cabello et al., 2013; Hairi et al., 2010; Trombetti et al., 2016). For all this, greater individual effects on functioning, quality of life, and mortality risk might be expected (Marengoni et al., 2011).

Age-related physiological changes, chronic diseases and multimorbidity are commonly know as geriatric syndromes, which results in the particular health states in older age (Inouye, Studenski, Tinetti, & Kuchel, 2007). One of these geriatric syndromes is frailty, which can be regarded as a progressive age-related deterioration in physiological systems that increases the risk of care dependence and death (Clegg, Young, Iliffe, Rikkert, & Rockwood, 2013). In Europe, around 15-35% of people aged 75 years or older need assistance in activities of daily living due to substantial losses in functioning (Beard et al., 2016). This condition is very common at age 50–64 years, increasing a 17% in people older than 65 years (Santos-Eggimann, Cuenoud, Spagnoli, & Junod, 2009). Therefore, due to the complexity of health states in older age, the concept of health could result inadequate to health in an older person. Rather than the presence or absence of disease, the most important consideration for an older person is likely to be their functioning (or intrinsic capacity), which could be defined as the physical and mental capacities that the individual can draw on at ay point in time (Beard et al., 2016). Of this same way, comprehensive assessments of functioning in older

age could be also much better predictors of survival instead of the absence/presence of diseases or comorbidities (Lordos et al., 2008).

No doubt that the increment in the number of older persons have implications for nearly all sectors of society and the preparing for the economic and social shifts associated with an ageing population is thus essential to fulfil the pledge of the 2030 Agenda for Sustainable Development that "no one will be left behind" (Nations United, 2017). Based on the survey of European Commission (2012), European Union (EU) Member States spend, on average, more than a quarter of their Gross Domestic Product (GDP) for the benefit of older people in the form of pensions, health and long-term care. However, with the economic crisis, ageing is perceived by many as a threat instead of one of our greatest achievements. This crisis has left us with large public deficits and a huge public debt burden at a time when the "baby boomers", which were born in the two decades after World-War II, start to retire from the labour market. However, this growing number of older people can have an important contribution in the society if allowing people to stay active during ageing. This is why the EU decided to designate 2012 as the "European Year for Active Ageing and Solidarity between Generations". Active ageing is not just about the participation of older workers in the labour market, it's about their active contribution to society through voluntary work, notably as family carers, or the possibility to live independently thanks to adapted housing and infrastructure. Therefore, it is crucial that healthy care systems work to promote healthy ageing, sus as prevent and treat non-communicable diseases and chronic conditions.

Physical activity is wide considered as a powerful tool for both the treatment and prevention of non-communicable diseases in every age group. In fact, there is a lineal relationship between activity level and health status (Sallis, 2015). Numerous studies suggest that the health risk of inactivity are higher that those of obesity (Blair, 2009). An inactive society has consequences on health care costs: active patients had almost 30% lower health

care costs than those who were inactive (Anderson et al., 2005). As inactivity is more common in older adults, the benefits of physical exercise in this population are strongest, having also a protective effect (World Health Organization, 2013). Compared to less active individuals, active old adults have lower rates of all-cause mortality, coronary heart disease, high blood pressure, stroke, type 2 diabetes, colon cancer, breast cancer, a higher level of cardiorespiratory and muscular fitness, healthier body mass and composition, and a biomarker profile that is more favourable for the prevention of cardiovascular disease, type 2 diabetes and a higher bone health (Paterson, Jones, & Rice, 2007; Paterson & Warburton, 2010; World Health Organization, 2008). In Figure 4, the recommendations of physical activity of World Health Organization (2010) are shown.

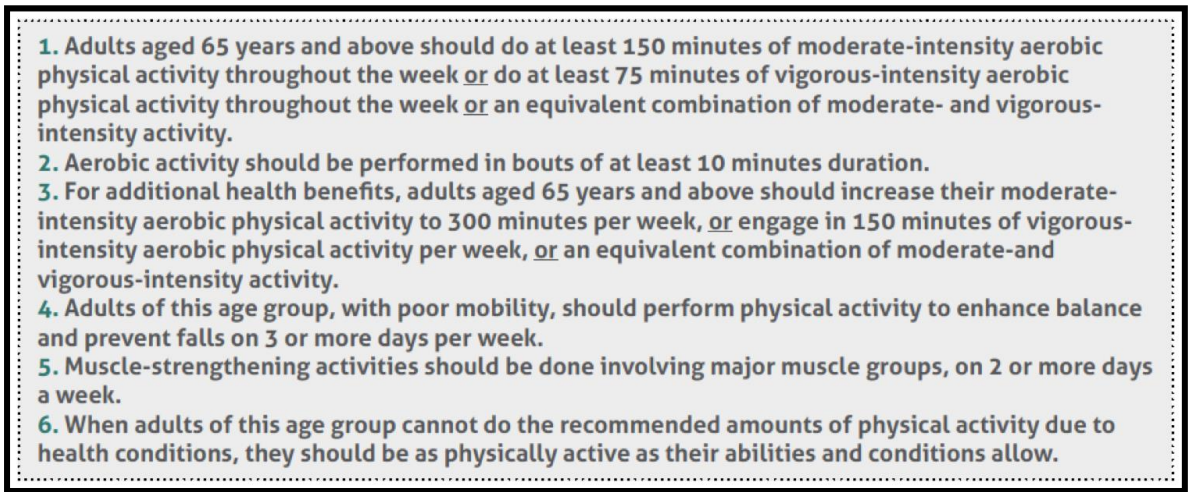

Figure 4 Global recommendations on Physical Activity for health address 65 years or over
Data source: (World Health Organization, 2010)

However, very few older adults are meeting the recommended levels of physical activity due to a large number of barriers, such as ill health, pain, and injury (Taylor et al., 2000). Heavy resistance exercises provide greater adaptation (Miller, Heishman, Freitas, & Bemben, 2018), but due to age-related factors, such as cardiac limitation, weak balance, or lack of sufficient motivation or interest, many elderly people are unable or unwilling to increase their level of physical activity (Rosenberg et al., 2018). In these sense, numerous investigations have proposed the whole-body vibration (WBV) training as a safe and

tolerable alternative for this population (Ko et al., 2017). Even though, this passive exercise could have effects on multiple body systems, the results of the scientific research are controversial and high frequency and duration of training may be necessary (Gomez-Cabello, Ara, Gonzalez-Aguero, Casajus, & Vicente-Rodriguez, 2012). In the research of new physical activity therapies, the therapeutic use of hypoxia has been recently suggested for elderly population or chronic diseases patients (Millet, Debevec, Brocherie, Malatesta, & Girard, 2016). Combing with exercise, hypoxic sessions could have a lower stress on the locomotor systems for a similar physiological strain than in normoxia (Pramsohler et al., 2017).

Therefore, the combination of effects of WBV training with hypoxic exposure could provide new approaches that are, first and foremost, suitable for enhancing physical fitness and health and are consequently feasible and comfortable for older adults.

1.2 Therapeutic Use of Hypoxia

Last years, hypoxic conditioning studies have increased considerably in both athletic performance and health fields. Several finding suggest that hypoxic exposure may lead to some adaptations of the human body, able to protect him form pathological conditions (Verges, Chacaroun, Godin-Ribuot, & Baillieul, 2015). However, an imprecise terminology is present in the field of hypoxia research. Different terms have been used to refer to the alteration of the concentration of oxygen searching an organism response: high altitude training (HAT), hypoxic conditioning (HC), intermittent hypoxia (IH), intermittent hypoxia training (IHT). This difficulty regarding terminology is marked by the both adaptive (beneficial) and maladaptive (detrimental) responses to hypoxia and their potential pathogenic and prophylactic roles. Hypoxic exposure can be implemented with different patterns and severity, mediating different molecular pathways (Xi & Serebrovskaya, 2012). An optimal dose in terms of duration, frequency and severity could range from no response at low intensity to a protected state or even negative adaptations after higher intensities or a further increase in stimulus (Verges et al., 2015). In this sense, some authors have distinguished between different hypoxia modes. “Sustained hypoxia” is characterised by a single episode of hypoxia, which is maintained during a prolonged stay. On the other hand, hypoxia can also be interrupted by reoxygenation periods, differing two new modes of hypoxia: (a) “intermittent hypoxia” that is characteristic of obstructive sleep apnoea syndrome (OSAS), where many short cycles of severe hypoxia lasting 15–30 s are followed by longer periods of reoxygenation; (b) “cyclical hypoxia”, where longer periods of moderate hypoxia (until 12 h) are followed by identical reoxygenation periods (Xi & Serebrovskaya, 2012). In this sense, previous reviews have considered the effects of sustained, intermittent and cyclical exposures on cardiovascular and respiratory physiology, health and overall quality of life (Basovich, 2013; Dempsey & Morgan, 2015; Navarrete-Opazo & Mitchell,

2014; Serebrovskaya & Xi, 2016). It has been shown as the "sustained" and "intermittent" hypoxia could lead to deleterious consequences by increasing oxidative stress and producing systemic inflammation (Garvey, Taylor, & McNicholas, 2009). On contrary, "cyclical" hypoxia may lead to a prolonged and sustained state of protection (Verges et al., 2015). It might be hard to find a better example for the duality in life science than the effects of hypoxic exposure on living organisms (Xi & Serebrovskaya, 2012). This cyclic mode is also known as HC. Conditioning is a procedure by which a potentially harmful stimulus is applied near to but below the threshold of damage to the organism (Verges et al., 2015). This procedure is based in the "hormesis" (Figure 5): unique ability of the cells to adapt, condition and optimize themselves for future insults when it are exposed to a low dose of deleterious stimulus, whereas a high dose of the same stimulus could cause detrimental effects (Mattson, 2008; Navarrete-Opazo & Mitchell, 2014; Rybnikova & Samoilov, 2015). Ischemia, hypoxia, hypothermia, and pharmacological agents are stimulus, which can induce a preconditioning response and modify the responses of the organism to subsequent stress conditions (Verges et al., 2015).

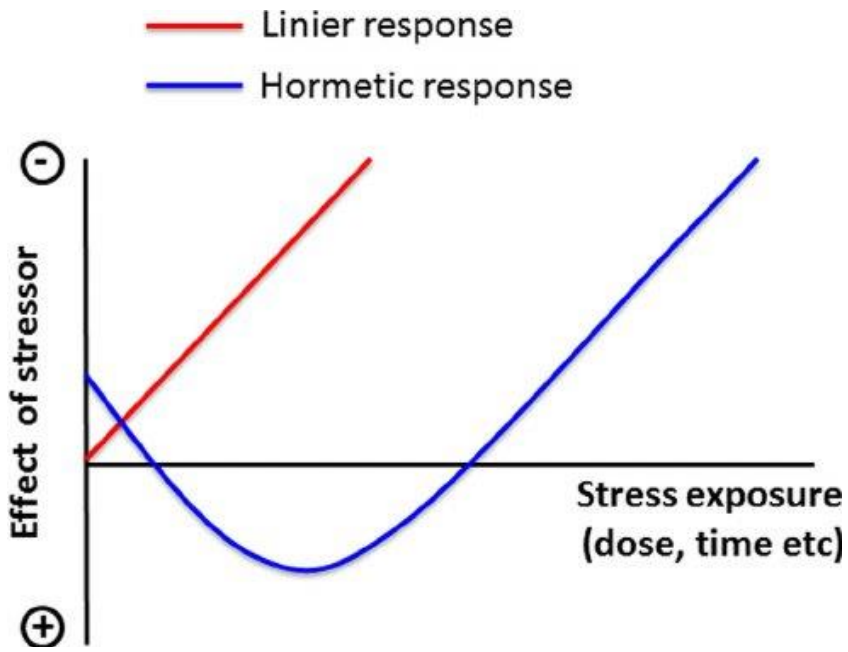

Figure 5 Hormesis Diagram
Source: (Merry & Ristow, 2016)

On the other hand, it is necessary to difference between passive and active hypoxic exposure. Active hypoxic exposure, knows also as hypoxia training, does not provoke the same acute responses compared with passive hypoxic exposure or when similar exercise are developed in normoxia (Millet et al., 2016). Specific responses are observed following exercising in hypoxia (Hoppeler, Klossner, & Vogt, 2008; Lundby, Calbet, & Robach, 2009). Furthermore, hypoxic training sessions could generate a lower stress on the locomotor systems for a similar physiological strain than in normoxia (Millet & Girard, 2017). Therefore, in spite of elderly individuals or patients the altitude is associated with increased health risks, combined hypoxia exposure with physical exercise could have beneficial synergistic effects and, therefore, exercising in hypoxia could be a valuable and viable "therapeutic strategy" (Millet et al., 2016).

1.2.1 Brief history of hypoxic conditioning

As indicated Serebrovskaya (2002), first investigations in the field of hypoxic conditioning (know as intermittent hypoxia) we developed in the Soviet Union and the Commonwealth of Independent States (CIS) in 1930s. Investigative reports related to the potential therapeutic effects of intermittent hypoxia were developed. In the intervening years to the present, intermittent hypoxia has been used extensively in the Soviet Union and the CIS to the treatment of a variety of clinical disorder, such as lung diseases and bronchial asthma in children and adults, hypertension, emotional disorders, diabetes mellitus, Parkinson's disease, inflammatory process, radiation toxicity and occupational diseases. However, because many of the scientific publications were in Russian or Ukrainian these research findings remained relatively unknown. Then, before World War II, Soviet pilots done stays and trained in high altitude camps and hypoxic chambers for several weeks as methods to increase the endurance to high altitude flights. As the transport of pilots to mountain environments or utilization of chambers proved expensive and inconvenient, as

early as 1938 some authors utilized inhalation of hypoxic gas mixture for training pilots. These studies led to improve the work capacity and endurance of paratroopers, athletes and spacemen. But it was not until the 1968 Olympics in Mexico City when this innovative strategy was wide researched. In this sport event, developed at 2,240 m above sea level, record low endurance event times were recorder and hypoxic training start to gain more recognition to increased athletic performance.

Currently, interest in hypoxic conditioning has increased and experts around the globe have contributed in promoting its clinical applications in diseases prevention and treatment.

1.3 Physiological mechanisms of Hypoxic Adaptations

Knowledge of the influence of hypoxia on human physiology has improved with the discovery of the hypoxia-inducible factor (HIF), which is upregulated and stabilised in many cell groups due to reduced availability of oxygen (Semenza, 2012). HIF family composed of three α subunits (HIF-1α, HIF-2α and HIF-3α) and one β subunit (Wan et al., 2010). HIF-1β subunit is constitutively expressed in the nucleus in an oxygen-independent manner (G. L. Wang & Semenza, 1993). All three subunits conserved Von Hippel–Lindau tumor suppressor (pVHL)-binding and oxygen-dependent degradation (ODD) domains and are consequently regulated by hypoxia in the same way (Weisener et al., 1998). ODD domain contains prolyl residues that are recognized and hydroxylated by specific prolyl hydroxylase domain (PHD) enzymes (Jiang, Zheng, Leung, Roe, & Semenza, 1997). As Figure 6 shows, under normoxia, prolyl hydroxylation by the ODD domain mediates the binding of the E3 ubiquitin ligase pVHL, a component of the complex that targets HIF-α for proteasomal degradation in presence of oxygen and iron (L. E. Huang, Gu, Schau, & Bunn, 1998). Under hypoxia conditions, the hydroxylation of HIF-α is inhibited and it accumulates in the cytoplasm. Then it translocates to the nucleus, where it dimerizes with the HIF-1β subunit, binding to a highly conserved hypoxia response element (HRE) within promoters of hypoxia-responsive genes (Bruick & McKnight, 2002). To date, more than 100 HIF downstream genes have been identified (Ke & Costa, 2006). Genes containing functional HREs encode proteins involved in multiple functions such as the angiogenesis, the maturation of red blood cells, the energy metabolism or cell proliferation and viability (Semenza, 2000). In spite of other non-hypoxic environmental factors such as inflammation, reactive oxygen species (ROS) or nitric oxide (NO) could induce HIF- α subunits accumulation and target gene expression, the hypoxic stimulus is the main cause (Ke & Costa, 2006).

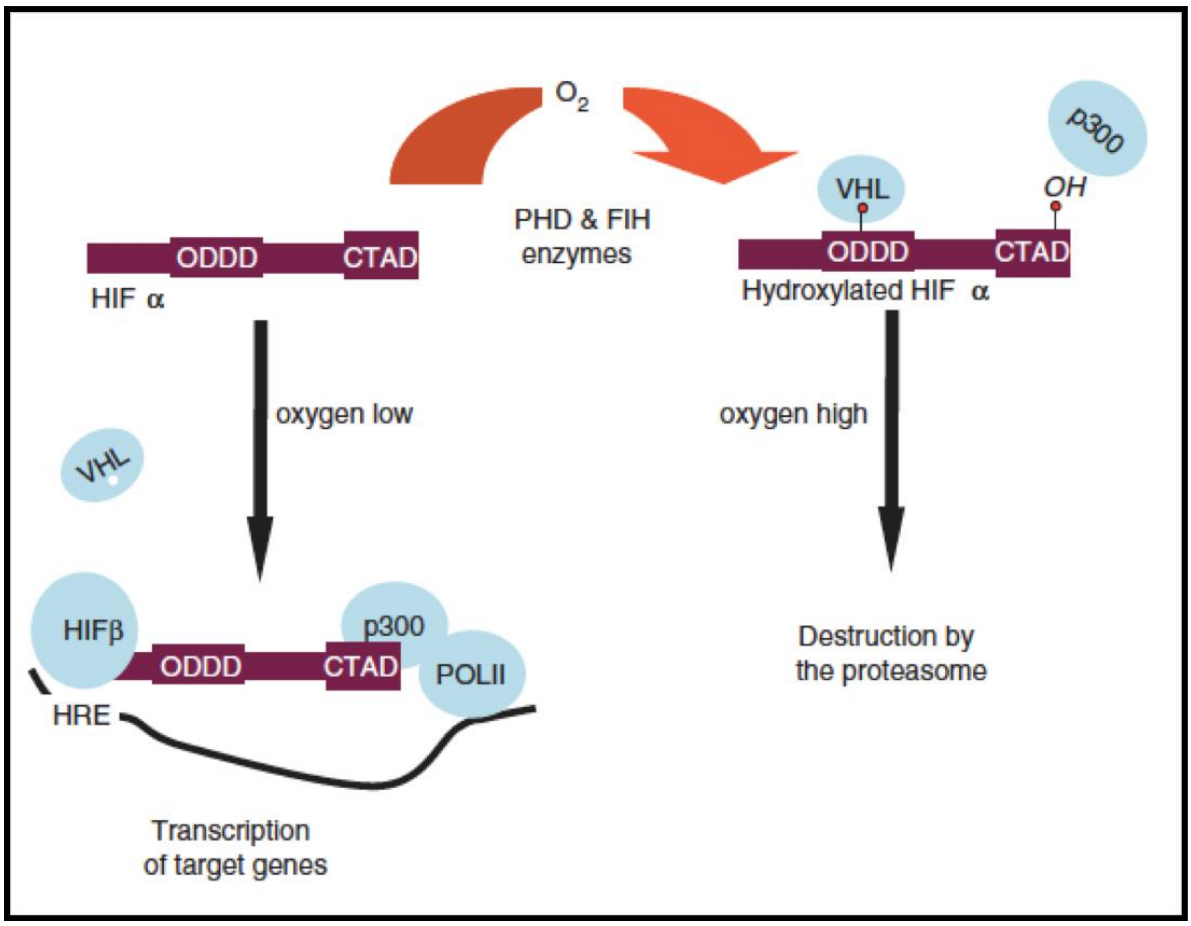

Figure 6 Regulation of the HIF complex by oxygen through hydroxylation of the α subunit
Source: (Maxwell, 2005)

1.3.1 Hypoxic Adaptations of bone metabolism

Hypoxia-driven pathways are vital in normal development and tissue homeostasis (Semenza, 2012), as the bone tissue. However, the influenced of hypoxia on bone remodelling is unclear and different mechanisms physiological could mediate in its effects.

Although little is known about the metabolic pathways used by bone cells, the glucose metabolism may play a prominent role during osteoblast differentiation (Li et al., 2016). Even in the presence of oxygen the bone cells metabolize glucose by a phenomenon known as the "Warburg effect" or "aerobic glycolysis" (Dirckx et al., 2018). Hypoxia-regulated transcription activates genes and pathways that reduce oxygen consumption and the cellular dependence on oxygen (Semenza, 2012). HIF mediated upregulation of glycolytic enzymes as pyruvate dehydrogenase kinase 1 (PDK1), lactate dehydrogenase A (LDHA) and glucose

transporters (GLUTs) compensate the energy inefficiency of glycolysis (Dirckx et al., 2018). Thus, hypoxia exposure might enhance bone formation promoting glycolysis as the main metabolic pathway.

On the other hand, HIF regulates bone remodelling involved genes as erythropoietin (EPO) vascular endothelial growth factor (VEGF) and osteoprogeterin (OPG). On account of lower oxygen concentration in the liver and kidney, EPO gene transcription is enhanced by HIF (Forsythe et al., 1996). It is well know that EPO controlled the production of erythrocytes, cells that transported the oxygen through the circulation. Moreover, it has been shown to stimulate bone formation and repair. Even though its role could be associated with increased in angiogenesis, osteoblastogenesis even osteoclastogenesis, your function in skeletal development remains largely unknown (Wu, Glaccia, & Rankin, 2014).

VEGF is a potent mitogen for vascular endothelial cells, which increases its expression in many different cell types in respond to hypoxia (Forsythe et al., 1996). In the bone system, VEGF increases the angiogenesis, promote the osteogenesis (Guner et al., 2013) and represents a key player in coupling of angiogenesis and osteogenesis (Dirckx et al., 2018). Based on working model of osteogenic-angiogenic coupling in trabecular bone (Figure 7), the osteoblast HIFα subunits transcript VEGF expression, assisting osteogenic-angiogenic coupling and trabecular bone formation (Towler & Hardie, 2007). But, bone formation is not dependent solely on osteoblast functions. It requires VEGF-mediated paracrine signals in bone that stimulate angiogenesis (Wang et al., 2007). In this sense, VEGF expands VEGFR2-expressing mesoangioblast numbers during angiogenesis (Cossu & Bianco, 2003), driving the bone formation trough the differentiation of microvascular smooth muscle cells known as pericytes (Esner et al., 2006), which have osteoprogenitors capacity, when placed in the correct microenvironment (Towler & Hardie, 2007). In this way, an increase in osteoblast numbers that promotes massive trabecular bone formation is produced

in the osteogenic marrow environment. Strategies that augment osteoblast HIFα/VEGF signalling (as low oxygen levels) may increase bone formation and enhance fracture healing (Towler & Hardie, 2007).

