Universidade da Maia

Impact of exercise on bone health in haemodialysis patients

Daniela Filipa Cardoso

Tese de doutoramento em
Ciências do Desporto - Exercício e Saúde

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Daniela Filipa Cardoso

Os Orientadores,

Dr. João Viana

(Universidade da Maia)

Dr. Diogo Leal

(Universidade da Maia)

Dr. Aníbal Ferreira

(Faculdade de Ciências Médicas Universidade Nova de Lisboa)

Dra. Alice Smith

(Universidade de Leicester)

Júri

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Publications

Journal Articles

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Presentations

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Diogo Vaz Leal, **Daniela Cardoso**, Pedro Martins, Manuel Aníbal A. Ferreira, Luke Baker, Alice Smith & João Viana (2023). Associations between physical activity, physical function and bone metabolism markers in haemodialysis patients: a preliminary cross-section study. Abstracts from European Renal Association-European Dialysis and Transplant Association (ERA-EDTA) Congress, Milan, Italy.

Diogo V. Leal, Pedro M. Martins, **Daniela Cardoso**, Manuel A. Ferreira, Kenneth R. Wilund & João L. Viana (2022). Implementation of a National Online Exercise Program in hemodialysis patients. Abstracts from Kidney Week Congress, Orlando, USA.

Daniela Cardoso, Diogo V. Leal, Pedro M. Martins, Manuel A. Ferreira, Luke Baker, Alice Smith & João L. Viana (2022). Effects of intradialytic progressive resistance and aerobic training on physical function. Abstracts from ERA-EDTA Congress, Paris, France.

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Abstract

Chronic Kidney Disease (CKD) is associated with mineral and bone disorders (MBD), which can induce bone loss in those patients. These bone comorbidities tend to aggravate with disease progression, leading to greater bone loss in later stages of kidney disease, particularly those on dialysis. Furthermore, dialysis patients tend to have low levels of physical function and physical activity (PA), that could contribute to greater bone loss. However, exercise has been pointing as a non-pharmacological adjunct strategy to improve bone health in e.g., individuals with osteoporosis; however, there is still little evidence regarding its effect in CKD patients, particularly those on haemodialysis (HD) treatment. Therefore, we investigated (i) the associations between bone biomarkers and markers of physical health; (ii) the effects of regular intradialytic aerobic and resistance exercise training in bone biomarkers and physical health outcomes; and (iii) the effects of a single bout of exercise (aerobic or resistance) in bone biomarkers and physical health outcomes in HD patients. As a results, according to the studies presented in the current thesis, 1) high physical function and muscle strength are associated with low concentrations of bone resorption biomarker (Tartrate-resistant Acid Phosphate 5b, TRAP-5b), as well as a decrease in osteoclastogenesis (Osteoprotegerin, OPG). In addition, increased PA levels are associated with high levels of a marker of bone formation (Osteocalcin, OC) and lower levels of a regulator of bone turnover (OPG); 2) non-exercise seems to reduce bone formation (OC), physical function and PA time; while 12 weeks of aerobic exercise seems to promote an increased in bone resorption and improve muscle strength and endurance. However, resistance exercise improved aerobic capacity when compared to aerobic exercise. Despite, regardless modality, exercise seems to improve overall physical function and muscle strength; 3) finally, a single session of HD treatment as well as a single bout of acute and chronic exercise promotes a decrease in bone formation and an increase in osteoclastogenesis in those patients. These results demonstrate that regular exercise may have an important role in improving overall physical function and activity; however, a single session of intradialytic resistance or aerobic exercise as well as 12 weeks

of both types of exercise seems to not be enough to promote an osteogenic effect in those patients.

Keywords: Chronic Kidney Disease, Bone Biomarkers, Exercise, Physical Function, Physical Activity.

Resumo

A doença renal crónica está associada a desordens minerais e ósseas, que por sua vez, poderão levar à perda óssea nestes pacientes. Estas comorbilidades ósseas normalmente agravam-se com a progressão da doença, levando a uma maior perda óssea em estadios mais avançados da doença, nomeadamente, em pacientes a realizarem diálise. Para além disto, os pacientes em diálise tendem a ter baixos níveis de aptidão física e atividade física, fatores estes que também contribuem para uma maior deterioração óssea. Contudo, o exercício físico tem sido demonstrado como uma terapia não farmacológica coadjuvante na melhoria da saúde óssea em e.g. indivíduos com osteoporose, mas ainda existe pouca evidência relativamente ao seu efeito em pacientes com doença renal crónica, nomeadamente nos pacientes em hemodiálise. Desta forma, investigamos (i) as associações entre os biomarcadores ósseos e os marcadores da aptidão e da atividade física; (ii) os efeitos do treino aeróbio e resistido nos biomarcadores ósseos e na aptidão e atividade física; e (iii) os efeitos de uma sessão de exercício aeróbio ou resistido nos biomarcadores ósseos em pacientes em hemodiálise. De acordo com os estudos presentes nesta tese: 1) elevada aptidão física e força muscular estão associadas a uma redução nas concentrações do biomarcador de reabsorção óssea (Tartrate-resistant acid phosphatase 5b), assim como a uma diminuição da osteoclastogénese (osteoprotegerina, OPG). Além disso, elevados níveis de atividade física estão também associados a um aumento do biomarcador de formação (osteocalcina) e níveis baixos de um regulador do turnover ósseo (OPG); 2) a inatividade física parece reduzir a formação óssea, a aptidão física e o tempo de atividade física diária, enquanto 12 semanas de exercício aeróbio parece promover um aumento na reabsorção óssea e na força muscular dos membros inferiores. Contudo, o exercício resistido quando comparado com o exercício aeróbio, melhorou a capacidade aeróbia. Mas, independentemente do tipo de exercício, há melhora da aptidão física no geral e a força muscular; 3) uma única sessão de hemodiálise, uma única sessão de exercício, assim como uma única sessão de exercício regular, promoveram a diminuição da formação óssea e um aumento da osteoclastogénese em pacientes em hemodiálise. Desta forma, estes resultados demonstram que o exercício regular pode ter um

papel importante na melhoria da aptidão e atividade física. No entanto, uma única sessão de exercício (resistido ou aeróbio) e 12 semanas de treino de ambos os tipos de exercício parecem não ser suficientes para promover um efeito osteogénico nestes doentes.

Palavras-chave: Doença Renal Crónica, Biomarcadores Ósseos, Exercício, Aptidão Física, Atividade Física.