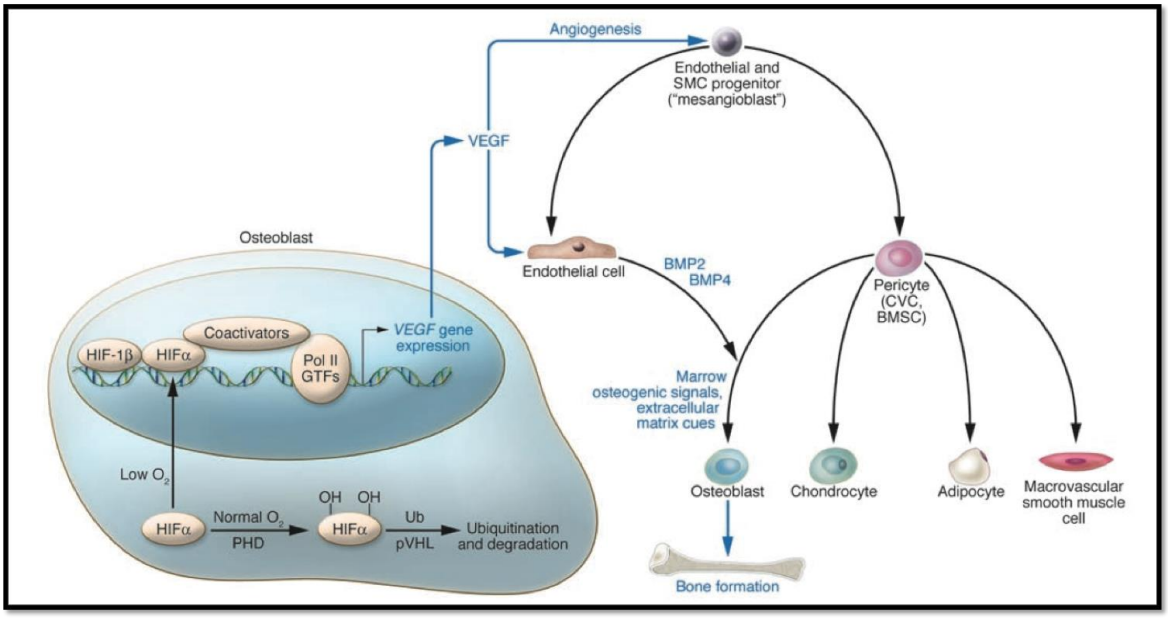

Figure 7 Working model of osteogenic-angiogenic coupling in trabecular bone
Source: (Towler & Hardie, 2007)

OPG has been recognized as a direct transcriptional target of HIF-2 α (Dirckx et al., 2018)It is the factor that inhibits osteoclastogenesis by counteracting Receptor Activator of Nuclear Factor κB Ligand (RANKL) and therefore, the bone reabsorption (Wu et al., 2014).

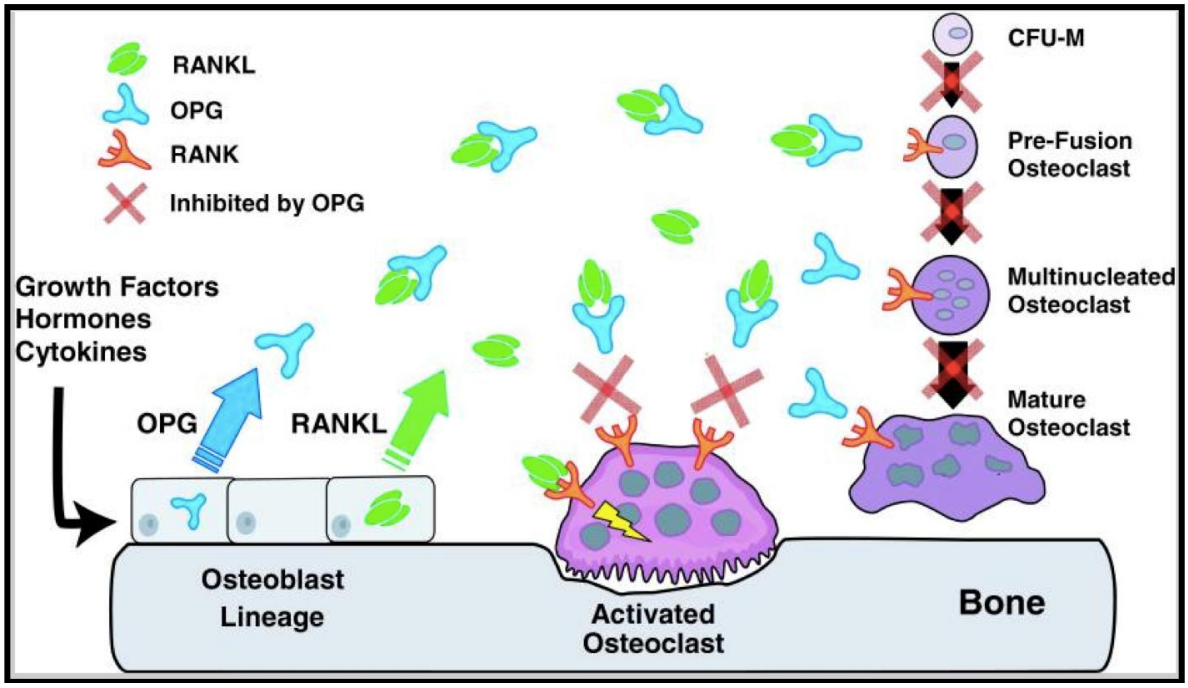

Figure 8 Mechanisms of action for OPG, RANKL and RANK by osteoblasts
Source: (Kearns, Khosla, & Kostenuik, 2008)

In the mid-to-late 1990s was discovered the RANKL/Receptor Activator of Nuclear Factor κB (RANK)/OPG signalling system (Figure 8), which supposed the major advance in the understanding of the regulation of bone modelling and remodelling (Boyce & Xing, 2007). According to this signalling system, under the control of various proresorptive growth factors, hormones and cytokines, osteoblasts and bone marrow stromal cells produce RANK. These cells produce also OPG, which binds to and thereby inactivates RANKL. In the absence of OPG, RANKL activates its receptor RANK and lead to preosteoclast recruitment, fusion into multinucleated osteoclasts, osteoclast activation, and osteoclast survival (Kearns et al., 2008). On the other hand, the hypoxia conditioning could also increase the levels of inflammatory cytokines, which has been shown to interact with RANKL and OPG and play important role in bone remodelling (Guner et al., 2013). As result of increased ROS production, pro-inflammatory cytokines induce the producing of NO in osteoblast and osteoclast (Damoulis & Hauschka, 1994). It is known that NO regulates osteoclast-mediated bone reabsorption, activating osteoblastic activity and inhibiting RANKL expression (Fan et

al., 2006; J. Wang et al., 2008). But NO exerts biphasic effects on bone cell activity; growth and differentiation of osteoblasts are inhibited by high concentrations of NO (Ralston et al., 1995). In this sense, the cytokine-induced release of NO might serve as a control mechanism to avoid excess bone resorption and prevent bone loss (Riancho, Zarrabeitia, Fernandez-Luna, & Gonzalez-Macias, 1995). Therefore, low-dose hypoxia could increase oxidant defence, reinforcing the immune system, while suppressing the production of proinflammatory mediators (Serebrovskaya, Nikolsky, Nikolska, Mallet, & Ishchuk, 2011). As a result of all these mechanisms, hypoxia conditioning may inhibit osteoclastic activity and/or stimulate osteoblastic activity.

1.3.2 Hypoxic Adaptations of neuromuscular system

Like other tissue, during the hypoxic exposure the skeletal muscle homeostasis is challenged (Lundby et al., 2009). One hour of systemic hypoxia is sufficient to increase HIF-1α protein expression in this tissue (Stroka et al., 2001). But even in normoxic condition this protein is highly expressed, suggesting its potential function in muscle homeostasis (Ameln et al., 2005; Pisani & Dechesne, 2005). Therefore, HIF-1α protein expression due to hypoxia could be marginally altered and little additional changes could be leave when the oxygenation is decreased (Lundby et al., 2009). It has been argued that hypoxic stimulus is not sufficient in order to induce adaptations alone, and that hypoxia has to be combined with other stressor, such as exercise (Jackson, Sillau, & Banchero, 1987).

Higher HIF-1α protein expression is the results of combining acute hypoxia and acute exercise (Ameln et al., 2005). This combination was originally studied by restricting blood flow, which showed increase muscle size and strength (Scott, Loenneke, Slattery, & Dascombe, 2015). However, as this strategy can only be applied to limb muscle, different authors begun to apply systemic hypoxia (Ho, Kuo, Liu, Dong, & Tung, 2014; Manimmanakorn, Hamlin, Ross, Taylor, & Manimmanakorn, 2013; Nishimura et al., 2010).

No doubt that the combination of load, sets, repetition of sets, rest and speed of movement are key factors in the physical and functional changes derived from specific training as well as the influence that hypoxia could have (Feriche, Garcia-Ramos, Morales-Artacho, & Padial, 2017). In spite of traditionally the hypoxic training been associated with endurance performance, it could also have advantages responses in the muscle performance.

Muscle strength and structural physiological changes could be achieved when resistance training is combined with hypoxic condition by enhances exercise-induced metabolic stress mechanisms (anabolic hormones, cytokines, reactive oxygen species and oxidative stress factor), which are crucial to muscle growth (Feriche et al., 2017; Ko et al., 2017; Scott, Goods, & Slattery, 2016). Metabolite accumulation in the cells results in a cellular swelling that may increase protein synthesis and decrease protein degradation contributing in net protein accretion and muscle hypertrophy (Loenneke, Wilson, & Wilson, 2010). Large endocrine responses have also been observed following strength training in hypoxia (Kon et al., 2010; Kon, Ikeda, Homma, & Suzuki, 2012; Yan, Lai, Yi, Wang, & Hu, 2016), but its importance for muscle hypertrophy has been questioned (Schoenfeld, 2013). Moreover, the resultant hypoxia-mediated increases in motor unit recruitment could stimulate a larger portion of the muscle (Scott, Slattery, Sculley, Lockhart, & Dascombe, 2017). Therefore, the increase of muscle strength under hypoxic conditions could be mediated by hypertrophic adaptations (Scott, Slattery, Sculley, & Dascombe, 2014) and/or neural adaptations (Inness et al., 2016).

Velocity and power improvements at altitude have also been reported (Hahn & Gore, 2001; Hamlin, Hopkins, & Hollings, 2015; Levine, Stray-Gundersen, & Mehta, 2008). Although the mechanisms are unclear, in response to resistance exercise in hypoxia, the motor unit recruitment patterns are modified due to increase anaerobic metabolism release (Billaut, Gore, & Aughey, 2012). As a result of metabolic stress induced by low oxygen

saturation arterial (SaO_2) a recruitment of fast twitch muscle fibers is show (Manimmanakorn, Manimmanakorn, et al., 2013), making the movement faster due to the intrinsic capacity of larger motor neurons to drive the impulses at higher speeds (Feriche et al., 2017). On the other hand, researchers have used the hypothesis of the approximate 22.9% difference in air density at moderate altitude (Levine et al., 2008) could contribute to making the movement faster than at normal altitudes (Peronnet, Thibault, & Cousineau, 1991). However, more studies are needed to analyse the influence of air pressure and composition.

Strength training under systemic hypoxia also causes a greater increase in skeletal muscle, VEGF and capillarization, which may potentially lead to increased muscular endurance (Kon et al., 2014), but little research has paid attention to the effects of exercise under hypoxia on muscular endurance and muscle fatigue. Therefore, the combined resistance training and hypoxic conditions could result in a large range of functional adaptations in skeletal muscle (Lundby et al., 2009).

1.3.3 Hypoxic conditioning as therapeutic strategy in elderly population

Therapeutic benefits of hypoxic training have been suggested for clinical populations, such as the elderly who could suffer musculoskeletal impairments (Millet et al., 2016). As was above-mentioned, a hypoxic session supposes a lower stress on locomotors systems for a similar physiological strain than in normoxia (Pramsohler et al., 2017). In this sense, hypoxic training may contribute significantly to improvements in exercise capacity of elderly adults up to 92 years, being a safe and non-invasive strategy (Bayer et al., 2017).

However, fewer studies to date have applied this innovative therapeutic strategy in old adults. Schega (2013) were the first supplying hypoxic training before a strength endurance exercise program to a group of elderly adults. They augmented a positive effect on quality of life in the elderly, but the 18 sessions of this treatment were not enough to improve muscle

strength. Later Bayer (2017) studied the effects on cognitive function and functional exercise in geriatric patients suffering from mild-to-moderate dementia with a mean age of more than 80 years old. Theirs short-term protocol did not result in improved cognitive function neither functional exercise capacity. Pramsohler (2017) applied an endurance training protocol in normobaric hypoxia with geriatric patients and an increase in maximum power output was shown.

1.4 Physiological mechanisms of Whole-body Vibration Training

In spite of the vibration has traditionally been regarded as only detrimental (Rittweger, 2010), the relevance of mechanical vibration for human being is well documented (Bemben, Stark, Taiar, & Bernardo-Filho, 2018). Transmission of mechanical vibrations from the environment to body of healthy subject occurs during simple daily activities (such as walking or running), the practice of different sports or even in some occupational activities (Cardinale & Wakeling, 2005; Du et al., 2018; Szczepaniak, Tanas, & Kromulski, 2014; Tarabini, Saggin, & Scaccabarozzi, 2015). Energy delivered during the vibrations is transmitted to the body through different biological systems, such as muscles, bones, cartilages, synovial fluids and joints with expected and unexpected consequences (Bemben et al., 2018). But if an individual cannot exposure naturally at these vibrations (individuals with disability and/or disease), a vibration platform could generate the mechanical vibrations to be transmitted to the body (Rittweger, 2010). In the past, few authors have postulated therapeutic effects by vibration stimuli. The first were Sanders (1936) and Whedon, Deitrick, & Shorr (1949), who used oscillating bed to improve cardiovascular and musculoskeletal systems. Later, Nazarov & Spivak (1985) begun to apply vibration to improve the athletic performance. In this moment, the scientific literature begins to interest in this emerging exercise modality and different companies stared to commercialize devices. It is becoming popular in health and fitness clubs as an alternative training method (Delecluse, Roelants, & Verschueren, 2003).

As defined Rittweger (2010), in the physical sense, the vibration is a forced oscillation, where energy is transferred from a vibration device to the human body or parts of it. Commonly the subject is standing on oscillating platform, which could be of two different types based on how the energy is transferred to the individual. Some models apply the

vibration to the right and the left foot in a side-alternating way. On other vibration platforms, the right and the left foot move up and down at the same time (Figure 9).

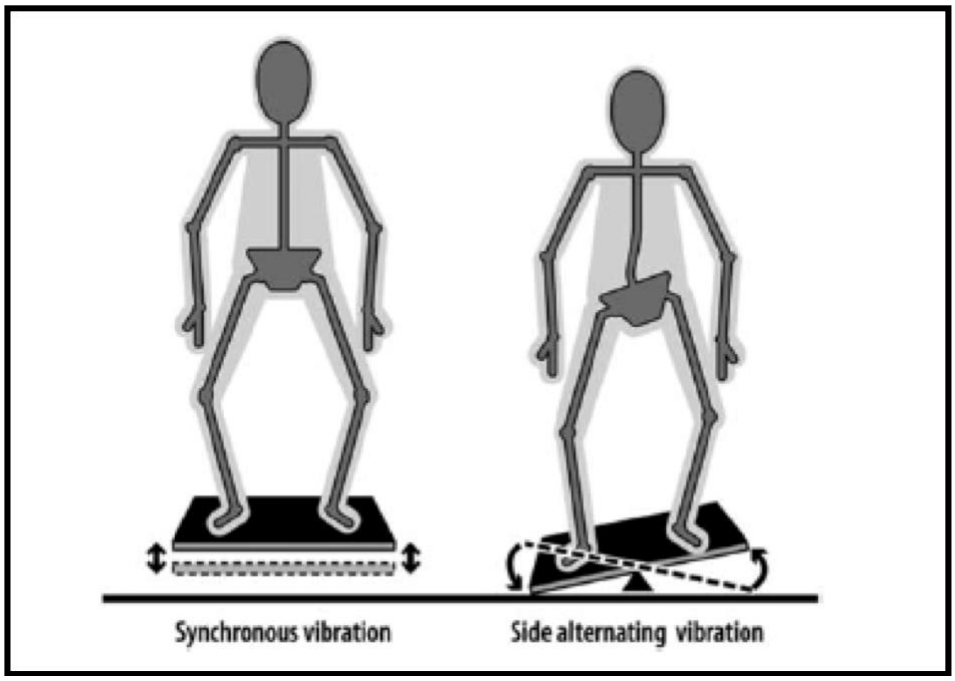

Figure 9 Modes of vibration transmit from two different vibrations platforms to body
Source: (Rittweger, 2010)

In the scientific literature, as recommend the International Society of Musculoskeletal and Neuronal Interactions (Rauch et al., 2010), it is necessary to provide information on frequency and the extent of the vibration. Regarding to extent of the vibration is important to difference between two concepts: peak-to-peak displacement, that expressed the displacement from the lowest to the highest point; or amplitude; that expressed the maximum displacement from equilibrium. In WBV treatment literature these terms are usually confused and it is preferable to use the term "peak-to-peak displacement" to indicate the extent of the vibration. Furthermore, it is recommended to provide also the acceleration levels associated with the vibration, defined as the peak acceleration in multiples of Earth's gravity. This term could be useful to compare between findings of different scientific studies.

1.4.1 Effects of Whole-Body Vibration Training on bone strength

Application of vibration for enhancement of bone strength could be justified by different theories (Rittweger, 2010). Firstly, the vibration could be as a tool to enhance bone

strength through the muscle contraction. Mechanical vibrations generated the contraction of the muscle, which causes the greatest forces that load our bones and accordingly our bone have to adapt to these forces (Schonau, Schwahn, & Rauch, 2002). In this sense, as the skeletal system seems to adapt to the current requirements it might be possible that a positive influence on bone can be achieved through functional muscle activation (Bemben et al., 2018). On the other hand, mechanical stimulation generated during WBV training, could be anabolic to bone (Zaki, 2014). It could create a fluid movement in the bone's structural network, which in turn generates shear stresses on the plasma membranes of resident osteocytes, bone lining cells, and osteoblasts, favoring the bone remodeling (Rubin et al., 2004, 2002).

Regarding to scientific investigations, several authors reported conflicting finding, which may be attribute to the variety of protocols used (Marin-Puyalto et al., 2018). In postmenopausal women and elderly population, only longest training durations to lead changes in BMD (Gomez-Cabello et al., 2012; C. Ma, Liu, Sun, Zhu, & Wu, 2016). High-magnitude (>1 g) and low frequencies (<20) could be the most effective to improve lumbar spine and hip BMD outcomes (Bemben et al., 2018) of postmenopausal women.

1.4.2 Effects of Whole-Body Vibration Training on neuromuscular system

Although not fully understood, it has been proposed that various neural and endocrine mechanisms have been implicated in WBV-induced increased muscle activity (Chen, Ma, Lu, & Ma, 2017). Most frequently cited mechanism underpinning the WBV response is a reflex muscular contraction termed the tonic vibration reflex. In this sense, the mechanical stimuli caused by the vibration stimulates the muscle spindles, sensory receptors that activation of the alpha-motoneurons (Couto et al., 2013; Zaidell, Mileva, Sumners, & Bowtell, 2013). As a result, a sequence of rapid muscle stretching is produced, which described as "tonic vibration reflex" (Hagbarth & Eklund, 1966). Other mechanisms of improved muscle function

following vibration include enhanced corticospinal excitability and intracortical processes (Pamukoff, Ryan, & Blackburn, 2014). Vibration produces also a sharp increase in blood levels of hormones such as testosterone and growth hormone (Bosco et al., 2000), which may explain the changes in lean mass after vibration training (Chen et al., 2017). A wide scientific literature has investigated the effects of WBV training on muscle strength/power in older people. WBV training might be useful for counteracting the loos of muscle strength and mobility associated with sarcopenia in older adults (Cristi, Collado, Marquez, Garatachea, & Cuevas, 2014; Machado, Garcia-Lopez, Gonzalez-Gallego, & Garatachea, 2010). Moreover, its interventions are beneficial on risk and incidence of falls because the balance and leg strength are improved following WBV training (Smith et al., 2016).

1.4.3 Whole-Body Vibration Training as therapeutic strategy in elderly population

Various beneficial effects have been associated from children to elderly individuals or even individuals with chronic diseases following to WBV training (Bemben et al., 2018). Increases in muscular strength/power, flexibility and gait speed, improvements in bone mineral density (BMD), balance, blood flow, cognition/executive functions, and quality life or decreased pain and risk of falls have been some of these beneficial effects, with side effects associated it (Nawayseh, 2018; Prisby, Lafage-Proust, Malaval, Belli, & Vico, 2008; Regterschot et al., 2014; Rittweger, 2010). A recent narrative review, which aimed to present the importance of the WBV training for the elderly individuals, shown as could be a suitable and efficient strategy with reduced cost for the management of several unhealthy age-related conditions (Bemben et al., 2018).

However, on the bone system, 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 in this population (Gomez-Cabello et al., 2012; C. Ma et al., 2016). Furthermore, the vibration effect does not affect significantly the upper body region and benefits have only

been found in the hip or lower body (Marin-Puyalto et al., 2018). Currently, there is a great challenge for future investigations to find a suitable training-protocol, which provokes the expected beneficial adaptations for such population. At our knowledge, there are not in the scientific literature investigations that had studied the combined effects of WBV training and HC.

Chapter 2. OBJECTIVES

Chapter 2. OBJECTIVES

Due to above exposed, this project contemplated the three general aims, dividing the last one in different specific objectives:

1 Reviewing current literature of bone metabolism and hypoxic stimulus.

2 To study the effects of passive hypoxia conditioning on bone mineral density of healthy elderly people.

3 To examined the effects of active hypoxia conditioning on healthy parameters of healthy elderly people.

3.1 To explore the combined effects of whole-body vibration training and hypoxia conditioning on bone mineral density of healthy elderly people.

3.2 To explore the combined effects of whole-body vibration training and hypoxia conditioning on muscle mass density of healthy elderly people.

3.3 To explore the combined effects of whole-body vibration training and hypoxia conditioning on muscle strength of healthy elderly people.

3.4 To explore the combined effects of whole-body vibration training and hypoxia conditioning on functional mobility of healthy elderly people.

Chapter 3. METHODS

Chapter 3. METHODS

3.1 Systematic Review

Regarding of first objective of this thesis, a systematic review was carried out following the Preferred Reporting Item for Systematic Reviews and Meta-Analyses (PRISMA) methodology (Liberati et al., 2009). The well-known electronic databases selected were Web of Science and PubMed. The selected articles were original articles published between 1900 and 2019. The search terms used were: "hypoxi*", "altitude", "oxygen deprivation", "bone remodelling", "bone metabolism", "osteog*", "bone tissue remodelling", "bone mineral density" "growth*" "pulmonary", "cancer" and "tumour". The search was finalized on the 1st of March 2019. Duplicate articles were deleted.

Inclusion and exclusion criteria that were defined for article selection were: (1) to be written in English; (2) to be an experimental study on bone remodelling parameters; (3) to have a sample of healthy organisms; (4) to apply a hypoxia treatment (modification of ambient oxygen). Studies were excluded if they were only presented once as a conference, congress or seminar. These criteria were evaluated firstly by the titles and abstracts. If they met the inclusion criteria or if the title and abstract did not provide enough information, full articles of these studies were obtained to apply the criteria to the full text.

Internal quality of each study was assessed using the Office of Health Assessment and Translation (OHAT) Risk of Bias Rating Tool (NTP, 2015). Eleven risk-of-bias questions using a 4-point scale ranging from low and high risk-of-bias options were assessed. For both in vivo and in vitro studies, an initial confidence rating was given, which is subsequently downgraded or upgraded according to factors that decrease or increase confidence in the results (Matta et al., 2019; Runkle, Flocks, Economos, & Dunlop, 2017)

In order to establish a credible link between hypoxic exposure and bone health effect, confidence ratings were assigned to individual study designs and were translated into a level

of evidence ("high", "moderate," "low," "evidence of no health effect," and "inadequate evidence").

Details were extracted regarding a: (a) sample: type (i.e. human or animal or cell), age and sample size; (b) study design: conditions, exposure type or timing (i.e. normobaric or hypobaric; expansion, proliferation or differentiation); protocol (i.e. sustained, cyclic or intermittent) and duration, frequency and hypoxic level; (c) effects of hypoxia on bone outcomes.

3.2 Participants

The inclusion criteria were: women and men aged 65 years or more; absence of participation in any other type of intervention based on physical exercise in last 6 months; absence of have been above 1500 m during the last 3 months; the ability to follow the protocol; free of disease or medication known to affect bone metabolism; estimated daily calcium intake of 500-600 mg/day, non-smoker; consumption of no more than four alcoholic beverages per week. The exclusion criteria will be mainly based on contraindications for WBV (severe cardiovascular diseases, ocular diseases that affect the retina, neuromuscular and heart diseases, stroke, implant, bypass, stent, arthritis and other joint disease or epilepsy) or a frequency of participation in the stipulated program lower than 80% (participants who missed more than 20% of training sessions were excluded).