List of abbreviations

ACR	Albumin:Creatine Ratio
ALP	Alkaline Phosphatase
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
BALP	Bone-specific Alkaline Phosphatase
BMC	Bone Mineral Content
BMD	Bone Mineral Density
BMI	Body Mass Index
BMU	Bone Metabolic Units
BV	Blood Volume
CIDESD	Research Center in Sports Sciences, Health Sciences and Human Development
CKD	Chronic Kidney Disease
CKD-MBD	Chronic Kidney Disease-Mineral and Bone Disorder
DKK1	Dickkopf WNT Signaling Pathway Inhibitor 1
CV	Coefficient Variation
DXA	Dual X-ray Absorptiometry
EDTA	Ethylenediaminetetraacetic Acid
ELISA	Enzyme-linked Immunosorbent assay
FGF-23	Fibroblast Growth Factor-23
GFR	Glomerular Filtration Rate
HD	Haemodialysis
HGS	Handgrip Strength
iAET	Intradialytic Aerobic Exercise Training

ISWT	Incremental Shuttle Walk Test
iPINP	Intact Procollagen Type I N-propeptide
iPRT	Intradialytic Progressive Resistance Exercise Training
iPTH	Intact Parathyroid Hormone
IQR	Interquartile Range
KDIGO	Kidney Disease: Improving Global Outcomes
MBD	Mineral and Bone Disorder
MRI	Magnetic Resonance Imaging
Non-RCT	Non-Randomized Controlled Trial
NOS	Newcastle-ottawa Scale
OC	Osteocalcin
OPG	Osteoprotegerin
OPN	Osteopontin
PA	Physical Activity
PBS	Phosphate Buffered Saline
PINP	Procollagen Type I N-propeptide
PTH	Parathyroid Hormone
PV	Plasma Volume
QCT	Quantitative Computerized Tomography
RANKL	Receptor Activator of Nuclear Factor Kappa-β Ligand
RCT	Randomized Controlled Trial
RCV	Red Cell Volume
RPE	Rate of Perceived Exertion
RPM	Repetition Maximum

SD	Standard Deviation
SOSt	Sclerostin
SPPB	Short Physical Performance Battery
STS-5	Sit-to-stand 5
STS-60	Sit-to-stand 60
TGF-β	Transforming Growth Factor beta
TMB	Tetramethylbenzidine
TRAP-5b	Tartrate-resistant Acid Phosphate 5b
TUG	Timed Up and Go
1-eRM	1 Estimated Repetition Maximum
5-eRM	5 Estimated Repetition Maximum

Chapter 1: General introduction

1.1. Introduction

CKD represents a worldwide burden with an estimated prevalence around 13% [1]. According to Kidney Disease: Improving Global Outcomes (KDIGO), CKD is defined as any present abnormalities in the kidney structure and/or function for at least 3 months [2], and can be classified into 5 stages bason upon glomerular filtration rate (GFR) with stage 5 of CKD requiring kidney replacement therapy (dialysis or kidney transplant) [2]. CKD-related complications are well documented [3]. Mineral and bone disorders are one of the main complications in CKD patients and is characterised as a syndrome comprising vascular calcification and biochemical, turnover, microarchitecture and strength bone abnormalities [4]. Changes in bone turnover increase with the disease progression impacting bone health of CKD patients [4,5].

HD patients mainly experience low bone turnover characterized as normal mineralization, microstructural changes and low to normal volume (the amount of bone per unit volume of tissue) [6]. Compelling evidence has demonstrated the positive impact of exercise on bone health in older adults [7], osteoporotic patients [8] and in postmenopausal women [9]. Additionally, recent evidence demonstrates that acute exercise bouts induce an increase in markers of bone resorption, while chronic exercise elicits an increase in bone formation markers in healthy subjects [10]. Despite the uprising volume of research on the effects of exercise in healthy and in osteoporotic populations [8], there is a lack of evidence regarding its effects on bone biomarkers in CKD patients [11]. Yet, the available evidence shows that resistance exercise may elicit a positive effect on bone health in CKD patients; however more trials with different exercise interventions are needed [11]. Additionally, CKD patients also experience low physical function and lower PA levels which impact their health-related quality of life as well as their bone health [12–14]. Despite, both types of exercise aerobic and resistance have also been shown to increase PA levels and to improve physical function traducing in better clinical-related outcomes in those patients [15,16].

Therefore, this present thesis focuses on the examination of 1) the overall effects of PA and exercise interventions on bone health-related outcomes in CKD patients existing in the literature; 2) to describe bone

biomarkers profile, examine relationship between them, and assess their association with the physical function and PA in HD patients and 3) the effects of intradialytic aerobic and resistance exercise on bone turnover markers in this same population. Structurally, this thesis is divided into six chapters: Chapter 1 outlines a general introduction and background that supports this research project; Chapter 2 focuses on the general methods regarding the experimental studies, and also describes the process of the development of the intradialytic resistance exercise training machine and the performed protocol; Chapter 3 show a systematic review of the impact of PA and exercise on bone health in patients with CKD (https://doi.org/10.1186/s12882-020-01999-z); Chapter 4 presents a cross-sectional study related to the associations between bone biomarkers, physical function and PA in HD patients; Chapter 5 and Chapter 6 are both experimental studies exploring the chronic and acute effects of intradialytic aerobic and resistance exercise on bone biomarkers in HD patients, respectively. Lastly, Chapter 7 provides a summary and an overall discussion of the main findings.

1.2. Background

Chronic Kidney Disease

1.2.1. Definition, classification, and epidemiology

CKD is a condition defined by the presence of either or both abnormalities of kidney function or structure, for at least 3 months [17]. The criteria for CKD is based on the presence of one or more markers of kidney damage such as: albuminuria, electrolyte and urine sediment abnormalities, structural abnormalities detected by imaging, abnormalities on histology and a history of kidney transplantation; or a decreased in kidney function observed through GFR lower than 60ml/min/1.73m^2 [17]. The current CKD classification is based on cause (clinical context), GFR and albuminuria category (Table 1.1. presented in the next page) [17]. There is evidence of a relationship between a reduced estimated GFR (eGFR) and an increase in albuminuria hallmarks [17]. Despite a variety of equations that have been developed to measure kidney

function [18,19], GFR is still the most adequate indicator of kidney function, and thus is frequently measured in CKD patients [17]. GFR indicates the total amount of fluid filtered through kidneys per unit of time (ml/min/1.73m^2) (21). CKD is classified into 5 stages according to GFR: stage 1 (≥90 mL/min/1.73m²), stage 2 (60−89 mL/min/1.73m²), stage 3a (45−59 mL/min/1.73m²), stage 3b (30−44 mL/min/1.73m²), stage 4 (15−29 mL/min/1.73m²) and stage 5 (<15 mL/min/1.73m²) [17]. Yet, it may also be characterised in terms of albuminuria levels, from which 3 stages are defined: A1 (<30 mg/g), A2 (30−300 mg/g) and A3 (>300 mg/g) [17].

Table 1.1. Stages of CKD based on GFR and albuminuria categories (adapted from KDIGO 2024 guidelines)

CKD STAGES	DESCRIPTION	GFR, ML/MIN/1.73M^2	ACR, MG/G
1	Normal or high kidney function	<90	-
2	Mildly decreased kidney function	60-89	-
3A	Mildly to moderately decreased kidney function	45-59	-
3B	Moderately to severely decreased kidney function	30-44	-
4	Severely decreased kidney function	15-29	-
5	Kidney failure	<15	-
A1	Normal to mildly increased in kidney damage	-	<30
A2	Moderately increased in kidney damage	-	30-300
A3	Severely increased in kidney damage	-	>300

ACR, Albumin: Creatine Ratio; CKD, Chronic Kidney Disease; GFR, Glomerular Filtration Rate; KDIGO, Kidney Disease: Improving Global Outcomes.