The participants were treated in accordance with the agreements of the Declaration of Helsinki and Bioethical and Biosecurity Commission of University of Extremadura approved the project (17/2016, see Appendix C).

3.2.1 Passive Hypoxia (objective 2)

Letters done the disclosure of this project and verbal communication requesting the voluntary participation of the old adults engaged in the Senior Universities and pensioners associations of Cáceres (Spain). All the volunteers (n=32) were informed about the study procedures. Eligible volunteers, which requested to sign a declaration that voluntarily consent to participate in this research (Appendix B), were randomly divided into groups: (1) Hypoxia group (HYP) received exposure to normobaric hypoxia, and (2) Control group was instructed to continue with their normal daily activities for the study development. Figure 10 shows the flow of participants through each stage of the trial.

3.2.2 Active Hypoxia (objective 3)

Letters done the disclosure of this project and verbal communication requesting the voluntary participation of the old adults engaged in the Senior Universities and pensioners associations of Évora (Portugal). All the volunteers (n=46) were informed about the study procedures. Eligible volunteers, were requested to sign a declaration that voluntarily consent to participate in this study (Appendix B), were randomly divided into groups: (1) Hypoxia Whole Body Vibration group (HWBV) which performed whole body vibration treatment under normobaric hypoxic conditions, (2) Normoxic Whole Body Vibration group (NWBV) which performed whole body vibration treatment under normoxic conditions, and (3) Control group which was instructed to continue with their normal daily activities for the study development. Figure 10 shows the flow of participants through each stage of the trial.

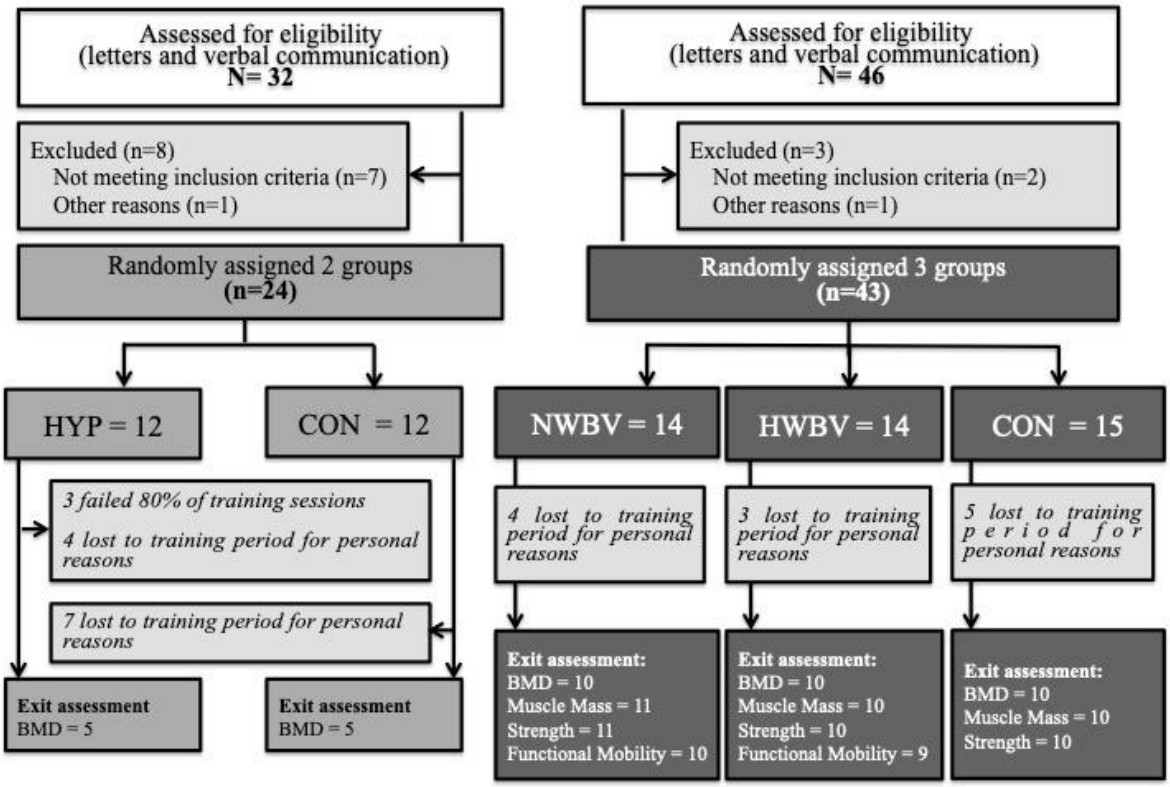

Figure 10 Flow of participants through each stage of the trial
HYP: hypoxia group; CON: control groups; BMD: bone mineral density; NWBV: Normoxia Whole Body Vibration; HWBV: Hypoxia Whole Body

3.3 Intervention Protocols

During 36 weeks the volunteers completed the intervention. The frequency of participation was twice a week; sessions were scheduled with at least one day of rest in between for optimal recovery and participants were requested to participate at the same time throughout the 36 sessions. All patients were assessed at two time points: at baseline (PRE) and reassessed after intervention (POST). During the protocol, participants were instructed to maintain theirs daily calcium intake and the absence of participation in any other type of intervention based on physical exercise and to stay 1,500 m above the sea level.

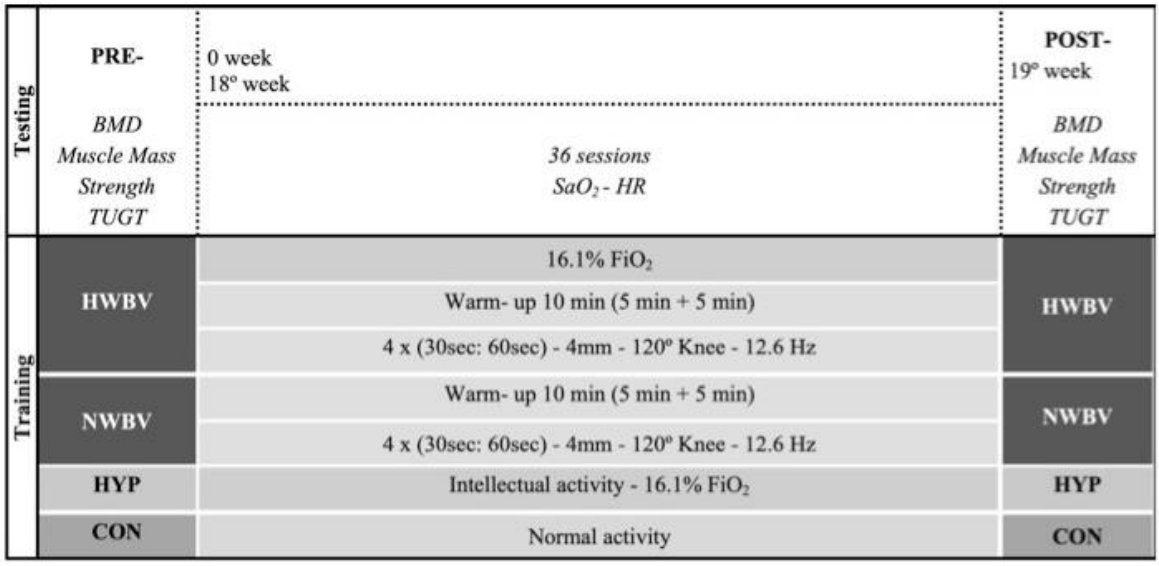

Figure 11 Study design
PRE-: baseline; POST-: reassessed after intervention; BMD: bone mineral density; TUGT: Time Up and Go Test; SaO_2: arterial oxygen saturation; HR: hear rate; HWBV: Hypoxia Whole Body Vibration group; NWBV: Normoxic Whole Body Vibration group; HYP: Hypoxia group; CON: Control groups.

3.3.1 Passive Hypoxia (objective 2)

During 16 minutes of session, HYP group performed an intellectual activity while they were exposed to normobaric hypoxic conditions in a hypoxic chamber (CAT 310, Lousiville, Colorado). They inspired oxygen fraction (FiO_2) set to 16.1% in order to simulate an altitude of 2,500m above the sea level. FiO_2 was controlled regularly with an electronic device (HANDI+; Maxtec, Salt Lake City, UT).

During several times, SaO_2 was controlled using a finger pulse-oximeter (Konica Minolta, Japan) and heart rate (HR) using a heart rate monitor (Polar team 2, Polar, Finland) to know the physiological challenge posed on the participants during the exposure. These parameters were measured in the second, fifth, sixth and eleventh minutes of exposure.

3.3.2 Active Hypoxia (objective 3)

Participants that performed the vibration exercise were placed in a standing position with feet side by side on a specific landmark on the board, barefoot to eliminate any damping of the vibration caused by footwear. The angle of flexion of the knee during the vibration exercise was set at 120º. They were allowed to hold the vibration platform with their hands for postural control. The vibration stimulus (12.6 Hz) was produced by a sinusoidal vibration platform (Galileo 2000; Novotec GmbH, Pforzheim, Germany) and the distant position formed by the axis of rotation was 4mm peak-to-peak. The effective acceleration was 2.54 g force (g represents the Earth's gravitational acceleration at 9.81 m/s^2). Subjects performed four sets of 30 seconds of vibration per session, separated by 60 seconds of rest. The total duration of the training session was about 16 minutes, which included a 10-minute warm-up with 5 minutes bicycling at 25–50W and 40–50 rpm and another 5 minutes of stretching exercises.

NWBV and HWBV groups performed the vibration exercise in a hypoxia chamber (CAT 310, Louisville, CO) placed in the laboratory. For safety reasons, the FiO_2 in the HWBV group was set at 16.1% to simulate an altitude of 2,500m above sea level. FiO_2 was controlled regularly with an electronic device (HANDI+; Maxtec, Salt Lake City, UT). To blind subjects to altitude, the system was also run for the NWBV group with normoxic airflow into the chamber (up to 1000 L/min) and produced the same audible noise as in the hypoxic condition. NWBV subjects inspired a FiO_2 of 21.0% to simulate an altitude of 459m

above sea level. Furthermore, all systems were covered with fabric to prevent participants from visually identifying the normoxic or hypoxic conditions.

During several times, SaO_2 was controlled using a finger pulse-oximeter (Konica Minolta, Japan) and heart rate (HR) using a heart rate monitor (Polar team 2, Polar, Finland) to know the physiological challenge posed on the participants during the exposure. These parameters were measured coinciding with the second minute of warm-up (second minute), the start (fifth minute), the second and third set (sixth minute) and the finish (eleventh minute) of whole-body vibration protocol.

3.4 Testing Protocols

Bone Mineral Density Assessment

BMD values from the whole body and right proximal femur (femur total, trochanter and intertrochanteric region) were assessed using DXA (Norland Excell Plus; Norland Inc., Fort Atkinson, USA). The same experienced technician performed all the scans. BMD was expressed in $g \cdot cm^{-2}$ and as the difference, expressed as standard deviations (SD), from the normal average value of peak bone mass at a participant's age. T-scores were calculated using the National Health and Nutrition Examination Study III BMD norms, as recommended by the WHO for the diagnosis of osteoporosis in clinical practice (Looker, Melton 3rd, Borrud, & Shepherd, 2012).

Body Composition

Body composition variables (percentage fat mass) were obtained using DXA (Norland Excell Plus; Norland Inc., Fort Atkinson, USA). The same experienced technician performed all the scans. Height (0.1 cm, SECA 769; seca gmbh & co.kg, Hamburg, Germany) and body mass (0.1 kg, SECA 769; seca gmbh & co.kg) were measured through recommended standardised techniques. Body mass index (BMI) was determined per the accepted method ($BMI = weight/height^2$, $kg \cdot m^{-2}$).

Lifestyle Questionnaires

A general questionnaire was administered to collect medical and demographic data to check the inclusion/exclusion criteria. Furthermore, prior and after the intervention, dietary intake was estimated using a food frequency questionnaire and physical activity level using the bone-specific physical activity questionnaire (BPAQ), that were considered as control variables. In BPAQ, the respondents will record type, frequency and years of physical activity involvement to know effects of mechanical loading on the skeleton (Weeks & Beck, 2008).

3.4.1 Active Hypoxia (objective 3)

Muscle Strength

Isokinetic leg muscle strength was evaluated using a Biodex isokinetic dynamometer (System-3; Biodex, NY) by performing unilateral tests in the right leg. Each subject was attached to the seat of the dynamometer so that the axis of their knee coincided with the axis of the dynamometer following standardised protocols. The procedure of the test was explained to all subjects before placing special emphasis on exerting maximal effort within each individual's tolerance threshold. At the start of each single test, the subject was asked to relax his/her leg to determine the effect of gravity on the limb. Testing was performed using a hard deceleration cushion. The motion ranged from 80º of knee flexion to full extension. Participants rested for 2 minutes between each trial. First, the maximal strength test was measured by performing a three-repetition unilateral test of knee extension (Ext) - flexion (Flex) (concentric/concentric) at 60º/s. To analyse the strength balance between the hamstrings (H) and quadriceps (Q), the H/Q ratio was calculated as the quotient between peak torque (PT) values of the Flex and the Ext (PTFlex / PTExt). Endurance strength was evaluated through a 20-repetition unilateral test of knee Ext-Flex (concentric/concentric) at 180º/s. Mean work (W) was calculated from the recorded force curves of these series of

exercises for each direction of movement. Muscle fatigue was determined in terms of the relative change in mechanical between the first and the last third of the repetitions: [(Wfirst – Wlast) / Wfirst] · 100.

Functional Mobility

Timed Up and Go Test (TUGT) was used to assess the participants' functional capacity. A chair with a seat height of 46 cm was used. Participants stood up from the chair, walked to the mark at 3 m, turned around, returned to the chair and sat down. The instructions given were "Walk as quickly and safely as possible to the marked line, turn through 180 degrees, walk back to the chair, and sit down again". After verbal instructions, the participant performed the trial. The starting command was "Get ready, go!" The time spent task accomplishment was measured.

3.5 Statistical Analysis

Statistical analyses were performed using the statistical analysis package SPSS v.20 for MAC (IBM, New York, USA). Standard statistical methods were used for the calculation of descriptive statistics. Compliance training was calculated as the number of sessions completed divided by the 36 possible sessions available per participant. Kolmogorov–Smirnov tests were conducted to show the distribution of the studied variables and Levene's test for homogeneity of variance. Based on this distribution and homogeneity of variance, parametric or nor parametric test were used. Chi-squared tests and one-way analysis of variance (ANOVA) were used to determine whether differences existed between groups in baseline.

3.5.1 Passive Hypoxia (objective 2)

To know the effects of passive hypoxia on bone mineral density, the statistical significance of the difference for paired samples was estimated with Wilcoxon test; for the difference between independent samples a Mann–Whitney test was performed. The effect

size (Cohen, 1992) was calculated for all variables between baseline and after 18 weeks of intervention. The magnitude of change considered was trivial (<0.5), small (0.5-1.25), moderate (1.26-1.99) or large (>2.00) (Rhea, 2004).

Additionally, absolute change and the percentage change from pre- to post-test were calculated for some variables for each group. Effects of the different interventions on bone mineral density parameters were evaluated by pre-specifying the minimum detectable change (MDC). The MDC is an absolute measure of reliability (measurement error), which accounts for various sources of variability in defining a confidence interval in units of the measure. These values are being increasingly used to assist in interpreting results and determining whether a change between repeated tests is random variation or a true change in performance (Haley & Fragala-Pinkham, 2006). An effect was considered relevant when the change was greater than MDC.

The $p<0.05$ criterion was used for establishing statistical significance.

3.5.2 Active Hypoxia (objective 3)

To know the changes of whole body vibration combined with normobaric hypoxia on bone mineral density and muscular parameters a mixed factorial ANOVA with Bonferroni post hoc tests were used to investigate the main effects and the interaction between group-factor (control vs. normoxia vs. hypoxia) and time-factor (pre-training vs. post-training), considering the sex as covariable. On the other hand, the statistical significance of the difference for paired samples was estimated with Wilcoxon test and for the difference between independent samples a Mann–Whitney test was performed to know the effects of active hypoxia on functional mobility,

The effect size was calculated for all variables between baseline and after 18 weeks of intervention. The magnitude of change considered was trivial (<0.5), small (0.5-1.25), moderate (1.26-1.99) or large (>2.00).

Additionally, absolute change and the percentage change from pre- to post-test were calculated for some variables for each group. Effects of the different interventions on bone mineral density parameters were evaluated by pre-specifying MDC. An effect was considered relevant when the change was greater than MDC.

The $p<0.05$ criterion was used for establishing statistical significance.

Chapter 4. RESULTS

Chapter 4. RESULTS

4.1 Systematic Review

The initial electronic database search resulted in a total of 39 citations in Web of Science and 236 in PubMed. After deleting 22 duplicates, title and abstract of 253 studies were analysed. A total of 167 studies were excluded and 86 potentially relevant studies were selected for full-text review. Fifty-two studies were identified in our systematic search from which risk of bias was described and data extracted. A flowchart of the search procedure can be found in the Figure 12.

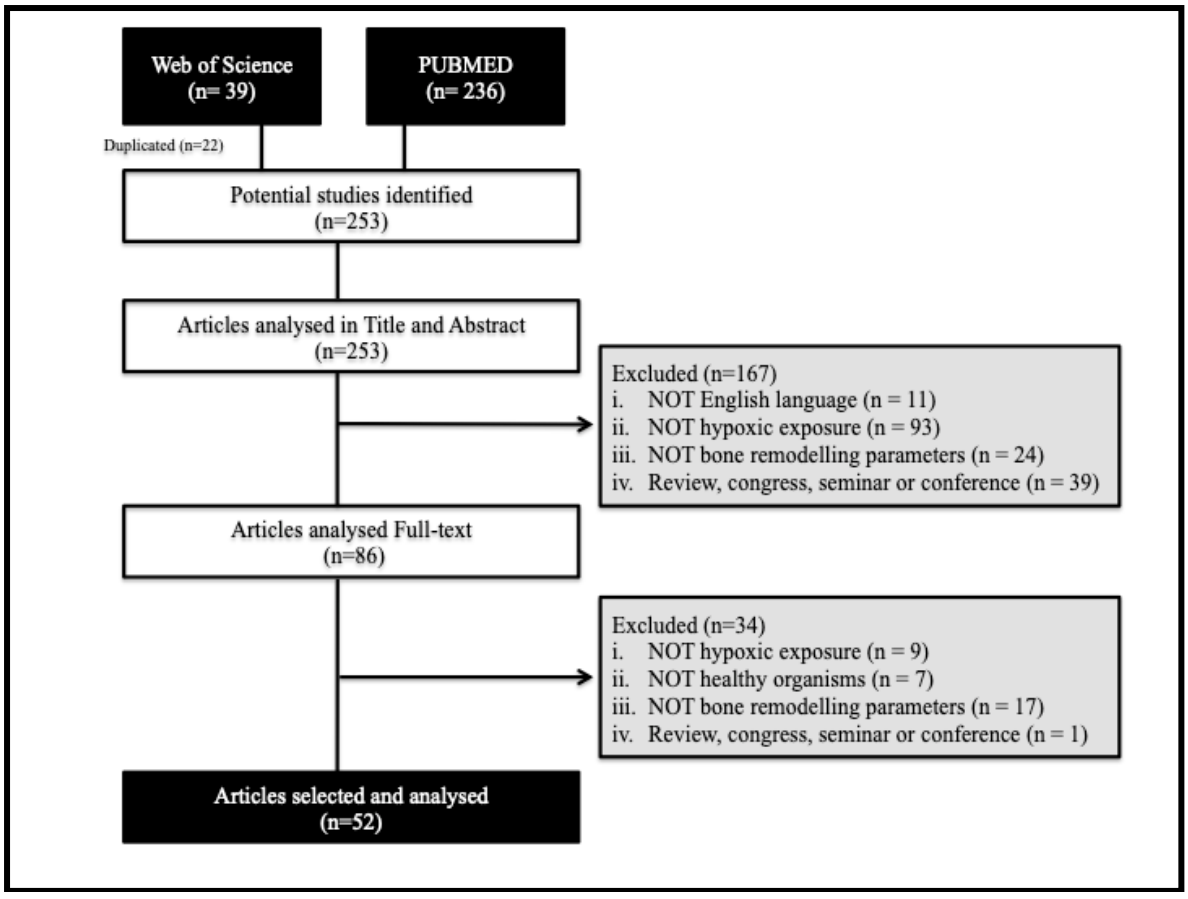

Figure 12 Flowchart of article searches and selection strategies of the systematic review

Results from the risk of bias assessment are shown in Appendix D and E. Evidence summarised from animal, human and in vitro studies suggested a range of likely high to definitely low level of confidence (see Table 1). Selective reporting was considered the most pertinent domain, rating from likely low and definitely low for animal and human in vitro

studies. Conversely, performance domain was identified at likely high or low level of confidence. This domain was likely high for all human studies.

Table 1. Summary risk of bias domain assessment for animal, human an in vitro studies

	Animal Studies			Human Studies			In vitro Studies		
Selection	++	+	-	+		-	++	+	-
Performance		+	-		-		++	+	-
Attrition/exclusion	++		-	++	+	-		+	-
Detection	++		+	++	+	-	++	+	-
Selective Reporting	++		+		++	+		++	+

++ definitely low (black colour)
+ probably low (light grey colour)
- probably high (not report; white colour)

In the association between hypoxic exposure and bone health effect (Table 2), moderate confidence ratings were assigned to epidemiological studies (human cohort and cross-sectional) whereas high confidence was established in experimental studies (in vitro, animal or human).

Table 2. Confidence rating for a health effect given strengths and weaknesses of a collection of animal and human studies

Type of study	*Level of confidence for health effect Bone Remodelling*
Experimental Animal	High
Human Controlled Trial	High
Human Cohort	High
Human Cross-Sectional	High
In vitro Studies	Moderate

++ ++ High confidence in the association between exposure to the substance and the outcome. The true effect is highly likely to be reflected in the apparent relationship

+ ++ Moderate confidence in the association between exposure to the substance and the outcome. The true effect may be reflected in the apparent relationship

++ Low confidence in the association between exposure to the substance and the outcome. The true effect may be different from the apparent relationship

+ Very low confidence in the association between exposure to the substance and the outcome. The true effect is highly likely to be different from the apparent relationship

Tables 3 and 4 summarise the data from 39 in vitro and 13 in vivo studies. Table 3 shows the results of the extraction data of the in vitro studies. Experimental details from the in vivo studies can be found in Table 4.

4.1.1 In Vitro Studies

Thirty-nine studies analysed the effect of sustained or cyclic hypoxia exposure on bone remodelling parameters in different cell models from animals (14 studies) or humans (34 studies). Most commonly used stem cells models were: bone marrow stromal cells (BMSCs; n = 26), adipose-derived stromal cells (ASCs; n = 8), placental mesenchymal stem cells (pMSCs; n = 1), tendon-derived stem cells (TDSCs; n = 1), umbilical cord perivascular cells (UCPVCs; n = 1) and cartilage endplate stem cells (CESCs; n = 1). Only three of the in vitro studies reviewed exposed osteoblasts or osteocytes to sustained hypoxia (Bouvard et al., 2014; H. P. Ma et al., 2014; A M Zahm, Bucaro, Srinivas, Shapiro, & Adams, 2008). Thirty-two studies included in this review applied sustained hypoxia protocols. Sustained hypoxia was administered from one to 28 days throughout the different timelines of cellular differentiation (expansion, n = 16; proliferation, n = 1; and differentiation, n = 27). Related to the hypoxia level, the dose ranged from 0.1 to 7% Inspired Partial Pressure of Oxygen (PiO_2). Severe hypoxia (<3% PiO_2) was applied more often (n = 33) than moderate hypoxia (>3% PiO_2; n = 11). Only one study applied cyclic hypoxia (two bouts of 3 min per day) over the course of 15 days at 1, 3, 5 and 10% PiO_2 in human BMSCs (D'Ippolito, Diabira, Howard, Roos, & Schiller, 2006). Runt-related transcription factor 2 (RUNX2; n = 15), alkaline phosphatase (ALP; n = 11), collagen type 1 alpha 1 (COL1A1; n = 6), osteopontin (OPN; n = 6) and osteocalcin (OC; n = 11) were the most studied genes. In terms of protein expression, RUNX2 (n = 5) and ALP (n = 14) were the predominant parameters. Mineralisation capacity was assessed using ALP activity (n = 17) and calcium deposits (n = 23).