CKD is a worldwide health problem with a huge rate of mortality, with around 1.2 million people dying from CKD all over the world in 2017 [20]. In addition, CKD is highly prevalent and the global prevalence of CKD in stages 1 to 5 was 13.4% in 2016 [1]. However, for CKD patients in stages 3 to 5 it was 10.6%, only and was predominantly higher in stage 3 of CKD (7.6%) [1]. It is well established that CKD prevalence rises with age [1], since GFR declines

with increasing ageing [21], thus, is highly prevalent in elderly [1]. In Portugal, the overall prevalence of CKD stages 1-5 was 20.9% in 2018, with a significant increase in older groups [22]. In addition, recent data of this country revealed that in 2020 there was around 2011.31 patients per million population in kidney replacement therapy [23]. Alongside, CKD seems to be more common in women than in men [1]. A long-life expectancy of women as well as a possibly overdiagnosis of CKD with an estimated GFR equations, can explain such differences [24]. The data from the Portuguese cohort are in line with previous literature with CKD being predominantly higher in Portuguese females rather than Portuguese males [22].

1.2.2. Aetiology and complications

CKD can result from a variety of distinct aetiologies [25–27]. However, the overall main causes of CKD are diabetes (corresponding to 30-40% of all CKD) and hypertension [25]. However, there are other risk factors related to the development of CKD, such as vascular disease, genetic factors, glomerulonephritis, renal fibrosis, obesity and low birthweight [25–27]. The initial progression of CKD, although often asymptomatic, impairs the quality of life of these patients due to complications associated with the disease [3,26].

The main CKD-related complications are briefly reported in Table 1.2, presented in the next page [3,26]. Of note that of the overall complications, metabolic acidosis that occurs at stage 3 [25] can induce muscle wasting, bone disease (including bone quantity loss) and CKD progression [5,26] may even lead to mortality [28]. As the disease progresses, the prevalence of metabolic acidosis increases getting worse in later CKD stages [29].

Table 1.2. A brief description of CKD complications (adapted from Bello et al. [3] and Webster et al. 2017 [26]).

SYSTEMS	COMPLICATIONS
CARDIOVASCULAR	Atherosclerosis, cardiomyopathy, and hypertension
ENDOCRINE AND METABOLIC	Menstrual disorders, sexual dysfunction, pregnancy disorders, infertility, electrolytes and mineral and bone disorders
GASTROINTESTINAL	Anorexia, nausea, emesis, and weight loss
HAEMATOLOGICAL	Anaemia, platelets disorders, coagulopathy, low cell count and infection risk
NEUROLOGIC	Neuropathy, seizures, and strokes
MUSCULOSKELETAL	Mineral and bone disorders, fractures, myopathy
INTEGUMENT	Dry skin, dermatitis, and pruritus
OTHER COMPLICATIONS	Fatigue, insomnia, impotence, cachexia, shortness of breath, cognitive impairment, itch, metabolic acidosis, and cramps

1.2.3. Kidney replacement therapies

Once in kidney failure stage (GFR<15 ml/min/1.73m^2), a kidney replacement therapy is required which may be dialysis (including HD or peritoneal dialysis) or kidney transplant [17,25]. Moreover, kidney failure with comprehensive conservative care may also be considered as an option for those patients who are not eligible to start kidney replacement therapy [17]. The purpose of this comprehensive conservative care is controlling the symptoms derived from their pathological condition [2,30]. Men are more likely to start kidney replacement therapy than women [24]. In Portugal, in 2020 61% of men have reached kidney replacement therapy comparing to 39% of women [23].

Dialysis treatment is the most common type of treatment among CKD patients in kidney replacement therapy. In 2019, the overall prevalence of HD and peritoneal dialysis treatment (from 34 countries analysed) was 63%, with HD being the most common therapy [26,31]. Dialysis treatment is essential for patients' survival and the main goal is the removal of uraemic solutes and fluid that are accumulated in kidney patients and cannot be removed by their kidneys, with this therapy ultimately leading to an improvement of their condition (34). CKD patients normally initiate dialysis when one or more of the following symptoms is present: signs or symptoms of kidney failure, malnutrition, incapacity to control volume status or blood pressure [2]. However, even in dialysis treatment mortality remains high (around 16-22%) comparing to the general population, and most of the deaths in CKD setting are linked to cardiovascular disease [32]. Nonetheless, the evidence shows that the mortality rate could be reduced with an earlier referral of patients to kidney replacement therapy [32].

1.2.3.1. Haemodialysis

In 2019, HD treatment was one of the most used treatment modalities corresponding to a total of 84% of all patients on kidney replacement therapy in Europe and countries bordering the Mediterranean Sea [31]. HD treatment is also the most common treatment for kidney failure in Portugal, with 1209.72 (in a total of 2011.31 patients) cases per million population [23]. This type of treatment is usually performed through a dialysis machine, 3 times per week for 3 to 5 hours per session [32]. The dialysis machine removes the fluids from the patients' blood trough a dialyzer [33]. In addition, to carry out the HD treatment, a vascular access on each patient (arteriovenous fistulae, arteriovenous grafts or central venous catheters) is needed [25,33]. Despite of HD treatment being crucial for patients' survival, there are some intradialytic complications associated that should be considered, such as: hypotension, cramping, nausea, vomiting, headaches, fatigue and arrhythmias [34].

1.2.4. Definition and pathophysiology

Mineral and bone disorder (MBD) is one of the main complications of CKD patients [26]. CKD-MBD is considered a syndrome that comprises a combination of bone and biochemical abnormalities and vascular calcification leading ultimately to mortality, fractures and cardiovascular morbidity [4,35]. Bone metabolism in CKD patients is highly affected as the disease progresses [26] resulting in biochemical abnormalities encompasses calcium, phosphorus, parathyroid hormone (PTH) and vitamin D metabolism changes [4].

Furthermore, CKD induces abnormalities in bone that include changes in bone turnover, mineralization, volume, linear growth or strength leading to bone pain or increased bone fragility [4]. Those changes commonly begin earlier, at CKD stage 3 [4,36,37], becoming worse in stages 4 and 5, especially in those undergoing dialysis [38], and may lead to a decline in the bone health of those patients [5].

The hyperphosphatemia (high phosphorus levels) experienced by CKD patients contributes to calcitriol deficiency, an increase of PTH secretion, and for the stimulation of fibroblast growth factor 23 (FGF-23) secretion. On the other hand, calcitriol deficiency accompanied by the decrease of intestinal calcium absorption leads to hypocalcaemia (low potassium levels) and consequently secondary hyperparathyroidism (increased PTH levels). In addition, CKD also induces an increased FGF-23 secretion, due to high phosphate levels and low GFR and klotho in parallel with an increase of the activin A (a multifunctional growth and differentiation factor) in circulation [39,40]. Activin A activation regulates RANKL levels promoting osteoclastogenesis leading to osteoclasts development and function in CKD patients [40]. All these factors together have an important impact on osteoblast function resulting in a high bone turnover characterized by an excess of bone resorption rates, skeletal frailty and consequently an increased fracture risk [5,41,42].

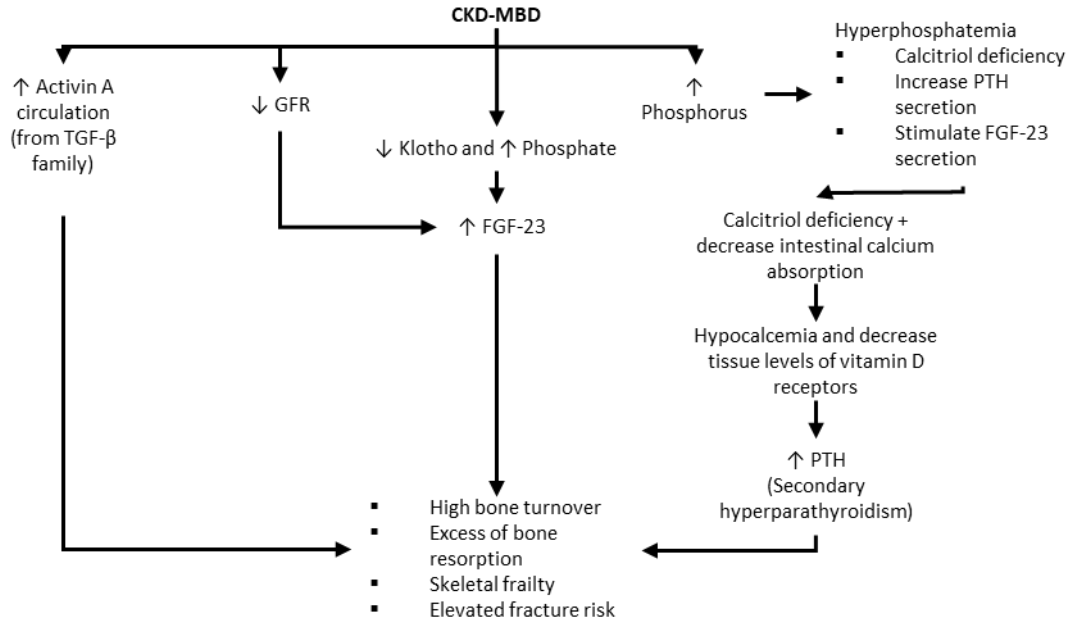

Figure 1.1. Mechanisms of CKD-MBD induced high bone turnover and bone fragility. FGF-23, Fibroblast growth factor 23; GFR, Glomerular Filtration Rate; PTH, Parathyroid Hormone; TGF-β, Transforming growth factor beta.

1.2.5. Bone turnover in chronic kidney disease

Bone is a dynamic tissue in continuously remodelling mediated by a group of cells called basic multicellular units that comprises osteocytes, osteoclasts and osteoblasts [43]. To maintain bone strength and mineral homeostasis, bone is renewed under a balanced and sequential activity of the following phases: activation, resorption, reversal, formation and mineralization [43]. The activation phase initiates the bone remodelling process after homeostasis changes or structural damages detection. Osteoclasts are activated starting bone resorption phase for 10 to 30 days [10]. Then, occurs the osteoclasts apoptosis, and osteoblasts initiate the mineralization process about 14 days in and taking around 3 months to be completed [10].

Bone remodelling is influenced by systemic (PTH, calcitriol, growth hormones, glucocorticoids, and sex hormone) and local factors, responding to mechanical loading applied, maintaining skeletal integrity [5]. While the bone remodelling process occurs during adulthood and includes all phases previously detailed for 3 to 6 months, this mechanism is related to the process of bone

formation and resorption at a specific site, that allows to shape skeletal elements and ensure the acquisition of the appropriate bone mass and morphology during growth [44,45].

CKD patients typically have bone abnormalities with an unbalance between bone formation and resorption leading to the loss of bone strength loss [5]. Loss of bone strength is associated with a reduction in bone quantity (bone mineral density (BMD)) and quality (microarchitecture, geometry and turnover) [46]. As described previously (page 29 in 1.2.4. section), changes in bone turnover are one of the main complications derived from CKD-MBD [35]. CKD patients can experience high bone turnover or low bone turnover/adynamic bone disease [5,47]. Adynamic bone disease is predominant in CKD patients, especially in those on dialysis (stage 5) and is characterised by low bone turnover, normal mineralisation and low to normal bone volume [6]. In turn, CKD-induced high bone turnover is characterized by high PTH levels and osteoclast activity, impaired mineralization and microstructural changes at cortical and trabecular bone with predominantly cortical bone loss [5,48].

Both abnormal mineralization and an increase in bone resorption rate could also lead to decreased bone mass in those patients [5]. According to the evidence, the severity of bone disease increase as the kidney function declines [4,5]. Thus, patients in later stages such as in HD treatment tend to have low bone mass mainly exacerbated by advanced age, low body weight and serum albumin as well as high serum alkaline phosphatase and PTH levels [49].

1.2.6. Diagnosis of bone turnover in chronic kidney disease

Bone health can be assessed throughout several methods such as dual-energy X-ray absorptiometry (DXA), trabecular bone score, quantitative computerized tomography, magnetic resonance imaging); however, bone biopsy is still considered the gold standard in the diagnosis and classification of CKD-MBD, reflecting bone turnover, volume, and mineralization at a define site [47,50]. Despite this, this invasive method is poorly performed in CKD patients unless if the result of the subsequent analysis will impact treatment decisions; or if CKD patients in stages 3 to 5 reveal biochemical abnormalities of CKD-

MBD and low BMD [47]. Alongside to this method, KDIGO recommend assess areal BMD (defined as bone mineral content (BMC) per unit area (g/cm2) and fracture risk of CKD patients through DXA which is based on T-scores [47,51,52].

According to KDIGO guidelines, DXA is only recommended to assess fracture risk in CKD stages 1 to 3 and in patients who do not show markers of CKD-MBD in their biochemical analysis [47]. For CKD stages 3 to 5, including those on dialysis, KDIGO suggests the use of DXA if the results may affect treatment management [47]. When this is the case, DXA can predict fracture risk through BMD at the forearm, hip and spine [52]. However, there are some limitations underlying their utilization. DXA cannot detect changes in bone microarchitecture and turnover, it does not distinguish cortical and trabecular bone, and it underestimates BMD in individuals with low structure [53]. Thus, some bone turnover markers have been suggested to be used in clinical practice [54], exactly because they reflect quick changes in bone turnover and represent the skeleton as a whole, in contrast bone histomorphometry, which only shows its activation at a definite site [55].

Despite some sources that may influence bone biomarkers concentrations (table 1.3. presented in the next page) [56,57], they are cost-effective, the samples which biomarkers are analysed are easy to collect, reveal rapid bone changes comparing to other clinical tests (e.g. longer pharmacological/exercise interventions periods are needed to observe changes in bone quantity measured by DXA), show acute effects and the turnover rate of the skeleton as a whole, and seem to be a very promising tool to evaluate bone turnover in CKD patients [54,56].

Table 1.3. Factors that may influence bone biomarkers concentrations [57].