4.1.2 In Vivo Studies

Three studies carried out in animal models implemented two different types of hypoxic exposure: sustained and cyclic hypoxia. All interventions involved daily exposure with a hypoxic level between 3,000 and 6,000 m over a prolonged period from 2 to 5 weeks (Guner et al., 2013; G. Wang et al., 2016; Y. Wang et al., 2012). Cyclic protocols adopted a pattern of exposure to hypoxia followed by the same time course of exposure to normoxia, lasting 4 h (G. Wang et al., 2016), and 5 h (Guner et al., 2013) per day. Six out of the ten studies in humans, included in this review, analysed the effect of sustained or cyclic hypoxia in healthy active (Basu et al., 2013, 2014; Martinez-Guardado et al., 2019; O'Brien et al., 2018; Ramos-Campo, Rubio Arias, & Jimenez Diaz, 2015) or sedentary adults (Rittweger et al., 2016). Three studies (Basu et al., 2013, 2014; O'Brien et al., 2018) implemented sustained hypoxia corresponding to periods of 16, 24 and 60 weeks. The hypoxic level during these stays ranged from 2,500 (moderate level) to 6,700 m (severe level). Whereas Martinez-Guardado (2019) and Ramos-Campo (2015) studied the effects after 7 and 8 weeks, respectively, of normobaric cyclic hypoxia training at 15% PiO_2 (2 days per week; 60 min per session) and Rittweger (2016) applied bed rest or ambulatory normobaric hypoxia during 21 days at 4,000 m of simulated altitude. Finally, four observational human studies analysed the effect of OSAS level (namely intermittent hypoxia) on bone remodelling parameters (Sforza, Thomas, Barthelemy, Collet, & Roche, 2013; Terzi & Yilmaz, 2016; Tng et al., 2018; Tomiyama et al., 2008). Healthy bone was evaluated with different parameters: bone volume (n = 2); trabecular number (n = 2); whole body BMD (n = 6) or spine (n = 3); speed of sound (SOS; n = 2) values of the radius, metatarsal and phalanx; and T-score of the radius and phalanx (n = 2). Additionally, bone formation and resorption markers as well as bone specific alkaline phosphatase (B-ALP; n = 2), ALP (n = 2), 25-hydroxy vitamin D3 (25-Vit D; n = 2), parathyroid hormone (PTH; n = 2), C-terminal propeptide of type I collagen

(CICP; n = 2), N-telopeptide of type I collagen (NTX; n = 2), C-terminal telopeptide (CTX; n = 2), urinary DPD (n = 2), creatine ratio (DPD/Cr; n = 2) and OPG (n = 1) were analysed to determine the effects of different hypoxia modes.

Table 3. Experimental details of in vitro studies included in the systematic review

Hypoxia Effects on Outcomes	Hypoxia Level (% PiO_2)	Duration, Frequency	References	Confidence Rating
Sustained Exposure				
↑RUNX2-g ↑ALP-g ↑ALP ↑ALP-activity ↑COL1A1-g ↑COL1A1 ↑Osteocalcin-g ↑Osteocalcin ↑Calcium deposit	**0.1%**	1 days	Huang et al. (2012)	Moderate
	1%	2 days	Kalinina et al. (2015)	Moderate
		12 days	Deschepper et al. (2011)	Moderate
		21 days	Gao, Ding, Xie, & Zhang (2013) Ding et al. (2014)	Moderate High
	2%	NR	Lee & Kemp (2006) Burian et al. (2017)	Moderate Low
		1 day	Bouvard et al. (2014)	Moderate
		2 days	Zhang et al. (2018)	Low
		3 days	Salamanna et al. (2018)	Low
		12 days	Ciapetti et al. (2016)	High
		14 days	Tsang et al. (2013)	Low
		21 days	Tsang et al. (2013)	Low
	5%	3 days	Gu, Gu, Shi, & Yang (2016)	Moderate
		14 days	Ding et al. (2014)	High
		49 days	Sengupta et al. (2010)	Low

↑RUNX2-g ↑ALP-g ↑ALP ↑ALP-activity ↑COL1A1-g ↑COL1A1 ↑Osteocalcin ↑Osteopontin-g ↑Osteopontin ↑Calcium deposit	**1%**	14 days	Jin et al. (2010)	Low
		21 days	Binder, Sagun, & Leach (2015) Ding et al. (2014)	Moderate High
	2%	5 days	Xu et al. (2007)	Moderate
		8 days	Xu et al. (2007)	Moderate
		12 days	Ciapetti et al. (2016)	High
		14 days	Tsang et al. (2013) Zhang et al. (2017)	Low Moderate
		21 days	Tsang et al. (2013) Lee, Lui, & Rui (2012)	Low Moderate
	3%	14 days	Holzwarth et al. (2010)	Low
	5%	NR	Russo, Yu, Belliveau, Hamilton, & Flynn (2014)	Moderate
		3 days	Gu, Gu, Shi, & Yang (2016)	Moderate
		21 days	Binder, Sagun, & Leach (2015)	Moderate
		49 days	Sengupta et al. (2010)	Low
	7%	NR	Iacono et al. (2018)	Low
	1%	NR	Lee et al. (2015) Hsu, Chen, & Wei (2013) Park et al. (2013)	Moderate Low Moderate
		2 days	Ma et al. (2014)	Moderate

↓ RUNX2-g **↓ RUNX22** **↓ ALP-g** **↓ ALP** **↓ ALP-activity** **↓ COL1A1-g** **↓ COL1A1** **↓ Osteocalcin-g** **↓ Osteocalcin** **↓ Osteopontin-g** **↓ Osteopontin** **↓ Calcium deposit**		21 days	Ding et al. (2014) Yao et al. (2017) Xu et al. (2013) Yang et al. (2011) Cicione et al. (2013)	High Low Moderate Low Moderate
	2%	NR	Burian et al. (2017)	Low
		3 days	Salamanna et al. (2018)	Low
		5 days	Xu et al. (2007) Huang et al. (2012)	Moderate High
		6 days	Pattappa et al. (2013)	Moderate
		7 days	Zahm et al. (2008)	Moderate
		8 days	Xu et al. (2007)	High
		14 days	Zhang et al. (2017)	Moderate
		21 days	Huang et al. (2012) Tsang et al. (2013) Malladi, Xu, Chiou, Giaccia, & Longaker (2006) (Lee et al. (2012)	Moderate Low Low Moderate
	3%	14 days	Holzwarth et al. (2010)	Low
	5%	NR	Russo, Yu, Belliveau, Hamilton, & Flynn (2014)	Moderate
		6 days	Pattappa et al. (2013)	High
		14 days	Inagaki et al. (2017)	High
		21 days	Hopper et al. (2015)	Low
		28 days	Merceron et al. (2010)	High

Cyclic Exposure				
□ALP activity **□Calcium deposit** **🡻 ALP** **🡻 RUNX2** **🡻 Osteocalcin**	**1%** **3%** **5%** **10%**	15 days 2 × 3 min/day	D'Ippolito et al. (2006)	Moderate

PiO_2: partial pressure of inspired oxygen; -g: genetic expression; RUNX2: runt-related transcription factor 2; ALP: alkaline phosphatase; COL1A1: collagen type 1 alpha1; NR: not reported.

Confidence Rating:

High confidence in the association between exposure to the substance and the outcome: The true effect is highly likely to be reflected in the apparent relationship.

Moderate confidence in the association between exposure to the substance and the outcome: The true effect may be reflected in the apparent relationship.

Low confidence in the association between exposure to the substance and the outcome: The true effect may be different from the apparent relationship.

Very low confidence in the association between exposure to the substance and the outcome: The true effect is highly likely to be different from the apparent relationship.

Table 4. Experimental details of in vivo studies included in the systematic review

Hypoxia Effects on Outcomes	Sample				Intervention			References	Confidence Rating
	Type	Age	Size	Conditions (*n*)	Hypoxia Level (Meters; % FiO_2)	Duration, Frequency	Exposure Type		
Sustained Exposure									
↓w-b BMD	Rats	12 weeks-old	NR	Normoxia (*n* = 4) Hypoxia (*n* = 4)	6000 m	3 weeks	Normobaric	Wang et al. (2017)	High
↓spine BMD	Healthy adults	24–58 years	5	NR	2,500 m	24 weeks	Hypobaric	O'Brien et al. (2018)	Moderate
↓SOS-R **SOS-T** **↓SOS-M** **↓SOS-P** **↓T-score-R** **↓T-score-P** **↑ALP** **↓BALP** **↑ Ca^{2+}** **↓25-Vit D** **↓PTH** **↓CICP** **↓NTX** **↓DPD/Cr**	Healthy adults	21–47 years	2600	Normoxia (*n* = 1300) Hypoxia (*n* = 1300)	3,450 m	16 weeks	Hypobaric	Basu et al. (2014)	High

SOS-R **SOS-T** **↓SOS-M** **↓SOS-P** **Z-score-R** **Z-score-T** **↓Z-score-M** **↓Z-score-P** **Ca^{2+}** **↑P** **↓ALP** **↓BAP** **↓25-Vit D** **↑PTH** **DPD/Cr**	Healthy adults	21–47 years	221	Hypoxia (*n* = 221)	3,000–3,754 m (24 weeks) + 5,400–6,700 m (16 weeks)	40 weeks	Hypobaric	Basu et al. (2013)	High
Intermittent Exposure									
spine BMD	Women with OSAS	56.3 ± 6.2 years	1201	NR	NR	NR	NR	Tng et al. (2018)	High
↑ CTX	Adults with OSAS	51 years	50	Control (*n* = 20) OSAS (*n* = 30)	NR	NR	NR	Terzi & Yilmaz, (2016)	High

↑ w-b BMD	Adults with OSAS	68.6 ± 0.8 years	833	Control (*n* = 373) OSAS (*n* = 459)	NR	NR	NR	Sforza et al. (2013)	High
CTX **RANKL** **OPG** **CTX** **RANKL** **OPG** **↑CTX** **↓RANKL** **OPG**	Adults with OSAS	51.0 ± 13 years	65	Control (*n* = 15) Mild OSAS (*n* = 10) Moderate OSAS (*n* = 12) Severe OSAS (*n* = 28)	NR	NR	NR	Tomiyama et al. (2008)	High
Cyclic Exposure									
↓w-b BMD **↓w-b BMC** **↓ w-b BMD** **↓ w-b BMC**	Rats	12 week-old	37	Normoxia (*n* = 6) Hypoxia (*n* = 7) Ovariectomized Normoxia (*n* = 12) Ovariectomized Hypoxia (*n* = 12)	3,000–5,000 m	2 weeks, 4 h/day	Normobaric	Wang et al. (2016)	High
↑ spine BMD	Rats	6 months-old	20	Normoxia (*n* = 10) Hypoxia (*n* = 10)	4,500 m	5 weeks 5 days/week 5 h/day	Hypobaric	Guner et al. (2013)	Moderate
↑ w-b BMD	Healthy adults	24.6 ± 2.8 years	28	Normoxia (*n* = 13) Hypoxia (*n* = 15)	15%	8 weeks 2days/week	Normobaric	Martinez-Guardado et al. (2019)	High

w-b BMD	Trained triathletes	27 years	18	Hypoxia Training (n = 9) Control (n = 9)	15%	7 weeks, 2days/week 60 min/day	Normobaric	Ramos-Campo et al. (2015)	High
↓ w-b BMD **↑ w-b BMD**	Healthy young	26.4 years	14	Normoxia Bed Rest (n = 14) Hypoxia Bed Rest (n = 14) Hypoxia Ambulatory (n = 14)	4,000 m	21 days	Normobaric	Rittweger et al. (2016)	High

FiO_2: fraction of inspired oxygen; w-b: whole body; BMD: bone mineral density; BMC: bone mineral, cortical; SOS-R: speed of sound at one-third of distal radius; SOS-P: speed of sound at the proximal third phalanx; SOS-M: speed of sound at fifth metatarsal; SOS-T: speed of sound at the mid-shaft tibia; ALP: alkaline phosphatase; BALP: bone specific alkaline phosphatase; Ca^{2+}: calcium deposit; 25-Vit D: 25-Hydroxy vitamin D3; PTH: parathyroid hormone; CICP: C-terminal propeptide of type I collagen; NTX: N-telopeptide of type I collagen; DPD/Cr: urinary DPD, creatinine ratio; CTX: carboxy-terminal collagen cross-links; RANKL: Receptor activator for Nuclear Factor κ B Ligand; OPG: osteoprotegerin; NR: not report; OSAS: obstructive sleep apnea syndrome.

Confidence Rating:

High confidence in the association between exposure to the substance and the outcome: The true effect is highly likely to be reflected in the apparent relationship.

Moderate confidence in the association between exposure to the substance and the outcome: The true effect may be reflected in the apparent relationship.

Low confidence in the association between exposure to the substance and the outcome: The true effect may be different from the apparent relationship.

Very low confidence in the association between exposure to the substance and the outcome: The true effect is highly likely to be different from the apparent relationship.

4.2 Passive Hypoxia

Baseline characteristics of the 10 healthy elderly are presented in Table 5. No significant differences between groups were observed in any variable. Similar results were also found for estimated calcium intake ($p>0.05$) in both groups between PRE (CON 1: 1263±249.89 mg/day; HYP: 1914±71.69 mg/day) and POST (CON 1: 1203±268.89 mg/day; HYP: 1908±44.30 mg/day) intervention. In the same way, no significant differences between groups were observed.

Table 5. Baseline characteristics of participants that were exposed to passive HC (HYP; n=5) and its related control group (CON ; n=5)

	HYP (Mean ± SD)	CON (Mean ± SD)	p
Age (years)	83.00±5.79	80.00±4.06	0.371
BMI ($kg \cdot m^{-2}$)	23.82±2.68	25.26±1.72	0.340
Fat Mass (%)	31.74±10.08	36.39±6.32	0.841
Calcium (mg/day)	1914±71.69	1263±249.89	0.556
Sex:			1.000 [b]
- Male [a]	3 (60.00%)	2 (40.00%)	
- Female [a]	2 (40.00%)	3 (60.00%)	

HC: hypoxia conditioning; BMI: body mass index. P values of the analysis of variance (ANOVA). [a] Values expressed as N (%).[b] P values of Chi-square analysis.

Bone Mineral Density Assessment

Results of bone parameters are showed in Figure 14 and Table 6. After hypoxia exposure the whole-body BMD had a significant increase compared to CON 1 group, whose values decreased ($p=0.008$). In the within group analysis, HYP group significantly increased theirs whole body BMD ($p=0.043$) and CON 1 group significantly decreased these parameters with an effect size of 1.18 ($p=0.042$). In the proximal femur, HYP group showed a significant decrease after intervention in the femoral neck ($p=0.041$).

Figure 13 shows the BMD individual responses after 18 weeks. Based on MDC, following hypoxia exposure bone parameters showed an increase in the different regions that reached this value: 20% of the subjects increased theirs whole body (MDC= MDC=0.104 g·cm-2), right leg (MDC= MDC=0.087 g·cm-2) and femoral (MDC= MDC=0.028 g·cm-2) BMD. Forty per cent of the subjects of this group increased also their trochanter BMD (MDC= MDC=0.030 g·cm^{-2}). Eighty per cent of subjects in both groups decreased femoral neck BMD, reached the MDC (HYP=-0.013 g·cm-2; CON 1= -0.016 g·cm-2). CON 1 group decreased also the rest of BMD values, reached the MDC: 60% of the subjects in whole body BMD (MDC= -0.051 g·cm-2), 40% in right leg BMD (MDC= -0.049 g·cm-2), 80% in femoral BMD (MDC= -0.017 g·cm-2) and 20% in trochanter BMD (MDC= -0.030 g·cm^{-2}).

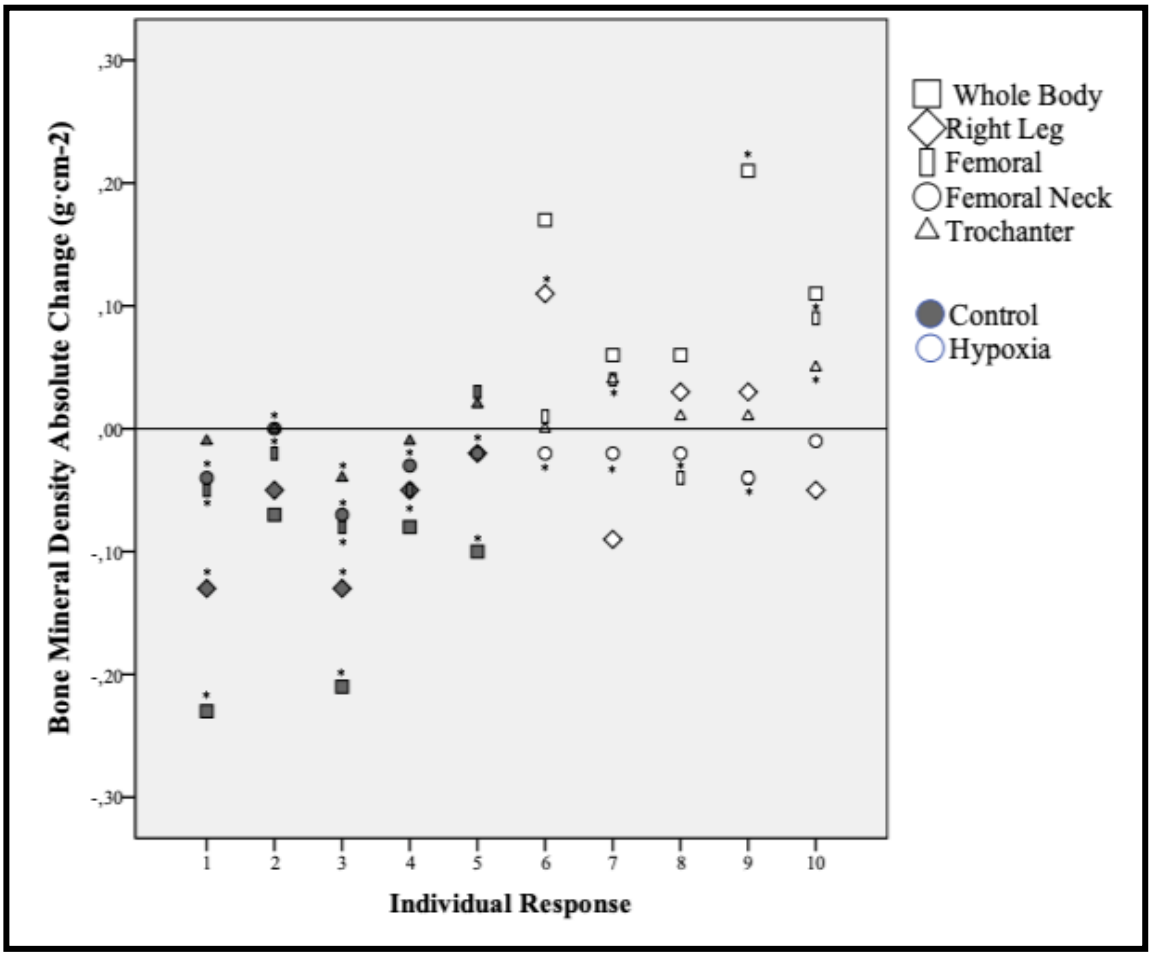

Figure 13 Individual Response of BMD absolute changes following passive HC exposure
*: intra-individual difference equal or greater than MDC (i.e., 0.10 g·cm-2 of whole-body BMD to Hypoxia group; BMD: bone mineral density

Table 6. Dual-energy X-ray absorptiometry (DXA) measurement at baseline (PRE) and reassessed after 18 weeks (POST) of passive HC (HYP; n=5) or its related control group (CON; n=5)

		PRE (Mean ± SD)	Δ (%)	POST (Mean ± SD)	ES	MDC (%)	Wilcoxon and Man Whitney U Test (p)	
							Time	Time x Group
Whole Body	HYP	0.880±0.145	7.04	0.942±0.205	0.35	11.42	**0.043**	**0.008**
	CON	0.986±0.053	-6.29	0.924±0.052	1.18	5.37	**0.042**	
Right Leg	HYP	0.908±0.149	0.44	0.912±0.186	0.02	9.55	0.892	0.310
	CON	0.994±0.071	-3.22	0.962±0.068	0.46	4.98	0.109	
Femoral	HYP	0.744±0.118	0.81	0.750±0.126	0.05	3.79	0.705	0.095
	CON 1	0.844±0.042	-1.18	0.834±0.051	0.22	2.06	0.129	
Femoral Neck	HYP	0.686±0.106	-2.92	0.666±0.108	0.19	1.96	**0.041**	1.000
	CON 1	0.752±0.067	-2.39	0.734±0.068	0.27	2.11	0.059	
Trochanter	HYP	0.568±0.097	3.17	0.586±0.093	0.19	5.20	0.109	0.056
	CON	0.654±0.064	-1.22	0.646±0.069	0.12	4.66	0.461	

HC: hypoxia conditioning; Δ: percentage of change between PRE and POST measurement outcomes; ES: effect size; MDC: minimum detectable change; p: p values.

4.3 Active Hypoxia

The characteristics of the participants (n = 31) at baseline are presented in Table 7. No significant pre-training differences were observed between the groups for any variable. Similar results were also found for BPAQ level ($p>0.05$) between PRE (NWBV: 10.44±7.93 score; HWBV: 8.03±6.07 score nor CON: 6.92±7.88 score) and POST (NWBV: 8.36±4.68 score; HWBV: 7.29±3.62 score nor CON: 7.74±7.69 score) intervention in all groups.

No significant differences were found for estimated calcium intake ($p>0.05$) in all groups between PRE (NWBV: 921.27±419.30 mg/day; HWBV: 1124.30±421.52 mg/day nor CON: 928.80±291.89 mg/day) and POST (NWBV: 848.00±229.81 mg/day; HWBV: 1186.80±468.97 mg/day nor CON: 969.80±392.73 mg/day) intervention.

Table 7. Baseline characteristics of participants that performed vibration exercise (NWBV; n=11 and HWVB; n=10) and its related control group (CON; n=10)

	NWBV Mean ± SD	HWBV Mean ± SD	CON Mean ± SD	p
Age (years)	70.18±6.39	73.50±4.74	73.40±5.02	0.296
BMI ($kg \cdot m^{-2}$)	29.46±4.80	28.86±4.20	28.85±3.50	0.929
Fat Mass (%)	38.02±14.50	37.02±8.66	38.26±6.66	0.537
BPAQ (score)	10.44±7.93	8.03±6.07	6.92±7.88	0.538
Calcium (mg/day)	921.27±419.30	1124.30±421.52	928.80±291.89	0.412
Sex:				0.232 [b]
- Male [a]	6 (54.54%)	3 (30.00%)	2 (20.00%)	
- Female [a]	5 (45.46%)	7 (70.00%)	8 (80.00%)	

BMI: body mass index; BPAQ: bone physical activity questionnaire. P values of the analysis of variance (ANOVA). [a] Values expressed as N (%).[b] P values of Chi-square analysis.