LIMITATIONS	DESCRIPTION
AGE	↓ concentration during the fourth decade of life in women
	↓ concentration during fifth decade of life in men
SEX	↑ concentrations in young men (<35 years old, comparing to women with similar age)
	↑ concentrations in postmenopausal women (comparing to men with similar age)
MENOPAUSE	↑ concentrations with menopause
LIFESTYLE	↑ concentrations with smoking
	↓ concentrations with alcohol consumption
FOOD INTAKE	↓ concentration of bone resorption after breakfast (around 50%)
CIRCADIAN RHYTHM	↑ concentrations of bone resorption markers at night
	↓ concentrations of bone resorption markers in the afternoon
EXERCISE	↑ concentrations of bone formation with intense exercise
	↓ concentrations of bone resorption with intense exercise

Bone turnover biomarkers have been validated and suggested as a surrogate of bone histomorphometry [56] as they reflect the metabolic activity of bone [57]. Bone biomarkers can be classified into markers of bone formation [bone-specific alkaline phosphatase (BALP), osteocalcin (OC), Procollagen Type I N-propeptide (PINP) and Procollagen type I carboxy-terminal propeptide], resorption (pyridinoline, deoxypyridoline, carboxy-terminal crosslinked telopeptide of type 1 collagen, amino-terminal crosslinked telopeptide of type 1 collagen, hydroxyproline, hydroxylysine, bone sialoprotein, osteopontin, tartrate-resistant acid phosphatase 5b (TRAP-5b) and cathepsin K) and regulators of bone turnover (receptor activator of nuclear factor kappa-B ligand (RANKL), dickkopf WNT signalling pathway inhibitor 1, osteoprotegerin (OPG) and sclerostin) [57–59].

Despite a huge variety of bone biomarkers, some of them have been pointed as promising biomarkers in CKD settings [54,60]. However, there is still

no consensus on which bone biomarkers should be assessed to evaluate bone turnover in CKD patients [54,56]. The selection of an ideal bone biomarker to assess bone turnover should depend on the clinical context, thus the same biomarker will not necessarily be used for all bone diseases (i.e., osteoporosis vs CKD) [61]. As an example, in CKD patients especially those on dialysis is important to measure biomarkers that are not cleared by the kidneys such as BALP and TRAP-5b [62], as the concentrations could not be influenced by the kidney function. KDIGO recommends measuring serum PTH or BALP to evaluate bone turnover in CKD stages 3 to 5 including those on dialysis [47]. Nonetheless, the evidence points towards other biomarkers that may be considered to assess bone turnover in CKD settings [60,62,63]. The following sections will briefly describe some bone biomarkers that have been recommended for bone turnover assessment markers in CKD patients.

1.2.7. Bone formation markers

1.2.7.1. Bone alkaline phosphatase

BALP is a bone-specific isoenzyme known for being specific for bone disease [64]. This bone formation biomarker is not cleared by the kidneys and is present in plasma or serum [64], with relatively low biological variability [65]. BALP has a reasonable correlation with bone histology [66] and a good sensitivity to distinguish high versus low bone turnover [54]. There is evidence of an association between high levels of BALP and mortality rate and fracture risk in dialysis patients [67,68]. According to the evidence, BALP levels may raise in clinical conditions with an increase in bone turnover such as osteoporosis and postmenopausal [69]. The same occurs with HD patients [66]. The concentration of this biomarker increases with high bone turnover and decreases with low bone turnover [66]. Additionally, BALP levels tend to increase over 24 months of dialysis, which may indicate an increased bone turnover [70].

1.2.7.2. Osteocalcin

OC is a bone-derived marker involved in the mineralization process [71]. This bone formation marker is released during both bone resorption and formation [56]. However, OC is released into the circulation in different fragments and is influenced by the kidney function due to a decrease in renal clearance [72], which may limit their usefulness as a marker of bone formation in the setting of CKD [56,60]. Despite this, significant correlations between OC and bone histomorphometric parameters (gold standard) in HD patients were observed [73], however, these findings may not be sufficient to use plasma OC as a biochemical marker of bone turnover in these populations. In postmenopausal osteoporotic women, OC levels are increased compared to non-osteoporotic women [58]. Additionally, these levels are also higher in premenopausal women (>40 years old) than in younger ones [72]. In CKD patients, OC concentrations are usually high [74–76], maybe due to uremic milieu that consequently leads to an increase in bone metabolism of these patients. Besides, OC concentrations decrease during the dialysis section, however, their levels tend to normalise within 6 hours [77,78].

1.2.7.3. PINP

PINP is a marker of bone formation that expresses bone metabolism, particularly bone collagen formation rate [10,54]. PINP is a stable bone biomarker that is not influenced by the kidney function and has a small variability [56]. PINP levels usually increase in osteoporosis and postmenopausal, which are associated with high bone turnover [58]. As described in section 1.2 (subsection 1.2.4), high bone turnover is associated with an increase in bone resorption. Thus, it explains why it occurs in osteoporosis and postmenopausal women. In CKD patients, intact PINP (iPINP) should be preferred instead of its monomeric form [71], as this is neither influenced by GFR nor cleared by the HD treatment [56]. In HD patients its concentration is higher in those patients with hyperparathyroidism and a single HD session seems to not change PINP levels [78]. Despite being a promising bone biomarker to assess bone formation in CKD patients, the literature about this biomarker in these populations is still scarce [56].

1.2.8. Bone resorption markers

TRAP-5b is considered an osteoclast-derived enzyme and a fairly relevant marker of osteoclastic activity in CKD patients [63]. This bone biomarker is released during bone resorption reflecting osteoclast number and activity [79], and it is known to have an important role in the diagnostic and prognostic of metabolic bone disease including renal osteodystrophy [80]. Evidence shows that a single HD session does not have a significant impact on TRAP-5b levels in HD patients [81]. In addition to the fact this biomarker is not influenced by kidney function, TRAP-5b also does not have a circadian rhythm and is not influenced by food intake [81], which makes it a potential biomarker of bone resorption in CKD patients. TRAP-5b has been shown to be correlated with histomorphometric parameters including cortical bone loss rate in HD patients [82]. However, despite their clinical utility in an HD setting, are not widely used to assess bone resorption in CKD patients [63,81]. The available assays of TRAP-5b not specific to bone may explain the limited use of this biomarker [71]. The mechanisms of TRAP-5b induced bone resorption are not fully understood [71]. In spite of this, serum TRAP-5b concentration has been reported to be higher in HD and pre-dialysis patients compared to healthy controls [76,81,83]. However, long-term HD treatment seems to not influence TRAP-5B concentrations. After one year of follow-up, TRAP-5b concentrations remain stable in HD patients [84].

1.2.9. Regulators of bone turnover

1.2.9.1. OPG and RANKL

OPG is a protein secreted by osteoblasts and is responsible for the inhibition of osteoclasts formation, function and survival through their linkage to RANKL [85]. Evidence shows that OPG deficiency could induce a decrease in BMD leading to a decrease in bone strength [86]. In healthy adults, OPG concentrations increase with age in both men (mean age: 50 ± 51.7) and women (mean age: 51.9 ± 51.3) [87]. In addition, there is a relationship between OPG levels and bone metabolism suggesting that high OPG levels are associated with a positive balance of bone formation [86]. However, OPG is three times higher in CKD patients, including HD patients, compared to healthy

controls [76,88,89]. The increase OPG levels in CKD patients could be explained by inflammation, high levels of FGF-23, and kidney function [90]. In addition, their concentration increases over CKD progression due to the reduction in GFR [85,90]; however, even with high OPG concentrations, these are lower in HD patients with low bone turnover compared to those with high bone turnover [5] inducing an increase in osteoclastic activity [91,92]. A single HD session could induce a significant 15% reduction in OPG levels [88].