SaO_2 was lower for the hypoxia groups in all measured points during the training sessions. Compared with NWBV group, HWBV group shown significant statistical

differences ($p < 0.01$). HR during the training sessions was not significantly different between groups (Figure 14).

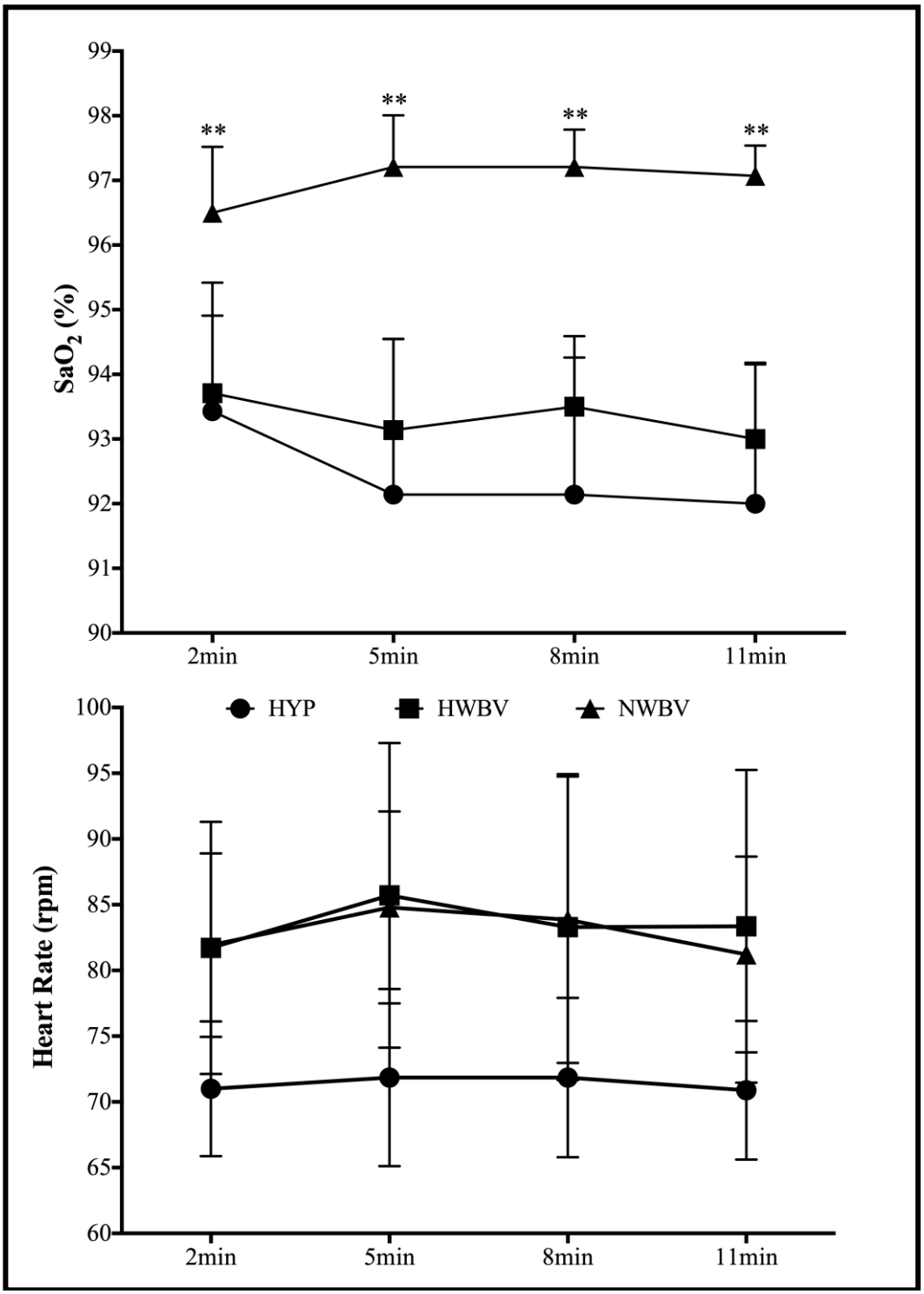

Figure 14 Physiological changes measured during intervention protocols

Bone Mineral Density Assessment

Results of bone parameter responses after 18 weeks of intervention are shown in Table 8. Because of the responses to the training showed no sex differences, results for male and female participants were combined and analysed together. In within-groups analysis, there was a significant increment in whole body BMD ($p=0.021$) after 18 weeks of combined hypoxia-whole body vibration exercise. In addition, T-score increased significantly with moderate effect size ($p=0.01$; ES= 1.35) in HWBV group. In the within-group analysis of the proximal femur, CON group showed a significant decrease in the trochanter BMD ($p=0.023$). Changes in whole body and proximal femur BMD were not significantly different between groups.

Table 8. Bone Mineral Density (BMD) measurement outcomes ($g \cdot cm^{-2}$) at baseline (PRE) and reassessed after 18 weeks (POST) of WBV training under normoxia conditions (NWBV; n=10), combined with HC (HWBV; n=10) and its related control group (CON; n=10)

		PRE (Mean ± SD)	Δ (%)	POST (Mean ± SD)	p	ES	MDC (%)	ANOVA (p)		
								Time	Group	Time x Group
Total	NWBV	1.112 ± 0.132	-1.17	1.099 ± 0.131	0.359	0.08	4.66			
	HWBV	1.061 ± 0.117	+3.58	1.099 ± 0.108	**0.021**	0.35	6.75	0.421	0.424	0.072
	CON	1.034 ± 0.093	+0.68	1.041 ± 0.095	0.813	0.11	2.38			
Total T-score	NWBV	-0.967 ± 0.569	+20.68	-1.167 ± 0.808	0.816	0.29	39.19			
	HWBV	-1.257 ± 0.428	-48.85	-0.643 ± 0.493	**0.001**	1.35	77.19	0.449	0.696	1.000
	CON	-0.950± 1.090	-10.53	-0.850 ±1.141	0.512	0.09	32.59			
Femoral	NWBV	0.889± 0.136	+1.57	0.903 ± 0.131	0.168	0.07	3.46			
	HWBV	0.837 ± 0.169	+1.19	0.847 ± 0.169	0.339	0.06	0.00	0.913	0.560	0.543
	CON	0.830 ± 0.143	0.96	0.838 ± 0.131	0.818	0.07	6.91			
Femoral T-score	NWBV	-0.744 ± 0.831	-13.44	-0.644 ± 0.763	0.218	0.13	26.63			
	HWBV	-1.070 ± 1.076	-3.74	-1.030 ± 1.082	0.648	0.04	15.60	0.877	0.658	0.684
	CON	-1.040 ± 1.064	-6.73	-0.970 ± 1.017	0.969	0.07	45.29			
Trochanter	NWBV	0.697 ± 0.126	+1.00	0.704 ± 0.123	0.134	0.00	4.67			
	HWBV	0.660 ± 0.145	+0.61	0.664 ± 0.145	0.673	0.00	7.57	0.126	0.527	1.000
	CON	0.651 ± 0.113	-2.00	0.638 ± 0.109	**0.023**	0.09	5.85			
Intertrochanteric	NWBV	1.048 ± 0.144	+2.48	1.074 ± 0.144	0.169	0.14	3.76			
	HWBV	0.977 ± 0.183	+1.43	0.991 ± 0.187	0.343	0.05	7.44	0.798	0.369	0.670
	CON	0.960 ± 0.160	+1.56	0.975 ± 0.144	0.895	0.07	8.92			

WBV: whole-body vibration; HC: hypoxia conditioning; Δ: percentage of change between PRE and POST measurement outcomes; ES: effect size; MDC: minimum detectable change; p: p values.

Whole body and right proximal femur BMD individual responses after 18 weeks of intervention are showed in the Figure 15, 16, 17 and 18. Based on MDC, 30% of the subjects of HWBV group (3/10) reached this value in whole body BMD (MDC=0.06 g·cm^{-2}, Figure 15).

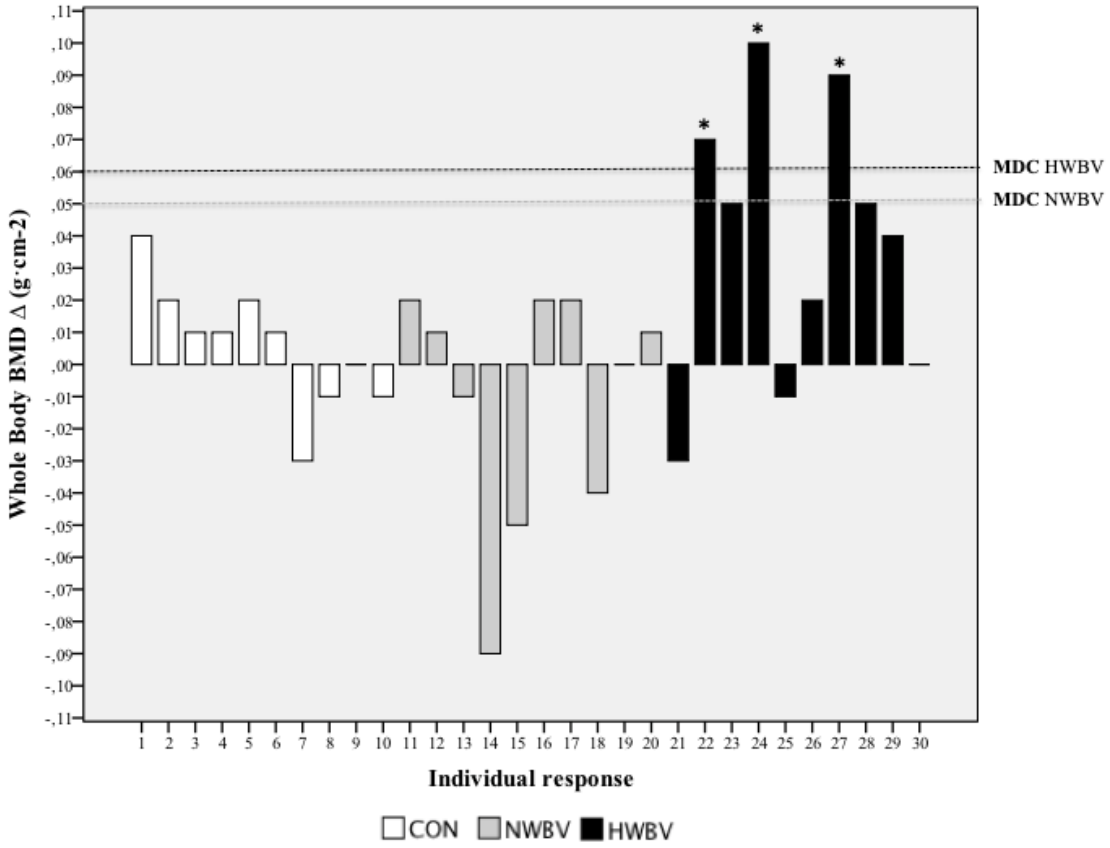

Figure 15 Heterogeneity of absolute change values in whole-body BMD following active HC exposure
Dark dash line: minimal detectable change (MDC) to HWBV group (whole body vibration in hypoxic conditions); light dash line: minimal detectable change to NWBV group (whole body vibration alone). *: intra-individual difference equal or greater than MDC. BMD: bone mineral density.

In the femoral BMD, 90% (9/10) of the subjects reached the MDC following HWBV (MDC=0.00 g·cm^{-2}) and 70% (7/10) following NWBV (MDC=0.03 g·cm^{-2}; Figure 16).

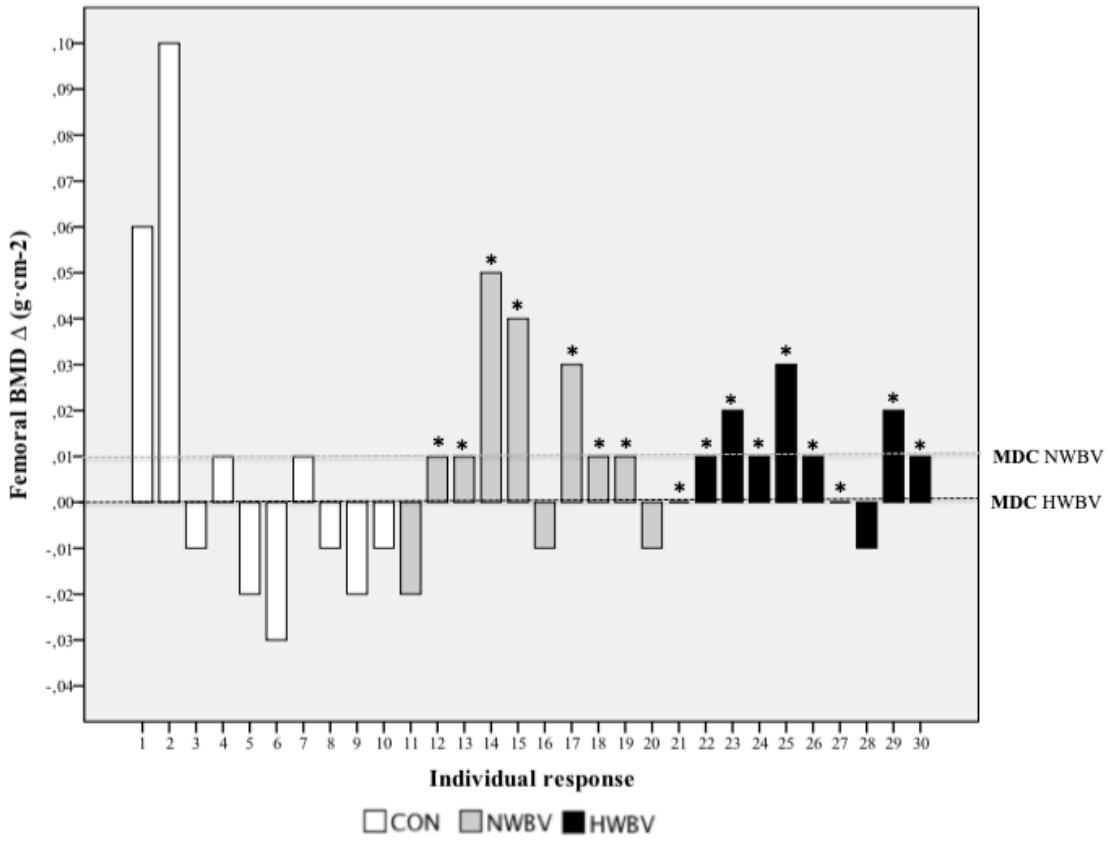

Figure 16 Heterogeneity of absolute changes values in femoral BMD following active HC exposure
Dark dash line: minimal detectable change (MDC) to HWBV group (whole body vibration in hypoxic conditions); light dash line: minimal detectable change to NWBV group (whole body vibration alone). *: intra-individual difference equal or greater than MDC. BMD: bone mineral density.

Only 20% of the subjects (2/10) showed trochanter BMD changes equal of MDC after HWBV (MDC=0.05 g·cm^{-2}; Figure 17).

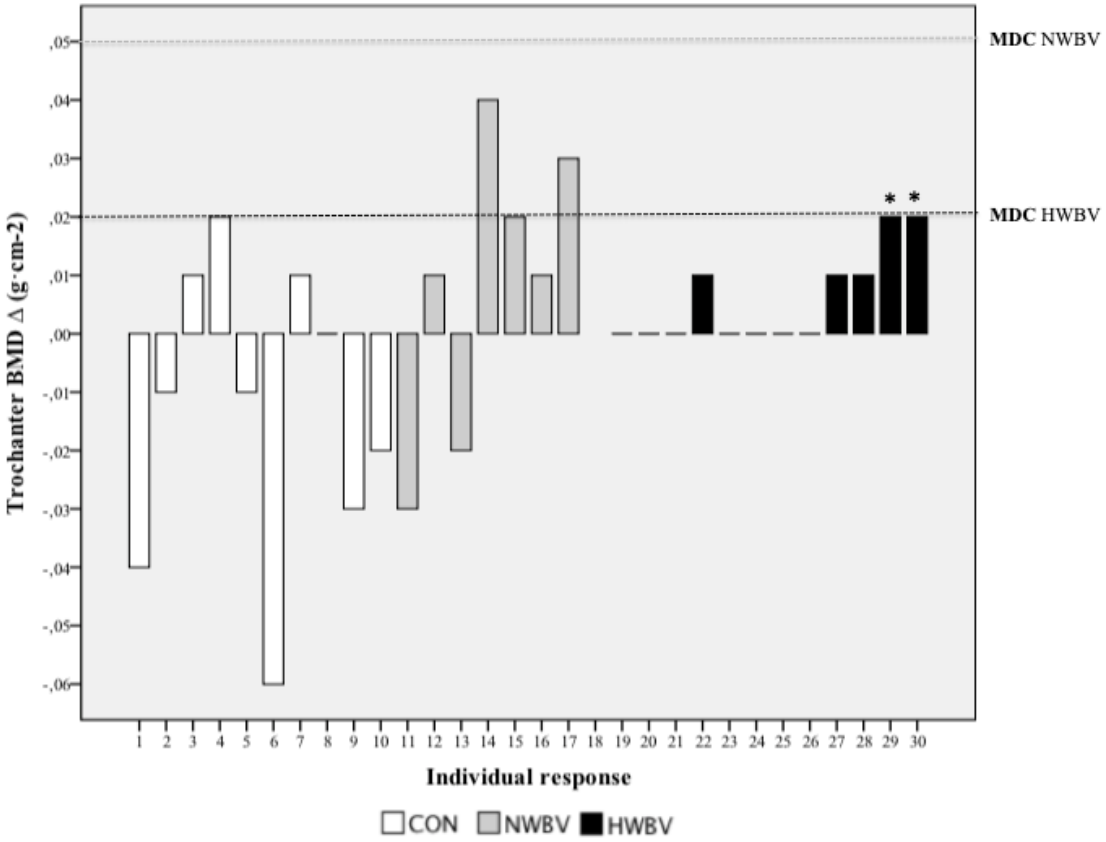

Figure 17 Heterogeneity of absolute changes values in trochanter BMD following active HC exposure
Dark dash line: minimal detectable change (MDC) to HWBV group (whole body vibration in hypoxic conditions); light dash line: minimal detectable change to NWBV group (whole body vibration alone). *: intra-individual difference equal or greater than MDC. BMD: bone mineral density.

Following HWBV (MDC=0.07 g·cm^{-2}) the 30% (3/10) of the subjects reached the MDC in the intertrochanteric region BMD and only a 10% (1/10) following NWBV (MDC=0.16 g·cm^{-2}; Figure 18).

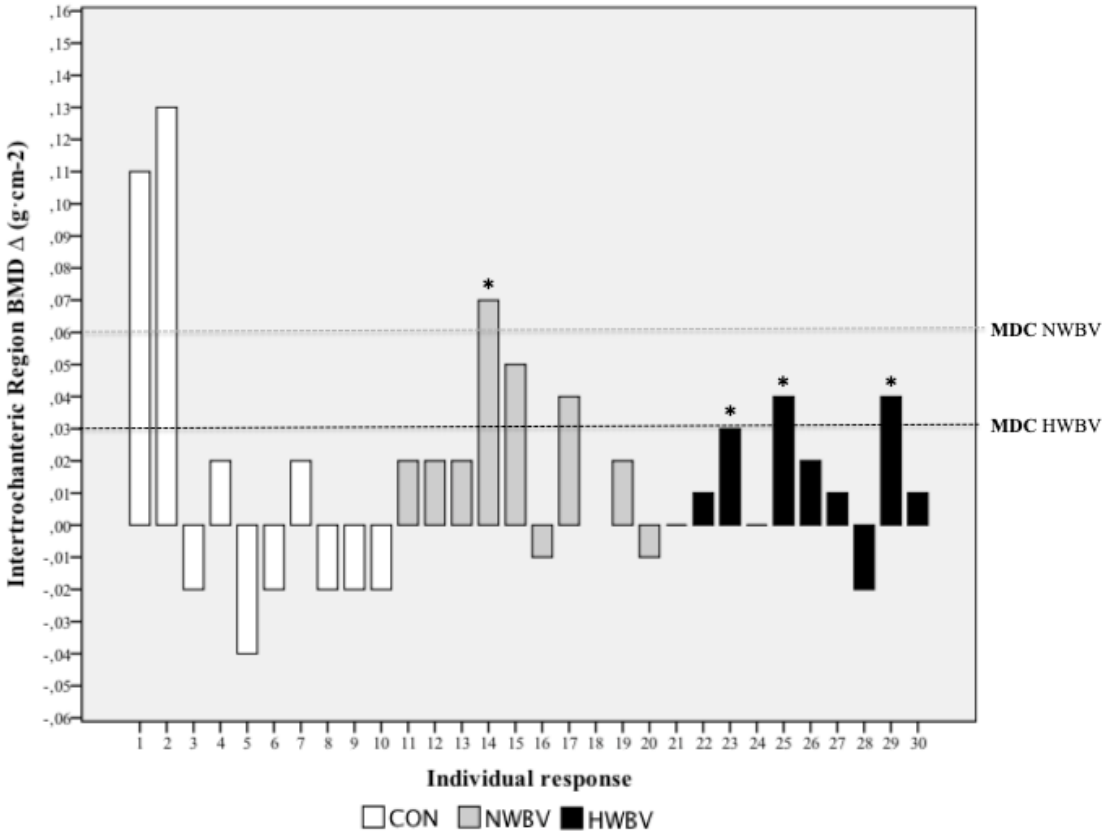

Figure 18 Heterogeneity of absolute changes values in intertrochanteric region BMD following active HC exposure

Dark dash line: minimal detectable change (MDC) to HWBV group (whole body vibration in hypoxic conditions); light dash line: minimal detectable change to NWBV group (whole body vibration alone). *: intra-individual difference equal or greater than MDC. BMD: bone mineral density.

Muscle Mass

Whole-body, trunk and right leg muscle mass percentages are presented in Table 9. Because of responses to the training showed no sex differences, the results for male and female participants were analysed together as aggregate data. There were no significant differences between groups for any of the three variables. In-group analysis did not show any significant differences in any of the variables among groups. Effect sizes were trivial in all body composition parameters.

Table 9. Muscle mass at baseline (PRE) and reassessed after 18 weeks (POST) of WBV training under normoxia conditions (NWBV; n=11), combined WBV training and HC (HWBV; n=10) and in the control group (CON; n=10)

		PRE (Mean ± SD)	Δ (%)	POST (Mean ± SD)	p	ES	ANOVA (p)		
							Time	Group	Time x Group
Whole Body (%)	NWBV	61.98±14.50	-1.28	61.19±16.42	0.105	0.05	0.182	0.257	0.337
	HWBV	62.97±8.66	0.18	63.08±8.34	0.983	0.01			
	CON	61.74±6.66	-0.60	61.37±6.42	0.411	0.06			
Trunk (%)	NWBV	62.55±10.21	-0.29	62.37±11.11	0.076	0.12	0.153	0.197	0.597
	HWBV	61.85±8.03	-0.65	61.45±7.15	0.752	0.05			
	CON	61.57±7.16	-0.82	61.06±6.71	0.577	0.07			
Right Leg (%)	NWBV	61.83±21.16	-0.36	61.61±20.10	0.705	0.01	0.346	0.386	0.957
	HWBV	63.36±12.36	-0.63	62.96±12.59	0.489	0.03			
	CON	59.12±7.06	-1.18	58.42±7.51	0.578	0.10			

WBV: whole-body vibration; HC: hypoxia conditioning; Δ: percentage of change between PRE and POST measurement outcomes; ES: effect size; MDC: minimum detectable change; p: p values.