The ratio of RANKL/OPG is commonly used to observe osteoclastogenesis [89]. RANKL is a protein produced by the osteoblasts, osteocytes and activated T-cells and has an important role in osteoclasts formation, differentiation, activation and survival [89]. Thus, RANKL can control the bone resorption process and mineral loss [89]. RANKL levels rise in postmenopausal osteoporosis [93]. Nevertheless, the concentration of this biomarker is significantly higher in HD patients than in healthy controls [76]. The RANKL/OPG ratio is increased in HD patients compared with healthy controls [94], indicating a high bone resorption [86].

1.2.9.2. Sclerostin

Sclerostin is derived from osteocytes and negatively regulates bone formation [56,95]. This biomarker is known to be negatively correlated with bone histomorphometric parameters of turnover (bone formation rate/bone surface and activation frequency) [96]. It is well known that serum sclerostin is increased in males and elderly subjects [97]. Additionally, it rises in women over age up to 45 years old and remains elevated in postmenopausal women [98]. Besides, according to the literature, sclerostin levels rise with a decrease in GFR with the greatest increase in dialysis patients [99], which may downregulate bone formation in those patients.

Table 1.4. Summary of the impact of CKD on bone biomarkers

Marker of bone formation	↑ BALP (high bone turnover) in HD [66] ↓ BALP (low bone turnover) in HD [66]
	↑ OC [14]
	↑ PINP (with hyperparathyroidism) in HD [78]
Marker of bone resorption	↑ TRAP-5b in HD [83]
Regulator of bone turnover	↑ OPG [90]
	↑ RANKL in HD [5]
	↑ Sclerostin [100]

BALP, Bone alkaline phosphatase; HD, Haemodialysis; OC, Osteocalcin; OPG, Osteoprotegerin; PINP, procollagen type I N propeptide; RANKL, Receptor Activator of Nuclear Factor Kappa B Ligand; TRAP-5b, Tartrate-resistant acid phosphatase 5b.

1.3. Exercise and chronic kidney disease

CKD patients frequently experience low levels of PA that tend to decline further with disease progression [13,101]. Moreover, HD patients experience the highest sedentary levels which is aggravated throughout dialysis vintage [13,101]. Wilkinson at el. reported that only 6% of HD patients were considered active, which consequently results in a total of 94% of inactive patients [101]. Despite current PA guidelines, CKD patients are far from reaching the recommendations, as it is well established that physical inactivity is highly prevalent across CKD stages [101]. According to the current guidelines, CKD patients, including those on dialysis, should perform 150min of moderate exercise intensity or 75min of vigorous activity per week or even a combination of both moderate and vigorous PA [102]. For those in HD, they also can exercise outside or during dialysis or a combination of both [102]. However, according to the evidence, HD patients only performed 23.9 ± 32.6min per day of moderate to vigorous PA [103]. To explain this low engage to PA there are multifaceted pathophysiological mechanisms that may contribute to induce exercise intolerance among CKD patients [104]. Among a variety of factors, skeletal muscle dysfunction and sarcopenia may determine exercise intolerance in those patients [104]. Additionally, CKD patients particularly those on dialysis have been reporting some barriers to exercise [105]. The most reported are

tiredness, muscle fatigue and body pain [105]. Moreover, there are some myths related to exercise in HD patients, including the belief that exercise induces muscle cramps, should be avoid in hypertensive and volume-overloaded patients, and that weightlifting exercises should be avoided in fistula arm [106]. Together may decreased PA engagement [107] and it is well known that lack of PA induces negative effects on health-related outcomes as well as on bone health in CKD patients [11,13]. Moreover, increased PA levels have been associated with a reduced mortality in end-stage kidney disease patients [108]. HD patients could exercise during HD treatment or out-of-clinic. Despite that, intradialytic exercise has some advantages, including time efficient, higher compliance, less patient burden and safety [109]. However, there is a limited choice of exercise types, intensity and mobility, potential hemodynamic instability and muscle catabolism [109]. According to recent evidence, 71% of exercise is prescribed during dialysis [110] which represents the majority of exercise interventions. Among this prescription, intradialytic aerobic and resistance exercise are the most prescribed types of exercise, of note that aerobic exercise is the most used type around 38.8% [110]. However, intradialytic exercises interventions are prescribed with low volume and intensity which may compromise positive effects on health in HD patients [111]. Thus, more studies looking for the exercise effects on health-related outcomes prescribing higher exercise volume and intensity are needed.

1.4. The role of exercise on bone-health related outcomes

The load applied by muscle on bone, known as stress, induces bone mechanical deformation. In turn, this mechanical deformation can be measured as strain. Osteocytes are the main cells that can detect mechanical loading and transmit the information to the osteoblasts and osteoclasts, controlling bone remodelling process [112]. However, to activate bone cells a minimum effective strain for bone remodelling is required and must be exceed approximately 3000 microstrain [113,114]. There is an association between the strain rate and bone formation response, thus an increase at the strain rate leads to an increase in bone formation [115]. Muscle contraction and ground-reaction forces are the main factors to initiate bone adaptation to exercise [112]. It is widely recognised

that exercise influences bone health contributing to maintain bone strength [112], whereas a lack of exercise has been associated with a higher loss of bone mass [113]. According to the evidence, mixed loading impact exercises improve BMD at the lumbar spine and femoral neck in older adults [7]. In addition, weight-bearing exercises help to maintain bone mass and consequently increase bone strength, thus this type of exercises are recommended during middle and older age [112]. However, not all types of exercise promote osteogenic effects on bone [116]. Of note that dynamic multidirectional exercises with high-intensity and impact should be performed in order to promote osteogenic responses [112]. In addition, exercises must include rest periods between loading cycles and sufficient intensity [116]. Exercise intensity should be highlighted, as is one of the most important factors for osteogenic response promotion [116].

1.4.1. The overall effects of exercise on bone biomarkers

The latest evidence shows that the bone response to exercise is influenced by exercise duration and volume [117]. Higher exercise duration and volume induce an increment in bone response in healthy subjects [117]. Markers of bone resorption seem to be more responsive to exercise [117]. However, the acute effects of exercise on bone formation markers are small and findings are still inconsistent [117]. Yet, some evidence highlighted that OPG and BALP increased after plyometric exercises in young boys (mean 10.2 years old) and men (mean 22 years old), suggesting that bone formation can be stimulated by one session of high mechanical stimuli [118]. However, OPG increased immediately after exercise, while BALP increased only after 24h of post-exercise [118]. Scott et al., reported an increment in OPG levels after endurance exercise; however, it was not intensity-dependent [119]. In addition, sclerostin levels increased after a single bout of high-impact (circuit of jumps) and resistance exercise composed of 3 sets of muscle-strengthening and core stabilization exercises [120,121].