Table 10. Maximal and endurance strength at baseline (PRE) and reassessed after 18 weeks (POST) of WBV training under normoxia conditions (NWBV; n=11), combined with HC (HWBV; n=10) or control group (CON; n=10)

			PRE (Mean ± SD)	Δ (%)	POST (Mean ± SD)	p	ES	ANOVA (p)		
								Time	Group	Time x Group
Maximal Strength										
Fmax (N·m)	Extensors	NWBV	94.81±27.80	17.34	111.25±32.96	**0.004**	0.54			
		HWBV	81.79±28.95	0.48	82.18±36.39	0.574	0.01	0.328	0.329	0.207
		CON	75.88±16.04	1.09	76.71±27.77	0.474	0.04			
	Flexors	NWBV	52.17±19.95	3.30	53.89±21.34	0.419	0.08			
		HWBV	44.27±18.86	-3.25	42.83±19.74	0.619	0.07	0.210	0.191	0.485
		CON	35.93±9.45	5.32	37.84±10.55	0.285	0.19			
H-Q Ratio		NWBV	0.55±0.12	-12.73	0.48±0.12	0.069	0.58			
		HWBV	0.55±0.13	-1.82	0.54±0.18	0.658	0.06	0.177	0.452	0.271
		CON	0.48±0.10	6.25	0.51±0.09	0.551	0.32			
Endurance Strength										
Wmean (J)	Extensors	NWBV	40.09±14.24	7.83	43.23±23.44	0.171	0.17			
		HWBV	45.55±16.17	10.19	50.19±17.84	0.063	0.27	0.030	0.519	0.793
		CON	45.15±15.73	8.57	49.02±16.19	0.082	0.24			
	Flexors	NWBV	18.14±14.20	11.30	20.19±12.91	0.438	0.15			
		HWBV	17.61±11.07	-11.81	15.53±14.05	0.328	0.17	0.247	0.970	0.455
		CON	18.40±13.37	-4.14	17.64±13.02	0.908	0.06			
Fatigue (%)	Extensors	NWBV	19.81±7.17	13.33	22.45±8.48	0.358	0.34			
		HWBV	13.25±10.13	47.62	19.56±7.82	0.127	0.70	0.492	0.290	0.601
		CON	22.82±12.43	5.78	24.14±8.43	0.273	0.13			
	Flexors	NWBV	25.30±16.27	36.68	34.58±19.04	0.056	0.53			
		HWBV	27.52±14.85	23.49	33.97±13.12	0.233	0.46	0.095	0.313	0.506
		CON	38.22±15.80	25.46	47.95±31.36	0.844	0.41			

WBV: whole-body vibration; HC: hypoxia conditioning; maximal Strength: 3-reps for 60º/second; Fmax: maximal force; H-Q ratio: hamstring-quadriceps ratio (maximal strength flexor/maximal strength extensor); Endurance Strength: 20-reps for 180º/seconds; Wmean: average work; Fatigue: muscle fatigue as % of Δ in20-reps; Δ: percentage of change between PRE and POST measurement outcomes; ES: effect size; MDC: minimum detectable change; p: p values.

Muscle Strength

Results of maximal and endurance strength are summarised in Table 10. Because of responses to the training showed no sex differences, the results for male and female participants were analysed together as aggregate data. There were no significant differences between groups in either maximal or endurance strength after 18 weeks of training. In-group analysis showed no significant differences in any of the variables of the HWBV group. The NWBV group showed significant improvements in the maximal strength of knee extensors with a small effect size ($p = 0.004$; $d = 0.54$). The H/Q ratio of the NWBV group decreased with a small effect size after 18 weeks of training (ES=0.58). HWBV group shown a small effect size in muscle fatigue of knee extensors (ES=0.70)

Functional Mobility

Table 11 shows the time spend of TUGT at baseline and after 18 weeks of intervention. There were no statistically significant differences in the test performances neither within group (NWBV, $p=0.241$; HWBV, $p=0.953$) nor between-groups analysis ($p=0.182$). At the end of the study, HWBV group improved the performance of this test (Δ=-0.44%). Participants in NWBV group needed more time at the end of the intervention to complete the TUGT regarding at baseline (Δ= 6.21).

Table 11. Time (s) spend of Time Up and Go Test (TUGT) at baseline (PRE) and reassessed after 18 weeks (POST) of WBV training in normoxic conditions (NWBV; n=10) or combined with HC (HWBV; n=9)

		PRE (Mean ± SD)	Δ (%)	POST (Mean ± SD)	ES	MDC (%)	Wilcoxon and Man Whitney U Test (p)	
							Time	Time x Group
TUGT (s)	NWBV	5.98± 0.74	+6.21	6.35 ± 0.94	0.44	4.87	0.241	0.182
	HWBV	6.36 ± 0.80	-0.44	6.33 ± 0.94	0.03	13.58	0.953	

Δ: percentage of change between PRE and POST measurement outcomes; ES: effect size; MDC: minimum detectable change; p: p values.

Chapter 5. DISCUSSION

Chapter 5. Discussion

Firstly, due to the lack of consensus regarding the response of bone metabolism to hypoxia, this project contemplated to review current literature to examine the impact of the different hypoxic modes on bone metabolism. Secondly, we aimed to know the passive and active normobaric hypoxia exposures effects on bone mineral density of healthy elderly people. The active exposure was based on the combination of WBV training and normobaric hypoxia and its effects on muscle mass and strength and functional mobility were investigated too.

Based on the results of our systematic review, hypoxia modes might have a different effect on skeletal health of animals and humans. As in other body systems, a sustained hypoxic environment could negatively influence bone mass and bone quality when tissue PiO_2 falls below 40 mmHg. Intermittent hypoxia (associated with OSAS) may have also unfavourable effects on bone metabolism and other organs and systems. However, short episodes with modest levels of hypoxia (9–16% PiO_2) could lead to benefits if it is administered repeatedly. In this sense, the present HC exposure to passive moderate hypoxia was enough to increase whole body BMD, but did not alter femur proximal BMD. When hypoxia was combined with WBV training seemed to increase whole body BMD and to decrease the prevalence of osteopenia/osteoporosis when compared to WBV alone or control group. But furthermore, the results indicate that the active hypoxic stimulus may cause an individualized response demonstrating effects in the proximal femur BMD after 36 sessions of our treatment.

However, combined WBV and normobaric hypoxic treatment do not have effects on muscle system. Body composition, especially muscle mass measured by DXA, of our older adults volunteers did not change after intervention. Regarding muscle strength, the findings of this project reject the aforementioned hypothesis with respect to the combination of WBV

training. As new treatment, our active HC exposure did not have any effect on maximal or endurance strength parameters. Similar results were obtained to functional mobility of this population. Despite the results show a slight improvement in HWBV in the TUGT, it has been observed that 36 sessions of this novel intervention do not have effects on functional mobility assessed through TUGT (P. Zhang et al., 2017).

5.1 Systematic Review

In spite of disparity in protocols, MSC sources and the composition of the culture medium (W. Y. Lee et al., 2012; P. Zhang et al., 2017) used have made difficult to establish the role of HC in osteogenic differentiation (P. Zhang et al., 2017). The results of our systematic review have shown how the hypoxic modes might have a different effect on skeletal health of animals and humans.

5.1.1 In vitro Studies

Sustained exposure

To evaluate the effects of hypoxia on osteogenic differentiation, genetic and protein expression of different biomarkers was evaluated. Sustained hypoxia was administered in mesenchymal stem cells (MSCs) derived from bone marrow, adipose tissue, placental, tendon, umbilical cord, or cartilage endplate from one to 28 days throughout the different timelines (expansion, proliferation, or differentiation).

While some studies showed decreases in RUNX2 gene expression (Ding et al., 2014; Hsu et al., 2013; Y. C. Huang et al., 2012; J. S. Lee et al., 2015; H. P. Ma et al., 2014; Merceron et al., 2010; Russo et al., 2014; Y. Wang et al., 2012; N. Xu et al., 2013; Yang et al., 2011; A M Zahm et al., 2008), expression was maintained in three studies (Sengupta et al., 2010; Tsang et al., 2013; P. Zhang et al., 2017). A shorter exposure (3–14 days), applied in two studies, increased RUNX2 gene expression (Salamanna et al., 2018; Tsang et al., 2013). RUNX2 protein expression decreased in the five reviewed studies (Ding et al., 2014; N. Xu et al.,

2013; Y. Xu et al., 2007; Yao et al., 2017; Adam M. Zahm, Bucaro, Srinivas, Shapiro, & Adams, 2008), which applied 5–21 days of sustained hypoxia at 1–2% PiO_2. Overall, data from mRNA expression analysis could differ from protein expression by the post-translational modification of osteogenic biomarkers that may be a critical step dependent on the duration of hypoxia (Ding et al., 2014). Osteogenic differentiation is controlled by RUNX2, a specific transcription factor that can promote or inhibit the expression of osteogenic differentiation-related genes (Ciapetti et al., 2016). Expression of RUNX2 can induce the synthesis of early (e.g., ALP and COL1A1) and late markers of osteoblast differentiation (e.g., OC) (P. Zhang et al., 2017). Thus, sustained hypoxic exposure of 1–5% PiO_2 for 2–28 days show contradictory results and so how this exposition affects to the osteoblastic differentiation cannot establish.

Sustained hypoxia protocols of 1–2% PiO_2 for 5–21 days showed lower ALP gene expression (Ding et al., 2014; J. Huang et al., 2011; Y. Wang et al., 2012; A M Zahm et al., 2008) while moderate hypoxia (2–5% PiO_2) for 3–14 days showed greater expression (Ciapetti et al., 2016; Gu et al., 2016; Tsang et al., 2013). Protein expression of ALP increased when lower severity (> 2% PiO_2) and length of exposure (2–21 days) were applied (Gao et al., 2013; J. Huang et al., 2011; Inagaki et al., 2017; J. H. Lee & Kemp, 2006; Salamanna et al., 2018; Tsang et al., 2013; J. Zhang et al., 2018). Finally, ALP activity decreased in 11 of 18 studies after severe hypoxia (1–2% PiO_2) was applied for 2–28 days (Hsu et al., 2013; Huang et al., 2012; Ma et al., 2014; Malladi et al., 2006; Merceron et al., 2010; Pattappa et al., 2013; Russo et al., 2014; Xu et al., 2013; Zahm et al., 2008; Zhang et al., 2018; Zhang et al., 2017) ALP is a biomarker of bone growth and development as it produces an alkaline environment allowing calcium to crystallise and strength to be achieved (Ding et al., 2014). Thus, it seems that exposures with greater severity (1–2%) and longer

duration (up to 28 days) could negatively affect ALP; however, protein expression increased when moderate hypoxia (< 2%) was applied for a shorter period of time (up to 21 days).

Similar to other genes, COL1A1 expression showed contradictory results. While expression of the COL1A1 gene was maintained at 5% PiO_2 (Gu et al., 2016; Sengupta et al., 2010), it decreased with 2% PiO_2 for 7 (Zahm et al., 2008) or 21 days (Huang et al., 2012), but increased with 2% PiO_2 for 12 days (Ciapetti et al., 2016). COL1A1 is an indicator of the efficiency of the final osteogenic potential (Huang et al., 2012). Therefore, a lower expression of COL1A1 would indicate a decreased efficiency in osteogenic potential (Salamanna et al., 2018). In this sense, moderate oxygen concentration (2–5%) with a moderate exposure time may promote bone formation.

During bone formation, late in the mineralisation process, non-collagenous glycoproteins such as OPN and OC are abundant in the bone matrix with biological and mechanical functions of bone (Bailey, Karsenty, Gundberg, & Vashishth, 2017). Decreases in OPN expression activate osteoclastic bone resorption and inhibit osteoblastic bone formation (Ishijima et al., 2001). A lower genetic expression of OPN was observed following severe hypoxia protocols (1–2% PiO_2) for 21 days (Ding et al., 2014; Hsu et al., 2013; Lee et al., 2012; Park et al., 2013; Xu et al., 2013), but OPN expression was maintained with 5% PiO_2 during cellular expansion and differentiation of human ASCs (Russo et al., 2014). Nine of the eleven studies that evaluated this parameter showed a lower expression of the OC gene when severe hypoxia protocols of 5–28 days were applied (Ding et al., 2014; Hsu et al., 2013; Huang et al., 2011; Lee et al., 2012; Park et al., 2013; Wang et al., 2012; Xu et al., 2013). Only two studies (Ciapetti et al., 2016; Gu et al., 2016) showed increased expression with a more moderate dose (> 2%) and lower exposure time (3–12 days).

Finally, in the present review, calcium deposits maintained the same values compared with the normoxia groups in four studies, after low oxygen availability between 1% to 5%

PiO_2 was applied for 14–21 days (Binder et al., 2015; Ciapetti et al., 2016; Holzwarth et al., 2010; Jin et al., 2010). In BMSCs, the most severe doses (< 2%) showed decreased calcium deposits (Burian et al., 2017; Cicione et al., 2013; Ding et al., 2014; Holzwarth et al., 2010; Huang et al., 2011; Lee et al., 2012; Park et al., 2013; Pattappa et al., 2013; Xu et al., 2013). Previous researchers have suggested that there may exist a basal threshold of tissue oxygenation that regulates the deposition of minerals in the extracellular matrix (Zahm et al., 2008). Thus, similar to osteocytes buried in mineralised bone, lower PiO_2 may result in low ALP activity and minimal mineralisation potential (Zahm et al., 2008).

Overall, it is difficult to clarify the role of sustained hypoxia on osteogenic differentiation (Zhang et al., 2017). The differences in reported effects on cellular behaviours may be due to disparity in protocols, MSCs sources from different species or tissues, and/or the composition of the culture medium (Lee et al., 2012; Zhang et al., 2017).

Different effects have been reported due to discrepancies in oxygen concentration and exposure time. It is notable that 2% may represent a critical concentration, and therefore, oxygen concentrations above 2% could promote osteogenic responses (Zhang et al., 2017). However, long-term or chronic exposure to hypoxia was reported to inhibit osteogenic differentiation. Effects of hypoxia on osteogenic differentiation may be time-dependent: osteogenesis could be accelerated in the early period, but sustained long-term hypoxia could result in poor osteogenesis (Ding et al., 2014). On the other hand, previous studies have reported that under low O_2 conditions, MSCs proliferate faster and for a longer period of time (Burian et al., 2017). However, maintaining the exposure during cellular differentiation could maintain the undifferentiated characteristics of these cells (Lee et al., 2012). Conversely, subsequent inductions under normoxic conditions during differentiation could maintain or improve the differentiation potential (Dos Santos et al., 2010). Thus, the timing of the hypoxic exposure of MSCs could be important in osteogenic differentiation.

According to the origins of different tissues, MSCs show altered differentiation in response to hypoxia (Yao et al., 2017). Osteogenesis may only be induced in periodontal ligament MSCs (Zhang et al., 2014) under sustained hypoxic conditions but inhibited in bone marrow and adipose MSCs (Khan, Adesida, Tew, Lowe, & Hardingham, 2010; Martin-Rendon et al., 2007; Merceron et al., 2010; Yang et al., 2011).

Cyclic exposure

In a study conducted by D'Ippolito et al. (2006), the effects of 15 days of different doses (1, 3, 5, and 10% PiO_2) of cyclic hypoxia (2 bouts of 3 min per day) during the differentiation of human BMSCs were studied. Compared with normoxic conditions, low oxygen concentration increased cell proliferation (especially at 3% PiO_2) but inhibited osteoblastic differentiation by decreasing RUNX2 and OC gene expression. Thus, the exposure time used was not sufficient to promote osteogenic differentiation.

5.1.2 In vivo Studies

Sustained exposure

Some reports have indicated that residency at altitude may cause a marked deterioration in different indices of skeletal health (Basu et al., 2014). Studies included in this review, which measured different indices of skeletal health in animals and humans, reported similar findings. At extreme altitude, healthy rats showed a decrement in whole body BMD and other bone parameters after 3 weeks of sustained simulated hypoxia at 6,000 meters (Wang et al., 2017). In addition, 14 healthy young adults were exposed to bed rest or ambulatory normobaric hypoxia for 21 days at 4,000 meters of simulated altitude (Rittweger et al., 2016). Whole body bone mineral content (BMC) decreased after bed rest protocols and increased after ambulatory hypoxic conditions. In addition, at high altitude, a group of 5 healthy active male adults (O'Brien et al., 2018), who participated in an expedition of 24 weeks at 2,500 meters of altitude showed a decrease in spine BMD values. Also, the Indian

army composed of healthy males stayed at high altitude (3,450 meters) for 16 weeks (Basu et al., 2013) and showed a decrease in bone strength (SOS values of the radius, metatarsal, and phalanx and T-score of the radius and phalanx). Thus, a sustained hypoxic environment could negatively influence the bone mass and bone quality when tissue PiO_2 falls below 40 mmHg. HIF could affect the activity of multiple skeletogenic cells involved in angiogenesis, extracellular matrix formation, and resistance to infection (Gordillo & Sen, 2003; Knowles, Cleton-Jansen, Korsching, & Athanasou, 2010). Nevertheless, hypoxic conditions could enhance the differentiation of osteoclasts (Utting, Flanagan, Brandao-Burch, Orriss, & Arnett, 2010) and modulate their binding to resorption sites (Arnett et al., 2003)

In addition to skeletal health, prolonged residency in a hypoxic environment is associated with changes in turnover of bone metabolism coupled with specific endocrine adaptations. Bone formation markers such as ALP, B-ALP, 25-Vit D; PTH, and resorption markers (e.g., CTX, DPD/Cr) were evaluated after a stay at extreme and high altitudes (Basu et al., 2013, 2014). The study reported that after 4 months at extreme altitude, ALP, B-ALP, and CTX were decreased and the DPD/Cr ratio did not show any significant change. These results indicate activation of the bone resorption process at extreme altitude. DPD/CR ratio, B-ALP, protein released by osteoblasts, and CTX were lower at high altitude. Decreased formation and expression of bone resorption markers reflected a lower bone turnover at high altitude.

PTH is the major hormone regulating calcium metabolism (Qin, Raggatt, & Partridge, 2004); this hormone aids in the production of bone-destroying osteoclasts and consequently speeds up bone remodelling and the release of calcium (Ca^{2+}) and other minerals in usable forms (Bingham, Brazell, & Owen, 1969). Whereas PTH levels are increased at extreme altitude and decreased at high altitude, serum 25-Vit D showed a significant decline at both high and extreme altitudes. Decline of 25-Vit D remains speculative but may be due to

declined conversion of 25(OH) to 1,25(OH) D3 under conditions of low oxygen (Basu et al., 2014). As a result, increased PTH may be required to increase this conversion to stimulate intestinal absorption of Ca^{2+}. Calcium levels are maintained at extreme altitude and significantly increased at high altitude. These studies suggest that sustained hypoxia is associated with a decline in bone turnover due to reduced formation and expression of bone resorption markers. Whether this decline in bone turnover can lead to an increase in Ca^{2+} deposition in bones during residency at high altitude remains to be determined (Basu et al., 2014). At extreme altitudes, more significant changes occur in hormonal and biochemical bone remodelling parameters.

Compared to *in vitro* studies, *in vivo* environments are much more complex, and more factors related to the hypoxic environment may be responsible for the impaired bone strength and quality (Wang et al., 2017). Weight loss (Bozzini et al., 2005), increased lean mass (Martinez-Guardado et al., 2019), lowered basal metabolic rate (Wang et al., 2017), decreased activity levels (Vuori, 2001), insufficient vitamin D levels (O´Brien, 2018) or dietary changes in Ca^{2+} (Kelly, Pocock, Sambrook, & Eisman, 1990), vitamin C (Aghajanian, Hall, Wongworawat, & Mohan, 2015) or vitamin D (Bischoff-Ferrari, Dietrich, Orav, & Dawson-Hughes, 2004) can also influence the BMD, playing an important role in healthy bone.

Intermittent exposure

Nocturnal breathing difficulty, specially, sleep apnea results in intermittently low oxygen levels by reductions of airflow while sleeping (Swanson, 2015). While some reports have studied the relationship between sleep apnea and bone health, it remains unclear. In the present review, observational human studies, which analysed the effect of OSAS level on bone remodeling parameters (Tng et al., 2018), observed similar values in spine BMD (Sforza et al., 2013) and higher in whole body BMD values compared with healthy adults.

These findings could be explained by osteogenesis-angiogenesis coupling phenomenon, induced by HIF secretion (Tng et al., 2018). HIF may promote osteogenic factors via VEGF or ALP expression (Tng et al., 2018). However, gender, comorbidities, ethnic group or age could lead conflicting results. Usual comorbidities, characteristics of OSAS patients, could affect to bone health and healthy controls could not be considered as a valid control. In this sense, others studies included in this review such as intermittent hypoxic expositions, which excluded OSAS patients with comorbidities, showed lower BMD in femoral neck (Terzi & Yilmaz, 2016). In addition, higher values of bone resorption markers (e.g., CTX) and similar OPG values were found in subjects with severe OSAS (Terzi & Yilmaz, 2016; Tomiyama et al., 2008). A previous review showed that OSAS's patients had increased circulating markers of systemic inflammation, which may contribute to the development of osteopenia (Kent, Mitchell, & McNicholas, 2011). Chronic hypoxia could reduce the expression of bone formation markers such as B-ALP or type I collagen (Arnett et al., 2003), and promote the function of osteoclasts by increasing cytokines such as interleukin-6 (Arnett et al., 2003; Orriss et al., 2009).

While some studies have shown that intermittent hypoxia may have a protective role in bone health (Tng et al., 2018), other studies with larger samples suggest that OSAS may have unfavourable effects on bone metabolism (Terzi & Yilmaz, 2016). In addition, intermittent hypoxia, which is associated with OSAS, causes an increase in oxidative stress with negative effects on other organs and systems (Barcelo et al., 2011; Destors, Tamisier, Baguet, Levy, & Pepin, 2014).

Cyclic exposure

Different findings have been reported following the application of HC protocols in humans and animals. Increases in the spine BMD of rats were observed after 5 weeks of cyclic normobaric hypoxia (5 days per week; 5 h per day) at 4,500 meters (Guner et al.,

2013). In addition, healthy active adults showed improved whole body BMD after 8 weeks of normobaric hypoxic training (Martinez-Guardado et al., 2019); however, in trained triathletes (Ramos-Campo et al., 2015), 7 weeks of normobaric cyclic hypoxia training at 15% FiO_2 (2 days per week; 60 min per session) resulted in no reported changes in whole body BMD. In another study, HC exposure was applied for 2 weeks, 4 h per day at 3,000 to 5,000 meters maintaining of whole body BMD and BMC level in healthy rats (Wang et al., 2016). The variations in the present findings may be partly explained based on previous findings that explain how the numbers of hypoxic episodes, severity, and duration of total exposure may result in different physiological responses (Serebrovskaya & Xi, 2016). In this sense, a small number of short episodes with modest levels of hypoxia (9–16% PiO_2) could lead to benefits (Navarrete-Opazo & Mitchell, 2014) administered repeatedly over days or weeks (Mateika, El-Chami, Shaheen, & Ivers, 2015).

Arterial hypoxemia has been postulated to cause systemic inflammation by activation of regulatory pathways and cytokines, thus causing bone loss (Kent et al., 2011). However, rats exposed to cyclic normobaric hypoxia for 5 weeks showed higher BMD (Guner et al., 2013). Increased ROS production will activate pro-inflammatory cytokines, which cause production of NO in osteoblasts and osteoclasts, among other cells. It is known that NO regulates osteoclast-mediated bone reabsorption, activating osteoblastic activity and inhibiting RANKL expression (Knapp, Blake, Spector, & Fogelman, 2001; Weiss, Ben-Shlomo, Hagag, & Rapoport, 2000). Inhibition of NO in these studies showed BMD levels significantly elevated as well, indicating that there are further mechanism(s) besides the NO-mediated effect in increasing BMD following cyclic hypoxic exposure, such as increased oxidative stress or a VEGF-mediated effect (Guner et al., 2013). On the other hand, no significant changes in the levels of Ca^{2+}, phosphorous (P), and PTH following hypoxic exposure could indicate a restrain of osteoclastic activity and/or stimulation of osteoblastic

activity, affecting bone metabolism via multiple mechanisms. Overall, cyclic modes may inhibit osteoclastic activity and/or stimulate osteoblastic activity; more research is needed to understand these mechanisms (Litovka, 2008).