The chronic effects of exercise on bone metabolism have also been reported [10]. Resting bone formation markers typically increase as an adaptive response to chronic exercise training [10]. Prolonged exposure to exercise

induced an increased in BALP and PINP levels [10]. According to the literature, PINP levels seem to increase after one [122] and four [123] months of endurance exercise training; whilst BALP only increased after two months of plyometric training [124] and two to five months of resistance exercise training [125–128]. However, strenuous exercise can impede bone formation perhaps due to inadequate individual rest that may inhibit reversal phase of the remodelling cycle [10]. OC increased after 4 weeks of endurance exercise [122], 24 weeks of combined exercise [129] and 9 weeks of plyometric exercise training [130]. However, one study reported a decreased in OC levels after 24 weeks remaining lower after 1 year of combined exercise [131]. The authors also shown a decreased in bone resorption biomarker namely carboxy-terminal crosslinked telopeptide of type 1 collagen levels [131]. In addition, the evidence shown a decreased in sclerostin levels after 1 year of resistance and plyometric exercise training [132]. Figure 1.2. depicts mechanical loading-induced acute and chronic exercise effects.

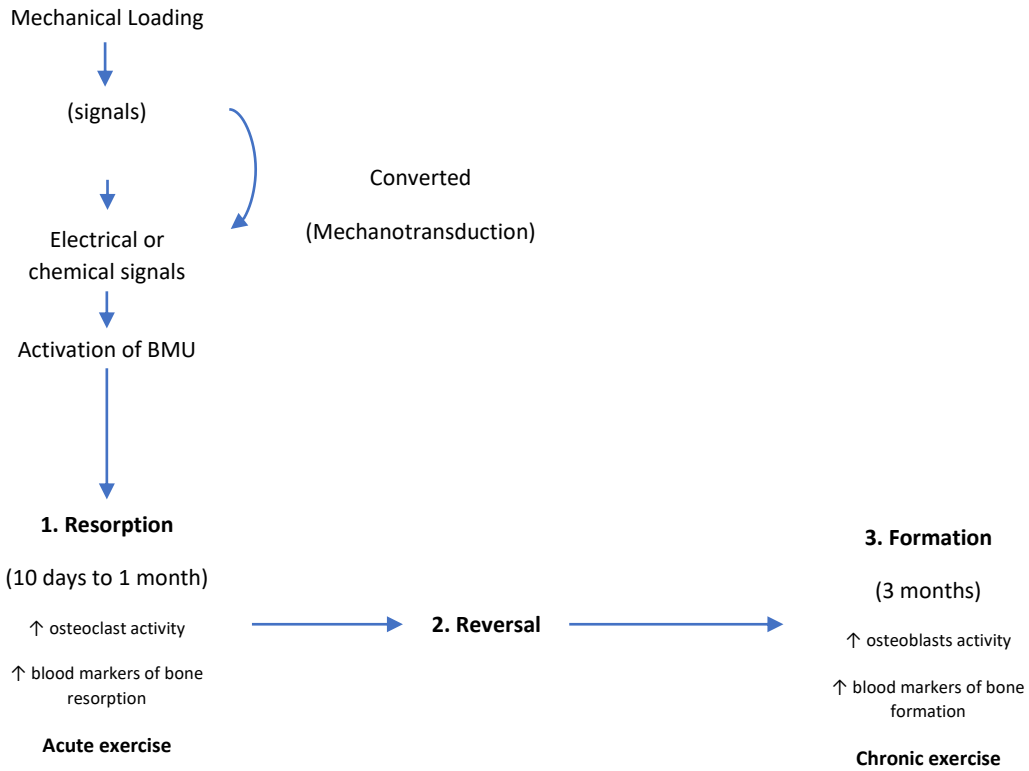

Figure 1.2. Mechanical loading-induced acute and chronic effects of exercise on bone metabolism. Figure adapted from Dolan et al. (2020) [10]. Mechanical load is applied on bone which induces bone tissue deformation. In turn, cells convert mechanical stimuli (mechanotransduction) in electric or mechanical

signals activating bone metabolic units (BMU) (osteoblasts, osteoclasts and osteocytes). Thus, the resorption phase starts taking 10 days to 1 month and is characterised with an increased in osteoclastic activity and an in increased in markers of bone resorption. Then, reversal phase will occur (period from bone resorption to new bone formation) and lastly, formation is the last phase that takes around 3 months with an increased in osteoblastic activity and in markers of bone formation. BMU, Bone Metabolic Units.

Chapter 2: General methods

2.1. Participants

The studies included in the current thesis were approval by Fresenius Medical Care Ethics Committee (CES_FMC_28.01.19) and were conducted in accordance with the Declaration of Helsinki. Additionally, all studies were developed in collaboration between the Research Center in Sports Sciences, Health Sciences and Human Development (CIDESD) at the University of Maia and Fresenius Medical Care, Nephrocare SA, Portugal. Patients older than 18 years, in HD treatment for, at least, 3 months, and willing to participate were recruited from three Nephrocare dialysis units (Vila Nova de Gaia, Braga and Oliveira do Bairro). Exclusion criteria include unstable or end-stage cardiac conditions or sudden cardiac death potentiators, unstable coronary heart disease, non-cardiac aneurysm with risk of rupture, uncontrolled arrhythmia and hypertension (systolic blood pressure >190mmHg and/or diastolic blood pressure >100mmHg), severe aortic stenosis, uncompensated heart failure or with an ejection fraction less than 30%, third degree atrioventricular block without a pacemaker, myocarditis for less than 6 months, pericarditis for less than 3 months, pulmonary thromboembolism for less than 1 month, acute thrombophlebitis, aortic dissection or rupture, vascular access with high risk of hematoma, acute myocardial infarction (<3 months), systemic infection, haemoglobin less than 8.5g/dl (monthly), untreated severe proliferative retinopathy and/or recent surgery with leisure treatment, acute thyroiditis, orthopaedic conditions that contraindicate physical exercise, electrocardiogram and/or echocardiogram and/or medical history of: hypertrophic cardiomyopathy, long QT syndrome or QT threshold, untreated Wolff Parkinson white, brugada syndrome, anomalous origin of the coronary arteries, arrhythmogenic right ventricular cardiomyopathy, mitral valve prolapse, cardiac sarcoidosis, chronic alcoholism and/or drug addiction, and pregnancy.

The recruitment for this research project was conducted by the author of the present thesis and Nephrocare Nephrologists from the three different Nephrocare clinics in Portugal; and it was completed in three years (2019-2021). Nephrologists were responsible for selecting patients that accomplished the inclusion criteria (previously described) for the project. After this, each patient was contacted by the author of this thesis for full written and verbal

information about the study protocol. After 24h, patients were contacted once again and those willing to participate in the project were required to provide their written informed consent (Attachment 1). Only patients who autonomously signed informed consent were included.

2.2. Research design

All HD adult patients included in the study performed a set of outcomes assessments (fully described in subchapter 2.4. Assessment). They were asked to maintain their usual routine, especially related to their PA level, during 6-week, determined as a run-in period. Following this period, all patients performed the same outcome assessments being subsequently randomly divided into a 12-week intradialytic aerobic (iAET) or resistance exercise training (also called iPRT) using a random block method (exercise protocols fully described in subchapter 2.3. Exercise training protocol). At the end of the 12-week intervention period, all patients repeated all assessment outcomes as the previous time points.