Hormonal factors could also have an influence on the achieved effects. A group of ovariectomised rats were exposed to the same dose of hypoxia showing a decrease in the assessed outcomes (Wang et al., 2016). This suggests that imbalanced bone remodelling caused by hypoxia occurs in female rats when oestrogen is deficient, leading to possible accelerated bone loss in postmenopausal women (Wang et al., 2016). Although long-term exposure to cyclic moderate hypoxia could have benefits without detrimental effects, establishing the optimal cyclical dose in terms of episode duration and time of exposure for the treatment of skeletal diseases with low oxygen concentrations requires substantial additional research (Mateika et al., 2015).

5.2 Passive Hypoxia

Bone Mineral Density Assessment

After 18 weeks of exposure to passive HC simulating 2,500 m above sea level (twice a week, 16 minutes per session), our finding is according to other previous studies, where values BMD after the HC exposure were maintained or increased. In the present project, whole body BMD increased significantly compared to the control group, whose levels decreased. Although the results indicate that it may cause an individualized response after 18 weeks of passive HC treatment was not enough to improve proximal femoral BMD.

Few in vivo studies have investigated the effects of HC on BMD. During 2 weeks, 4 hours per day at a altitude of 3,000 to 5,000 meters, healthy rats were exposed to HC maintaining BMD levels throughout the body (Wang et al., 2016). In another study, increases in dorsal spine BMD were observed after 5 weeks of exposure to CH (5 days per week, 5 hours per day) at 4,500 meters (Guner et al., 2013). Therefore, a certain number of short

episodes with a modest level of hypoxia (9-16% of PiO2) could generate benefits if administered repeatedly for weeks (Mateika et al., 2015). As a result, passive HC exposure may inhibit osteoclastic activity and/or stimulate osteoblastic activity. However, future research should confirm these effects and elucidate the underlying mechanism and signaling pathways.

5.3 Active Hypoxia

Bone Mineral Density Assessment

Previous authors have suggested that hypoxic training could enhance the physiological experience of training (Scott et al., 2014). Based on the results of the present study, the WBV combined with normobaric cyclic hypoxia treatment increased whole body BMD and to cause a greater individualized response regarding changes in the proximal femur BMD. So, hypoxic stimulus and WBV training could have synergic effects about bone metabolism. Unfortunately, there is limited research regarding the effect of cyclic hypoxic training on bone metabolism. Only two previous studies have investigated the effects of hypoxic training on BMD. As in the results of this investigation, healthy active adults showed improvements in whole body BMD after 8 weeks of normobaric hypoxic training (Martinez-Guardado et al., 2019). However, in trained triathletes 7 weeks of normobaric cyclic hypoxia training at 15% PiO_2 (2 days per week; 60 min per session) resulted in no reported changes (Ramos-Campo et al., 2015). In this sense, applied hypoxic protocol as well as the population that participated in the study could explain these different responses.

Regarding to WBV doses, our findings are similar to those reported by other authors (Merriman & Jackson, 2009). While a control group suffered a decrease in the bone parameters, the WBV training remained unchanged its BMD values. Therefore, WBV training could be considered as a protective agent (Marin-Puyalto et al., 2018). Although BMD maintenance can be considered positive due to the fact that elderly adults face rapid

bone loss (Karakiriou, Douda, Smilios, Volakis, & Tokmakidis, 2011), three times per week of WBV training could be necessary to obtain improvements in the hip region (Gusi, Raimundo, & Leal, 2006). A recent review concluded that long training periods are necessary in order to obtain results of minor clinic relevance in this population (Marin-Puyalto et al., 2018). Therefore, a higher amount of vibration exposure may likely cause larger effects (Gomez-Cabello et al., 2012) with WBV training alone. In spite of this could be a valid alternative for subjects unable to perform other types of training, the combination of WBV training with HC exposure could decrease the training volume for a population, which is unable or unwilling to increase significantly their level of physical activity (Rosenberg et al., 2018).

Muscle mass

Regarding muscle mass, our results showed no effects after 36 sessions along of WBV combined with hypoxic exposure. Previous studies have reported significantly increases in lean mass after strength training in hypoxia (Martinez-Guardado et al., 2019; Yan et al., 2016). These previous findings suggest that combining exercise, being of low to high intensity resistance, with a hypoxic stressor would play a role in slowing sarcopenia (Millet et al., 2016). However, Ramos-Campo, Scott, Alcaraz, & Rubio-Arias (2017) showed no significant changes in lean body mass after training in neither hypoxia nor differences between hypoxic and normoxic treatments. These authors affirm that hypoxia may affect to a different way when it is combined with different exercise types or training structures. So, disparate results could be found in the scientific literature. Baseline characteristics of the participants could also have an influence on effects, even when the WBV training is developed alone (Gomez-Cabello et al., 2013). In NWBV group effect on body composition were not reported either, as it was expected due to the results shown in previous studies (von Stengel, Kemmler, Engelke, & Kalender, 2012). A recent meta-analysis about the effects of

WBV training on lean mass demonstrated that this could lead to improvement in lean mass or muscle mass in younger adults, but not in other populations (Chen et al., 2017).

Muscle strength

Previous studies have shown that strength training in hypoxia seems to cause functional adaptations in skeletal muscle (Lundby et al., 2009). Moderate hypoxia (13%–16% FiO_2) increasing the metabolic stress that is caused by the exercise performed under hypoxic conditions (Kon et al., 2012; Ramos-Campo, Rubio-Arias, et al., 2017; Ramos-Campo, Scott, et al., 2017; Scott et al., 2017). Increased motor unit recruitment has been reported after strength training in hypoxia, which stimulated a larger portion of the muscle during exercise (Scott et al., 2017). However, in the elderly, hypoxia could be ineffective with regard to strength-endurance measurement, and strength training may go beyond the effect of hypoxia (Levine, 2002). In a study by Schega et al. (2013) a group of elderly adults was supplied with intermittent hypoxic training before a strength-endurance exercise program. Analysing the strength-endurance capacity, there were no significant differences between the intermittent hypoxic training group and the control group. It is also possible that a high level of hypoxia may result in performance decrements during training, which could limit any hypoxia-mediated benefits derived from such training (Scott et al., 2016). Ramos-Campo, Scott, Alcaraz, & Rubio-Arias (2017) reported lower muscle performance during two sets of half-squat exercises due to limited oxygen availability.

When exercising in hypoxia, the decrease in oxygen availability increases the contribution of anaerobic metabolism rather than aerobic energy production (Bowtell, Cooke, Turner, Mileva, & Sumners, 2014). An increase in intracellular acidosis due to glycolytic pyruvate production results in higher concentrations of blood lactate, which may contribute to muscular fatigue (Bowtell et al., 2014) and a decline in performance (Balsom, Gaitanos, Ekblom, & Sjodin, 1994). In addition, limited oxygen availability and brief rest intervals

affect the muscle's ability to maintain the balance between adenosine triphosphate (ATP) breakdown and ATP production, thereby limiting cellular recovery after each exercise set (Hogan, Richardson, & Haseler, 1999). In the present study, SaO_2 was significantly lower in the HWBV group when compared with the NWBV group during all training sessions. Decreases in SaO_2 during the four sets of WBV training in hypoxia could be associated with greater anaerobic energy production (Alvarez-Herms, Julia-Sanchez, Gatterer, Viscor, & Burtscher, 2015). Thus, strength performance in the HWBV group could be due to an inadequate supply of ATP to meet the demand of vibratory exercise with the resulting accumulation of metabolic products and ionic imbalance (Scott et al., 2017), which in turn involves higher levels of muscular fatigue and a decrease in voluntary effort during the training. Previous studies suggest that resistance training in hypoxia was perceived as more difficult than in normoxia (Alvarez-Herms et al., 2015; Ramos-Campo, Rubio-Arias, et al., 2017). Therefore, it has been observed that the training protocol and the level of hypoxia used during the strength sessions could cause different adaptations (Ramos-Campo, Rubio-Arias, et al., 2017; Scott et al., 2016). In addition, the specific work/rest ratio and the accumulated volume to achieve metabolic overload could also be dependent on the prior training status of each individual (Scott et al., 2016). Participants in the present study were untrained elderly people, so the combination of WBV training and exposure to hypoxia may have provoked high stress during the session, which could limit their capacity for improvement.

In the present study, the participants who underwent WBV training under normoxic conditions significantly increased the maximal strength of knee extensors. Consequently, a decrease in the H/Q ratio in this group was shown after 18 weeks of WBV training alone. However, as was above mentioned, had no effect on body composition were reported and therefore neuronal mechanisms may predominantly contribute to the observed strength gains in the NWBV group. WBV training consists of two components: reflex muscle contraction

induced by the vibration stimulus and the unloaded exercises performed on the platform (Rees, Murphy, & Watsford, 2007; Roelants, Delecluse, & Verschueren, 2004). This method of training generates simulated mechanical vibrations, activates the neuromuscular control system, increases the level of excitement and the number of motor units recruited, and coordinates synergistic and antagonistic muscles during rapid muscle contractions, thus enhancing muscle function (Delecluse et al., 2003). Intervention has to last for at least 8 weeks to improve muscle strength (Mikhael, Orr, & Fiatarone Singh, 2010) and the volume must be set at around 12–15 minutes of vibration per training session to obtain maximum gains (Marin & Rhea, 2010). Longer and/or more intensive WBV exercise sessions might result in higher motor unit activation, thus achieving better training effects (Delecluse et al., 2003). Also, it appears that the vibration stimulus, while standing with bent knees or squatting on a platform, may exhibit nonlinear adaptations in strength (Rees et al., 2007). Therefore, the standing position, which was adopted on the platform in this study, only allowed the improvement of knee extensor strength, which might have resulted in the strength imbalance between knee extensors and flexors. Adding dynamic exercises (such as deep squats or wide stance squats) to traditional standing with bent knees may facilitate the delivery of vibration to the muscles that would not have been otherwise stimulated (Delecluse et al., 2003; Rees et al., 2007). Although further research is necessary to ascertain this, WBV training, including dynamic exercises, could potentially benefit mobility and functional performance in an older population.

Functional mobility

Although the effects of WBV training on functional performance of elderly people have been reported in the scientific literature (Bemben et al., 2018), to our knowledge, this study is the first testing the changes of a combined WBV training and hypoxia exposure. In the previous study of Schega et al. (2013), an intermittent hypoxic training was applied prior

to a strength-endurance exercise program in a group of elderly adults. Results of this study together with our data may show the inefficiency of training in hypoxia regarding physical measure in the elderly. Little is known regarding the optimal combination between physical activity and hypoxic components, which adapt to the aged-related changes of organism.

TUGT is useful to assess the functional mobility (Sitja-Rabert et al., 2015). Cut off value of greater than 30 s is stated to predict functional dependence (Podsiadlo & Richardson, 1991). Participants of this study showed lower values than 20 s so that they could be considered independent seniors. Similar to previous studies (Beaudart et al., 2013; Buckinx et al., 2014), neither NWBV group nor HWBV group of the present study improved the functional mobility after the low frequency WBV protocol. Beneficial effects could depend of initial values of the participants and people with poor levels benefited most from WBV training (Sitja-Rabert et al., 2015).

5.4 Strengths and Limitations

To the best of our knowledge, this is the first study reviewing and evaluating the possible effects that HC may have on bone metabolism. During the last 30 years, hypoxic interventions with different patterns and severity have enhanced physical and mental functions and contributed to the prevention of ageing and different diseases (e.g., hypertension, Parkinson's Disease) in 2 million patients. Previous reviews have considered the effects of sustained, intermittent and cyclical exposures on cardiovascular and respiratory physiology, health and the overall quality of life. However, potential mechanisms remain unclear and consensus has not been reached on the bone metabolism response to hypoxia. Hence, the contributed knowledge in this work could be relevant for the development of new therapies and treatments for skeletal diseases. Moreover, little is known regarding the optimal combination between physical activity and hypoxic components, which adapt to the aged-

related changes of organism. In this sense, this project also contributed to the increase of knowledge in this field.

Several limitations deserve comment. Most important, the sample size is relatively small and might explain why we were not able to detect significant changes between groups. The difficulty to recruit sample of this age to take part in this kind of studies limits the potential of these researches (Bolam, van Uffelen, & Taaffe, 2013). In this sense, both men and women were included in this study, resulting in different numbers of males and females. Anyway, chi-square tests showed that groups were homogeneous at baseline regarding sexes; furthermore, as no sex by training interactions were found, the results for male and female participants were combined and analysed together. Unfortunately, our sample size was also limited due to logistic conditions (availability of rooms, devices, etc.). Therefore, the results of this study should be interpreted with caution and future research should have into account the inclusion of more participants to test these results in larger cohorts.

Additionally, we did not measure the serum and urinary levels of bone metabolism markers, which could offer more information about the effect of normobaric cyclic hypoxic exposure and WBV training on bone metabolism. Assessment of molecular signalling pathways during HC exposure could also help to elucidate mechanisms responsible for adaptations. In the same way, metabolic stress markers, such as blood lactate concentrations, cortisol or muscle deoxygenation (Formenti et al., 2018) were not measured either, which could have offered more information about the metabolic stress during WBV training in hypoxia.

Regarding to measurement techniques, although DXA is the most widely used bone densitometry technique among the reported studies, the ability of peripheral quantitative computed tomography to assess bone geometric properties may prove advantageous in evaluating the effects of treatment on bone health (Gomez-Cabello et al., 2012). However,

the sensitivity of those instruments in detecting subtle exercise-induced changes in bone geometry has not been exhaustively tested. DXA-derived BMD could monitor bone geometry and this technique could provide appropriate information to design optimal therapeutic exercise programmers (Harding & Beck, 2017). On the other hand, different characteristics of the open-chain approach of isokinetic testing versus the closed-chain one of the WBV exercises (squat positions). Although some studies have reported on the relationship between isometric and isokinetic strength measures and other functional measures (Broekmans et al., 2013; Burnfield, Josephson, Powers, & Rubenstein, 2000), their validity in isokinetic measuring tests is not conclusive. In this sense, other functional tests could help to know if hypoxia training about physical performance in this population. In addition to TUGT, 6min walking and Short Physical Performance Battery (SPPB) have been the most commonly used instruments for measuring physical performance in population studies of aging (Bustamante-Ara, Villarroel, Paredes, Huidobro, & Ferreccio, 2019). SPPB, for example, have a high predictive value for different health consequences such as mobility or disability (Gomez, Curcio, Alvarado, Zunzunegui, & Guralnik, 2013).

Finally, results were obtained in the context of an 18-week trial, and more long-term clinical effects remain unknown. Research on the effects of the training concluded that regarding bone mass among elderly people, continued exercise training is needed to maintain bone mass gain (Gomez-Cabello et al., 2012). Other authors proposed that between 2 and 90 days and most long-lasting protocols on consecutive or alternating days could be necessary to achieve potentially beneficial effects (Navarrete-Opazo & Mitchell, 2014). However, as intervention length remains unclear, an intermediate and detraining measurement might be appropriate to understand kinetic changes.

Chapter 6. CONCLUSIONS

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1 Reviewing current literature of bone metabolism and hypoxic stimulus.

Different modes of hypoxia may lead to different impacts on bone metabolism in both in vivo and in vitro models. While sustained and intermittent hypoxia might inhibit osteogenic differentiation and promote osteoclast function, cyclical hypoxia (know as hypoxia conditioning) has been presented as a promising strategy to beneficially impact bone metabolism. In this sense, moderate oxygen concentration (above 2% in vitro and 9–16% in vivo) administered repeatedly over days or weeks may promote mineralization potential, inhibit osteoclast activity and/or stimulate osteoblast activity. However, additional research is necessary to establish the optimal dose in terms of oxygen concentration and exposure time (episode duration, number of exposures per day and length).

2 To study the effects of passive hypoxia conditioning on bone mineral density of healthy elderly people.

Passive exposure to HC at 16.1% PiO_2 for 36 sessions is enough to significantly increase whole body BMD in older adults, but not in a specific region such as proximal femoral.

3 To study the effects of active hypoxia conditioning on healthy parameters of healthy elderly people.

3.1 To explore the combined effects of whole-body vibration training and hypoxia conditioning on bone mineral density of healthy elderly people.

3.2 To explore the combined effects of whole-body vibration training and hypoxia conditioning on muscle mass density of healthy elderly people.

3.3 To explore the combined effects of whole-body vibration training and hypoxia conditioning on muscle strength of healthy elderly people.

3.4 To explore the combined effects of whole-body vibration training and hypoxia conditioning on functional mobility of healthy elderly people.

Combined with WBV training, hypoxic stimuli tended to generate positive effects on whole body and proximal femur BMD of elderly population. Although changes did not differ significantly between groups, the benefits observed only within the WBV plus hypoxia group may be considered promising but have to be confirmed in future large-scale studies. WBV training generates shear stresses on the bone cells, which might be enhanced by effects related to the hypoxic stimuli. Future research will provide deeper understanding of mechanisms responsible for the effects of normobaric hypoxia combined with WBV training.

Active hypoxia does not cause any effect on muscle mass nor strength of older adults. Functional mobility neither changed following an active hypoxia protocol. Our finding suggests that the work/rest ratio and the accumulated volume could be important factors when untrained elderly people are subjected to WBV training in hypoxic conditions. High stress during the session could limit their capacity for improvement.

However, little is known regarding the optimal combination between physical activity and hypoxic components, which adapt to the aged-related changes of organism.

Chapter 7. REFERENCES

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Chapter 8. APPENDIX

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A. Results Divulgation

    i. Conferences Proceedings

    ii. Research Stays

B. Informed Consent to participate in this project as volunteer

C. Bioethical and Biosecurity Commission Approval of University of Extremadura

D. Risk of bias and confidence rating for in vitro studies

E. Risk of bias and confidence rating ratings for in vivo studies

MARATÓN DE INVESTIGACIÓN JOVEN

II CONGRESO MULTIDISCIPLINAR DE JÓVENES INVESTIGADORES DE EXTREMADURA

El Vicerrectorado de Infraestructura y Servicios Universitarios junto con la Oficina de Responsabilidad Social Universitaria de la UEx certifican que:

Marta Camacho Cardeñosa

Ha presentado como **comunicación oral** el trabajo titulado:

Efectos del ejercicio vibratorio corporal en hipoxia normobárica sobre la densidad mineral ósea.

Y para que conste, se firma en Cáceres a 10 de Marzo de 2016.

D. Antonio Díaz Parralejo
Vicerrector de Insfraestructura y Servicios Universitarios de la UEx

Dª. Dolores Gallardo Vázquez
Directora de la Oficina de Responsabilidad Social Universitaria de la UEx

D. / Dª. Marta Camacho Cardeñosa

Con DNI o Pasaporte nº: 53579268D

Ha asistido al I CONGRESO INTERNACIONAL:

"SALUD Y CALIDAD DE VIDA"

Actividad Acreditado por la Dirección General de Investigación y Gestión del Conocimiento de la Consejería de Salud de la Junta de Andalucía, con 1,5 créditos.

"Los créditos de este programa de actividades no son aplicables a los profesionales que participen en la misma y que estén formándose como especialistas en Ciencias de la Salud"

Celebrado en la Universidad de Almería (Almería) desde 11/10/2018 a 19/10/2018.

Almería a 20 de octubre de 2018.

Rubén Fernández

Código de acreditación: X180249_00

UNIVERSIDAD DE GRANADA

I CONGRESO VIRTUAL INTERNACIONAL EN SALUD Y CALIDAD DE VIDA

Dr. Rubén Fernández García y Dr. Félix Zurita Ortega, directores de este congreso,

CERTIFICAN

que los autores/as: **CAMACHO-CARDENOSA, MARTA; CAMACHO-CARDENOSA, ALBA; BRAZO-SAYAVERA, JAVIER**

han participado con la comunicación titulada "**ENTRENAMIENTO VIBRATORIO CORPORAL EN HIPOXIA NORMOBÁRICA PARA LA MEJORA DE LA MOVILIDAD DE PERSONAS MAYORES**" en el "I CONGRESO INTERNACIONAL ONLINE SALUD Y CALIDAD DE VIDA", organizado por la Asociación de Docentes e Investigadores Jóvenes en Educación y Salud "ADDIJES", el Departamento de Didáctica de la Expresión Musical, Plástica y Corporal de la Universidad de Granada y el grupo de investigación "PSICOLOGÍA, SALUD Y EDUCACIÓN (HUM-760) de la Universidad de Almería celebrado del 11 al 19 de Octubre.

Y para que conste a los efectos oportunos se expide la presente certificación correspondiente a la actividad, en Almería a 19 de Octubre de 2018.

ADDIJES
Asociación de Docentes e Investigadores Jóvenes en Educación y Salud
CIF - G19530383

Fdo.: Rubén Fernández García

Director del I Congreso de Salud y Calidad de Vida

Fdo.: Félix Zurita Ortega

Departamento de Didáctica de la Expresión Musical, Plástica y Corporal
Universidad de Granada

ADDIJES
Asociación de Docentes e Investigadores Jóvenes en Educación y Salud

Área de Humanidades
Universidad de Almería
Grupo de Investigación HUM-760

Área de Didáctica de la Expresión Corporal
Universidad de Gramada
Grupo de Investigación HUM-238

POLITÉCNICA INEF NSCA® SPAIN NATIONAL STRENGTH AND CONDITIONING ASSOCIATION

Madrid, Spain

December 14-15, 2018

XI INTERNATIONAL SYMPOSIUM IN STRENGTH TRAINING

We certify that

Timón, R., Camacho-Cardeñosa, A., Martínez-Guardado, I., Olcina, G., Leal, A., Camacho-Cardeñosa, M. have presented the Poster entitled: **"Effect of hypoxic training and whole-body vibration on muscle strength in elderly"**, at the XI International Symposium in Strength Training held in **Faculty of Physical Activity and Sports Sciences (INEF), Technical University of Madrid,** Madrid (Spain) from 14 to 15 December 2018.

Madrid, 15th December 2018.

Pedro J. Benito Peinado
President of the
Organizing Committee

Ana B. Peinado Lozano
President of the
Scientific Committee

Madrid, España

14 y 15 de Diciembre de 2018

XI SIMPOSIO INTERNACIONAL DE ACTUALIZACIONES EN ENTRENAMIENTO DE LA FUERZA

Se certifica que

Timón, R., Camacho-Cardeñosa, A., Martínez-Guardado, I., Olcina, G., Leal, A., Camacho-Cardeñosa, M. han presentado el Póster titulado **"Effect of hypoxic training and whole-body vibration on muscle strength in elderly"** en el XI Simposio Internacional de Actualizaciones en Entrenamiento de la Fuerza realizado en la **Facultad de Ciencias de la Actividad Física y del Deporte (INEF)** de la Universidad Politécnica de Madrid, entre los días 14 y 15 de diciembre de 2018.

Madrid, 15 de diciembre 2018.

Pedro J. Benito Peinado
Presidente del
Comité organizador

Ana B. Peinado Lozano
Presidenta del
Comité Científico

University of Central Lancashire

International Office
University of Central Lancashire
Preston PR1 2HE
Telephone +44 (0)1772 895026
Fax +44 (0)1772 895030
www.uclan.ac.uk

25th May 2015

Marta Camacho Cardeñosa
C/Padre Leocadio
10002 Cáceres
Spain

Dear Marta

It is my great pleasure to invite you under the auspices of ERASMUS+ to participate in the ERASMUS+ Work Traineeship Scheme at the University of Central Lancashire (UCLan) for the period from 2nd June to 2nd September 2015. Your traineeship will be supported by the School of Sport, Tourism and the Outdoors, and you are expected to collaborate with Dr Darrell Brooks on the project entitled Intermittent High Intensity Training Hypoxia in Obese Adults.

UCLan will not cover the costs of your travel, accommodation and subsistence expenses related to your traineeship as these are expected to be covered by yourself or funded by ERASMUS+. UCLan does not provide insurance services, and this is a responsibility of all students to have a comprehensive travel insurance including the medical cover.

We look forward to welcoming you to UCLan, Preston and the UK.

Yours sincerely,

Shirley Russo
Study Abroad Team
International Office
studyabroad@uclan.ac.uk
Tel: 00 44 1772 896365

University of Central Lancashire

International Office
University of Central Lancashire
Preston PR1 2HE
Telephone +44 (0)1772 895026
Fax +44 (0)1772 895030
www.uclan.ac.uk
www.uclan.ac.uk/international

Marta Camacho Cardeñosa
C/Padre Leocadio

10002 Cáceres
Spain

Friday, 02 October 2015

To Whom It May Concern:

I confirm that Marta Camacho Cardeñosa was an ERASMUS+ Work Traineeship student here at the University of Central Lancashire, Preston, UK for Sem 2 of the 2014/15 Academic Year in the subject area of **Sports Science** as per the following dates: **2th June – 2nd September 2015**

Her period at UCLan was as an ERASMUS+ Work Traineeship under the supervision or Prof Darrell Brooks, in the School of Sports, Tourism and Outdoors.

If you require any further information please do not hesitate to contact me.

Yours sincerely,

Shirley Russo
Study Abroad Advisor
International Student Support Team
studyabroad@uclan.ac.uk

Tel: 00 44 1772 896365

Dept. of Sport Science,
Medical Section
Fürstenweg 185
A-6020 Innsbruck
Austria

Prof. Martin Burtscher
Tel.: *43 512 507 45896
Martin.Burtscher@uibk.ac.at

To
Marta Camacho Cardeñosa
Faculty of Sport Science
Avda. Universidad, s/n
10003 Cáceres
Spain

February 20th, 2017

Dear Marta Camacho-Cardenosa,

It's my pleasure to invite you under the auspices of ERASMUS+ to participate in the ERASMUS+ Work Traineeship Scheme at the University of Innsbruck (Austria) for the period from 1st June to 1st September 2017.

Your traineeship will be supported by myself (Prof. Martin Burtscher) and you will also collaborate with and will be supported by my co-workers.
You are expected to work on our Project "Effects of hypoxia preconditioning" conducted at our institute (Sport Science, Medical Section) at the university of Innsbruck (Austria).

The university of Innsbruck will not cover the costs of your travel, accommodation and subsistence expenses related to your traineeship as these are expected to be covered by yourself or funded by ERASMUS+. The university of Innsbruck does not provide insurance services, and this is a responsibility of all students to have a comprehensive travel insurance including the medical cover.

We look forward to welcoming you to our institute at the University of Innsbruck (Austria).

Your sincerely,

Prof. Martin Burtscher

Dept. of Sport Science,
Medical Section
Fürstenweg 185
A-6020 Innsbruck
Austria

Prof. Martin Burtscher
Fax: *43 512 507 2838
Tel.: *43 512 507 4496
Martin.Burtscher@uibk.ac.at

Innsbruck, 29. August 2017

TRAINEESHIP CERTIFICATE

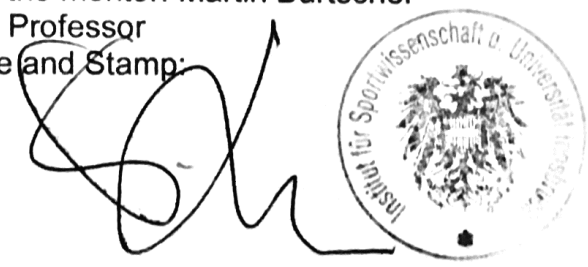
Name of the trainee: MARTA CAMACHO CARDEÑOSA
Personal ID nº: 53579268D
Project Nº: 2015-1-ES01-KA103-014379

Hereby we declare that the above mentioned person has completed a traineeship under the mobility project "BECAS QUERCUS+", funded by the European Programme ERASMUS+, within our organisation, Dept. of Sport Science, Medical Section, University of Innsbruck, located in Fürstenweg 185, Innsbruck, AUSTRIA.

The traineeship began on 01/06/2017 and ended on 01/09/2017 with an overall length of 3 weeks.
The trainee has fulfilled the traineeship programme proposed in the Learning Agreement.

FOR THE HOST ORGANISATION

Name of the mentor: Martin Burtscher
Position: Professor
Signature and Stamp:



25 Marzo 2018
Marta Camacho Cardeñosa
C/Padre Leocadio, 1
10002 Cáceres
España

IMIBIC
INSTITUTO MAIMÓNIDES DE INVESTIGACIÓN BIOMÉDICA DE CÓRDOBA

Estimada Marta Camacho Cardeñosa,

Tenemos el placer de invitarle a realizar una estancia de investigación en el Instituto Maimónides de Investigaciones Biomédicas de Córdoba durante el período del 02 de Abril de 2018 al 30 de Agosto de 2018. Su estancia será desarrollado en el grupo de investigación GC17 Fisiopatología de la Vitamina D. Biotecnología y envejecimiento, colaborando con el Investigador Antonio Casado Díaz en el proyecto titulado "Effects of whole body vibration under normobaric hypoxia conditions on bone mineral density"

Ni el Instituto Maimónides de Investigaciones Biomédicas de Córdoba ni el grupo de investigación GC17, cubrirá gastos de viaje, alojamiento ni otros gastos de subsistencia relaciones con el período de estancia, ya que se espera que estos sean cubiertos por usted o por su grupo de investigación de origen. Del mismo modo, tampoco ofrecerán servicios de seguro, siendo responsabilidad del estudiante tener un seguro que incluya cobertura médica.

Esperamos poder darle la bienvenida en nuestro centro próximamente.

Un cordial saludo,

En Córdoba a 25 de Marzo de 2018

Fdo. Antonio Casado Díaz

IMIBIC
INSTITUTO MAIMÓNIDES DE INVESTIGACIÓN BIOMÉDICA DE CÓRDOBA

D. Antonio Casado Díaz, investigador del Centro de Investigación Biomédica en Red Fragilidad y Envejecimiento Saludable, perteneciente al grupo de investigación GC17 del Instituto Maimónides de Investigación Biomédica de Córdoba

EXPONE:

Que Dña. **Marta Camacho Cardeñosa** con DNI 53579268D, ha realizado una estancia de investigación con el grupo de investigación GC17- Fisiopatología del sistema endocrino de la vitamina D. Biotecnología y envejecimiento en el Instituto Maimónides de Investigación Biomédica de Córdoba entre los días 02 Abril y 30 Agosto de 2018.

El trabajo desarrollado durante la estancia ha versado sobre *"Effects of whole body vibration under normobaric hipoxia conditions on bone mineral density"*

Córdoba a 30 de Agosto de 2018

Fdo. Antonio Casado Díaz

UEX RELACIONES INTERNACIONALES

LETTER OF ACCEPTANCE

We hereby declare to accept Mr/Mrs Marta Camacho Cardeñosa from the University of Extremadura (Spain), as a trainee student in the framework of the ERASMUS+ Programme, for a placement of 3 months, starting on 9th January until 9th March.

The student will be under the direct supervision of Mr/Mrs Pablo Tomás Carús.
Our Company/Institution will offer (please mark your choice if applicable):

- ☐ Accommodation
- ☐ Local Travel paid
- ☐ Meals Paid
- ☐ Others: ____
- ■ None

Company/Institution's Name:	Departamento de Desporto e Saúde. University of Evora
Legal Identification Number:	Erasmus CODE: P EVORA01
Address:	Prolongamento da Rua de Reguengos de Monsaraz, 14 7000-
Contact person:	Pablo Tomás Carús
Position:	Professor
Email:	ptc@uevora.pt
Phone:	+351 266 769 522
Internet site:	http://www.dds.uevora.pt/

Coordinator's name and function: Professor Pablo Tomás Carús Date: 16-Nov-18

Pablo Carús

Coordinator's signature:

UNIVERSIDADE DE EVORA DEPARTAMENTO DE DESPORTO E SAÚDE

In order to host this student for a traineeship, we need you to be registered as host organisation online into our platform http://www.becasquercusplus.net/, through which the UEx ERASMUS+ Placement program is managed. The platform is only for this purpose, it is not a public site and no external persons can see / have access to your data, only our institution.

Hereby you are informed and give your authorization to the management unit of the program to incorporate the data of your entity on your behalf.

☐ **Click only if you disagree**

Erasmus+

Avda. de Elvas, s/n. 06071 E - Badajoz
Tel.: (+34) 924 289 373 Fax (+34) 924 289 374 ori@unex.es
Plaza de Caldereros, 2. 10071 E - Cáceres
Tel.: (+34) 927 257 016 Fax: (+34) 927 257 017 relint@unex.es
UNIVERSIDAD DE EXTREMADURA
www.unex.es/relint

UEX

ESCOLA DE CIÊNCIAS E TECNOLOGIA
DEPARTAMENTO DE DESPORTO E SAÚDE

Évora, 09 March 2019

TRAINEESHIP CERTIFICATE

Name of the trainee: MARTA CAMACHO CARDEÑOSA
Personal ID nº: 53579268D

Hereby we declare that the above mentioned person has completed a traineeship under the mobility Project "BECAS QUERCUS+", funded vy the European Programme ERASMUS+, within our organisation, Dept. Desporto e Saúde, University of Évora, located in Prolangamento da Rua de Refuengos de Monsaraz, 14, 7000, PORTUGAL.

The traineeship began on 09/01/2019 and ende don 09/03/2019 with an overall lenght of 2 months.
The trainee has fulfilled the traineeship programme propotsed in the Learning Agreement.

FOR THE HOST ORGANISATION

Name of the mentor: Pablo Tomas-Carus

Position: Professor

Signatura and Stamp:

UNIVERSIDADE DE ÉVORA

Escola de Ciências e Tecnologia
Departamento Desporto e Saúde

FACULTAD DE CIENCIAS DEL DEPORTE
UNIVERSIDAD DE EXTREMADURA
Avda. Universidad s/n
10003 Cáceres (España)

CONSENTIMIENTO INFORMADO

El propósito de este documento es informarle y solicitar su participación voluntaria en una investigación titulada **"Efectos del ejercicio vibratorio corporal en hipoxia normobarica sobre la densidad mineral ósea"** desarrollado por el doctorando Marta Camacho Cardeñosa y llevado a cabo en la Facultad de Ciencias del Deporte de Cáceres (Universidad de Extremadura).

El **objetivo** de esta investigación es conocer los efectos que la exposición a hipoxia normobárica tiene sobre la densidad mineral ósea y las capacidades funcionales (fuerza, equilibrio y agilidad).
La hipoxia normobárica es una disminución de presión parcial de oxígeno, situación similar a la que ocurre en altura (a partir de unos 1500m. de altitud sobre el nivel del mar), que tiene múltiples beneficios sobre el organismo.

PROCEDIMIENTO Y DURACIÓN DEL ESTUDIO

A lo largo de 18 semanas (de Enero a Mayo de 2017, con una evaluación final en el mes de Octubre para ver efectos a largo plazo) los participantes serán sometidos a diferentes actividades y pruebas de valoración, a saber:

- Realización actividades lúdico-recreativas en cámara de hipoxia, simulando 2000m. de altitud (en Residencia Ciudad Jardín "Parque del Príncipe").
- Realización de un test sobre el consumo de medicamentos, en 1 ocasión.
- Realización de test sobre nivel de actividad física y consumos de calcio y alcohol, en 3 ocasiones.
- Valoración de la composición corporal y la densidad mineral ósea mediante la densitometría ósea (DXA), en 3 ocasiones (se realizará en clínica privada "Alejo Leal")
- Valoración de la fuerza muscular del tren inferior, mediante dinamometría isocinética en 3 ocasiones (a realizar en la Facultad de Ciencias del Deporte)
- Valoración del equilibrio en plataforma vibratoria, en 3 ocasiones (a realizar en la Facultad de Ciencias del Deporte)
- Realización de un test para valorar el equilibrio y la fuerza muscular, en 3 ocasiones (a realizar en la Facultad de Ciencias del Deporte).

RIESGOS

Los riesgos asociados son mínimos, propios de cualquier tipo de actividad física a la que puede someterse. Toda la investigación será controlada por personal titulado y cualificado. En caso de detectar, mediante las pruebas, cualquier tipo de incompatibilidad entre el evaluado y el programa de actividades, el equipo de evaluación le informaría y se suspenderían las pruebas o participaciones restantes.

COSTES

La participación en el proyecto NO será recompensada económicamente. Al finalizar el estudio se le informará del resultado global del mismo si usted así lo desea. El investigador principal, Marta Camacho Cardeñosa puede ser contactado en cualquier momento en el siguiente teléfono, 686 862 365 a fin de recabar información acerca del proyecto y en la siguiente dirección:

Grupo de Avances en el entrenamiento deportivo y acondicionamiento físico
Facultad de Ciencias del Deporte
Av. Universidad s/n
10003 Cáceres
927 25 78 94

BENEFICIOS

Al finalizar el estudio los participantes serán informados mediante un informe de los resultados obtenidos tras el programa de entrenamiento. A parte de lo comentado anteriormente, se estima que el desarrollo del estudio en el que participará comportará beneficios a medio y/o largo plazo sobre parámetros que puedan ayudar a prevenir y/o tratar enfermedades que afectan al metabolismo óseo como es la osteoporosis.

CONFIDENCIALIDAD DE LOS DATOS

Los datos obtenidos serán usados con fines científicos para la mejora del conocimiento. El protocolo de recogida de datos será archivado, y a cada participante se le asignará una clave de tal modo que no pueda relacionarse la

información obtenida con la identidad del sujeto de acuerdo con la Declaración de Helsinki y la ley 14/2007, de Investigación Biomédica.

Para todo lo no previsto en este documento, se aplicará la legislación vigente sobre protección de datos de carácter personal (Ley 41/2002, de 14 de noviembre, básica reguladora de la autonomía del paciente y de derechos y obligaciones en materia de información y documentación clínica, BOE 274 de 15 de noviembre de 2002; Ley Orgánica 15/1999, de 13 de diciembre, de Protección de Datos de Carácter Personal; BOE 298 de 14 de diciembre de 1999; Real Decreto 1720/2007, de 21 de diciembre, por el que se aprueba el Reglamento de desarrollo de la Ley Orgánica 15/1999, de 13 de diciembre, de protección de datos de carácter personal, BOE 17 de 19 de enero de 2008), sobre investigación biomédica (Ley 14/2007, de 3 de julio, de Investigación biomédica; BOE 159 de 4 de julio de 2007) y cualquier otra que resultara aplicable.

Los resultados del estudio pueden ser publicados en revistas científicas o publicaciones de carácter general. No obstante, la información concerniente a su participación será mantenida como confidencial.

FACULTAD DE CIENCIAS DEL DEPORTE
UNIVERSIDAD DE EXTREMADURA
Avda. Universidad s/n
10003 Cáceres (España)

DECLARACIÓN DEL PARTICIPANTE

Don/Doña _____, con DNI _____,

comprendiendo las características, ventajas e inconvenientes de la investigación expuesta y habiendo podido preguntar todo aquello que he considerado oportuno, conociendo que la única finalidad para el que se utilizarán mis datos serán de investigación sin ánimo de lucro, conociendo que en toda actividad física puede existir riesgo (por ejemplo, molestias por cansancio o derivadas de patologías previas; agujetas, etc.), entendiendo que soy libre de abandonar el programa en cualquier momento sin necesidad de justificar mi retiro y solicitar información sobre los resultados, ACEPTO LIBREMENTE COLABORAR CON EL PROGRAMA Y ESTUDIO MENCIONADO ANTERIORMENTE.

Así mismo, estoy de acuerdo con el desplazamiento a las instalaciones ajenas a la Residencia Ciudad Jardín "Parque del Príncipe" para la valoración de la densidad mineral ósea y de parámetros de fuerza y equilibrio en las 3 ocasiones en las que se desarrollarán: Enero – Mayo – Octubre.

Y para que así conste, firmo el presente consentimiento.

Firma:

Nombre y apellidos: _____DNI:_____

Cáceres, a _____ de _____ de _____

Firma de investigador principal que obtiene el consentimiento

---

Fecha ____/____/_____

UNIVERSIDAD DE EXTREMADURA

VICERRECTORADO DE INVESTIGACIÓN, TRANSFERENCIA E INNOVACIÓN

Campus Universitario
Avdª de Elvas s/nº
06071 BADAJOZ

Tel.: 924 28 93 05
Fax: 924 27 29 83

NºRegistro: 17/2016

Dª Mª ANGELES TORMO GARCIA, SECRETARIA DE LA COMISION DE BIOÉTICA Y BIOSEGURIDAD DE LA UNIVERSIDAD DE EXTREMADURA.

INFORMA: Que una vez analizada, por esta Comisión la solicitud de Proyecto de Tesis Doctoral al titulado " Efects of whole body Vibration under normobarix Hipoxic conditions on bone mineral density".cuyo Investigador Principal es D/Dª Marta Camacho Cardeñosa , ha decidido por unanimidad valorar positivamente el proyecto por considerar que se ajusta a las normas éticas esenciales cumpliendo con la normativa vigente al efecto.

Y para que conste y surta los efectos oportunos firmo el presente informe en Badajoz a 6 de abril de 2016

V°B°

Fdo.: Fernando Henao Dávila
Presidente por Delegación de Comisión
de Bioética y Bioseguridad

APPENDIX D. Risk of bias and confidence rating for in vitro studies

Reference	Risk-of-bias questions											Confidence rating
	1	2	3	4	5	6	7	8	9	10	11	
Bouvard, 2014	-	-	NA	NA	+	-	-	+	+	++	+	*Moderate*
Binder, 2015	-	-	NA	NA	+	-	+	+	+	++	+	*Moderate*
Burian, 2017	-	-	NA	NA	+	-	-	+	+	+	+	*Low*
Ciappeti, 2016	++	-	NA	NA	+	-	-	+	-	++	+	*High*
Cicione, 2013	-	-	NA	NA	+	-	-	+	+	++	+	*Moderate*
D'Ippolito, 2006	-	-	NA	NA	+	-	-	+	+	++	+	*Moderate*
Deschepper, 2011	-	-	NA	NA	-	-	-	-	+	++	+	*Moderate*
Ding, 2014	-	-	NA	NA	++	-	-	++	+	++	+	*High*
Gao, 2013	-	-	NA	NA	+	-	-	+	+	++	+	*Moderate*
Gu, 2016	-	-	NA	NA	-	-	-	+	+	++	+	*Moderate*
Holwarth, 2010	-	-	NA	NA	+	-	+	+	+	+	+	*Low*
Hopper, 2015	+	-	NA	NA	+	-	-	-	+	+	+	*Low*
Hsu, 2013	-	-	NA	NA	-	-	-	-	+	+	+	*Low*
Huang, 2011	-	-	NA	NA	+	-	-	-	+	+	+	*Low*
Huang, 2012	-	-	NA	NA	+	-	-	+	+	++	+	*Moderate*
Iacono, 2018	-	-	NA	NA	+	-	-	+	+	+	+	*Low*
Inagaki, 2017	-	-	NA	NA	+	++	-	+	+	++	+	*High*
Jin, 2010	-	-	NA	NA	+	-	-	+	-	+	+	*Low*
Kalinina, 2015	-	-	NA	NA	+	-	-	+	+	++	+	*Moderate*
Lee, 2006	-	-	NA	NA	+	-	-	+	+	++	+	*Moderate*
Lee, 2012	-	-	NA	NA	+	-	-	+	+	++	+	*Moderate*
Lee, 2015	-	-	NA	NA	+	-	-	+	+	++	+	*Moderate*
Ma, 2014	-	-	NA	NA	+	-	-	+	+	++	+	*Moderate*
Malladi, 2006	-	-	NA	NA	+	-	-	+	+	+	+	Low
Merceron, 2010	-	-	NA	NA	++	-	-	++	+	+	+	*High*

Park, 2013	-	-	NA	NA	++	-	-	+	+	+	+	*Moderate*
Pattapa, 2013	++	-	NA	NA	++	-	-	+	+	+	+	*High*
Russo, 2013	-	-	NA	NA	+	-	-	++	-	+	+	*Moderate*
Salamanna, 2018	-	-	NA	NA	+	-	-	+	-	+	+	*Low*
Sengupta, 2010	-	-	NA	NA	+	-	-	+	+	+	+	*Low*
Tsang, 2013	-	-	NA	NA	+	-	+	+	+	+	+	*Low*
Wang, 2012	-	-	NA	NA	+	-	-	+	+	+	+	*Low*
Xu, 2007	-	-	NA	NA	++	-	-	+	+	+	+	*Moderate*
Xu, 2013	-	-	NA	NA	++	-	-	+	+	+	+	*Moderate*
Yang, 2011	-	-	NA	NA	+	-	-	+	+	+	+	*Low*
Yao, 2017	+	-	NA	NA	+	-	-	+	+	+	+	*Low*
Zham, 2008	-	-	NA	NA	+	-	-	++	+	++	+	*Moderate*
Zhang, 2017	-	-	NA	NA	++	-	-	+	+	+	+	*Moderate*
Zhang, 2018	-	-	NA	NA	-	-	-	+	-	+	+	*Low*

++: definitely low; +: probably low; -: probably high (not report); --: definitely high; NA: not applicable.

++ ++ High confidence in the association between exposure to the substance and the outcome. The true effect is highly likely to be reflected in the apparent relationship

+ ++ Moderate confidence in the association between exposure to the substance and the outcome. The true effect may be reflected in the apparent relationship

++ Low confidence in the association between exposure to the substance and the outcome. The true effect may be different from the apparent relationship

+ Very low confidence in the association between exposure to the substance and the outcome. The true effect is highly likely to be different from the apparent relationship

APPENDIX E. Risk-of-bias and confidence rating ratings for in vivo studies

Reference	Risk-of-bias questions											Type of study	Confidence rating
	1	2	3	4	5	6	7	8	9	10	11		
Wang, 2016	+	-	NA	NA	-	-	++	+	++	++	+	EA	*High*
Wang, 2017	++	-	NA	NA	+	-	-	+	+	++	+	EA	*High*
Guner, 2013	-	-	NA	NA	+	-	+	+	-	++	+	EA	*Moderate*
Tomiyama, 2008	NA	NA	+	++	NA	NA	-	-	+	++	+	HCr-Se	*High*
Basu, 2013	NA	NA	++	+	NA	NA	+	+	-	++	+	HCr-Se	*High*
Basu 2014	NA	NA	++	+	NA	NA	+	+	-	++	+	HCo	*High*
Sforza, 2013	NA	NA	+	++	NA	NA	-	+	++	++	+	HCo	*High*
Terzi, 2015	NA	NA	--	++	NA	NA	-	++	++	++	+	HCo	*High*
Tng, 2008	NA	NA	--	++	NA	NA	-	+	++	++	+	HCo	*High*
O'Brien, 2018	-	-	NA	NA	NA	-	-	+	+	++	+	HCT	*Moderate*
Martínez-Guardado, 2019	++	++	NA	NA	NA	++	+	+	++	++	+	HCT	*High*
Ramos-Campos, 2015	+	-	NA	NA	NA	-	+	++	++	++	+	HCT	*High*
Rittweger, 2016	-	-	NA	NA	NA	-	++	++	+	++	+	HCT	*High*

++: definitely low; +: probably low; -: probably high (not report); --: definitely high; NA: not applicable; EA: experimental animal; HCT: human controlled trial: HCo: human cohort; HCr-Se: human cross-sectional.

++ ++ High confidence in the association between exposure to the substance and the outcome. The true effect is highly likely to be reflected in the apparent relationship

+ ++ Moderate confidence in the association between exposure to the substance and the outcome. The true effect may be reflected in the apparent relationship

++ Low confidence in the association between exposure to the substance and the outcome. The true effect may be different from the apparent relationship

+ Very low confidence in the association between exposure to the substance and the outcome. The true effect is highly likely to be different from the apparent relationship