The diagram of the research design is depicted in Figure 2.1. From this research design, 3 studies were developed (fully described in Chapter 4: Associations between bone biomarkers with physical health outcomes in haemodialysis patients: a cross-sectional study; Chapter 5: Effects of intradialytic resistance versus aerobic exercise training on bone biomarkers and physical health of haemodialysis patients: a randomized clinical trial; Chapter 6: Acute effects of resistance versus aerobic exercise on bone biomarkers in haemodialysis patients: a secondary analysis of a randomized clinical trial).

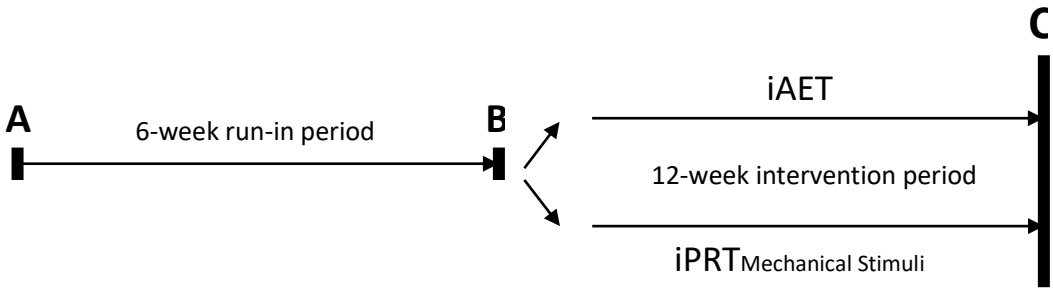

Figure 2.1. Research Design. A, Baseline; B, pre-exercise; C, post-exercise; iAET, intradialytic aerobic exercise training; iPRT, intradialytic progressive resistance exercise training.

2.3. Exercise training protocol

a) intradialytic Aerobic Exercise Training (iAET)

iAET was performed on a cycle ergometer (as shown on figure 2.2.) during the first 2h of HD sessions. Each exercise session consisted of a 5 min warm-up at 50 rotations per minute (rpm) on a rating of perceived exertion (RPE) between 6-11 (Borg scale 6-20 [133]). Then, load was increased to 50-70 rpm on a RPE between 12-15 rpm for 30 minutes. This time increased 10 minutes at each four weeks (week 4 and week 8) over 12 weeks of exercise intervention. The cool-down consisted of 5min at 50 rpm, with a RPE between 6-11.

Figure 2.2. Intradialytic Aerobic Exercise Training (photos taken from Nephrocare Vila Nova de Gaia Clinic, Portugal).

b) intradialytic Progressive Resistance exercise Training (iPRT)

The protocol of the iPRT has been published in DOI: 10.1159/000531973. it was performed in a new exercise training machine developed to exercise during HD (figure 2.3.).

Figure 2.3. Intradialytic progressive resistance training (iPRT) device (ProSport, Portugal). Taken from Cardoso et al. (2023) [134].

iPRT includes dynamic and resistance exercises within a specific intensity range that may be adjusted in accordance with the principle of progressive overload. Patients are encouraged to complete 12 repetitions at 65%-75% of their estimated 1-repetition maximum (1-eRM) of each exercise, for all three HD days. Throughout the 12 weeks, the exercise load progressively increased from 65% to 75% at each 4 weeks. The eight exercises in this programme, developed to be completed during an HD session within a maximum of 50 minutes, comprise three unilateral pushing and pulling upper body actions, i.e., chest press, rowing, and shoulder press and five lower body actions, i.e., hip flexion, leg press, leg curl, hip abduction, and hip adduction (figure 2.4 and 2.5; all pictures below were taken with patient written consent). Exercises were performed considering a full range of motion with moderate velocity contractions (1s concentric + 3s eccentric) in order to increase the rate of strength gains. All exercises were performed using the iPRT machine, except for the shoulder press, which was completed using free-weight dumbbells

(figure 2.4. B and 2.6. B). Any exercises using the vascular access arm were performed before the start of each HD session (figure 2.6.).

Both exercise interventions (iAET and iPRT) lasted 12 weeks, totalling 36 exercise sessions. All sessions were supervised by an exercise physiologist, or by an appropriately trained health professional.

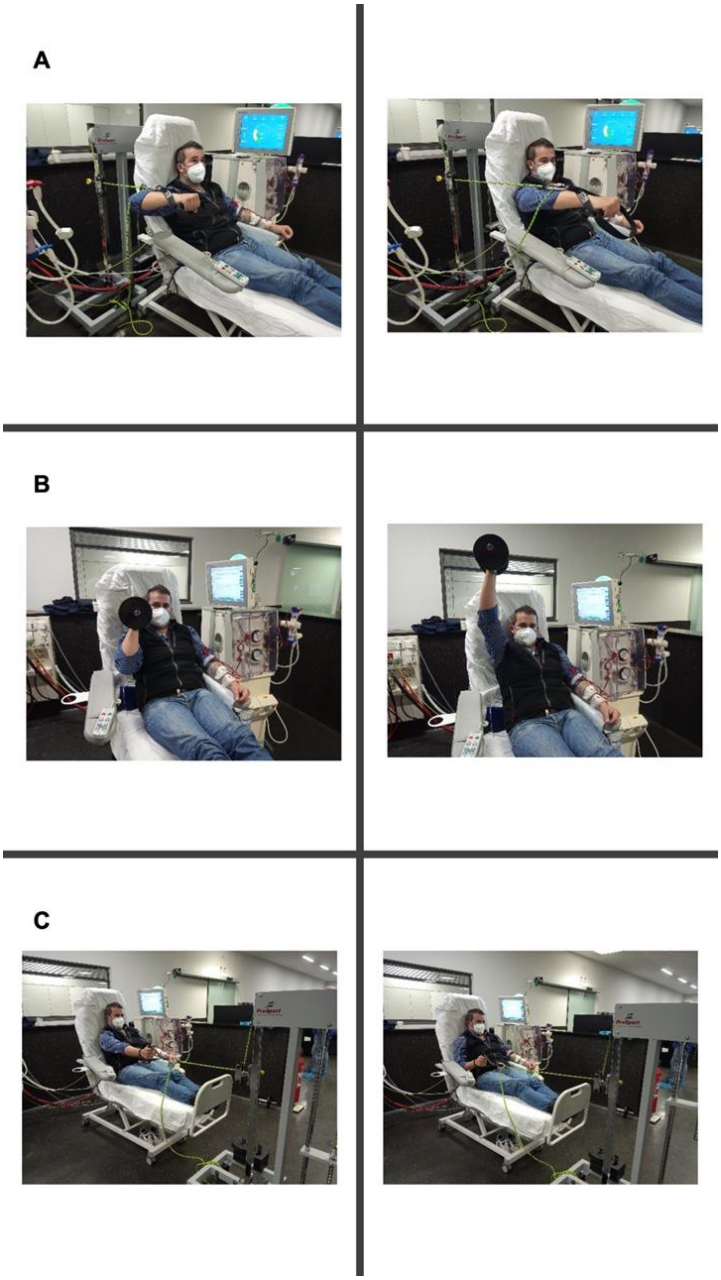

Figure 2.4. Intradialytic unilateral upper body exercises. A) Unilateral Chest Press; B) Unilateral Shoulder Press; C) Unilateral Rowing. Taken from Cardoso et al. (2023) [134].

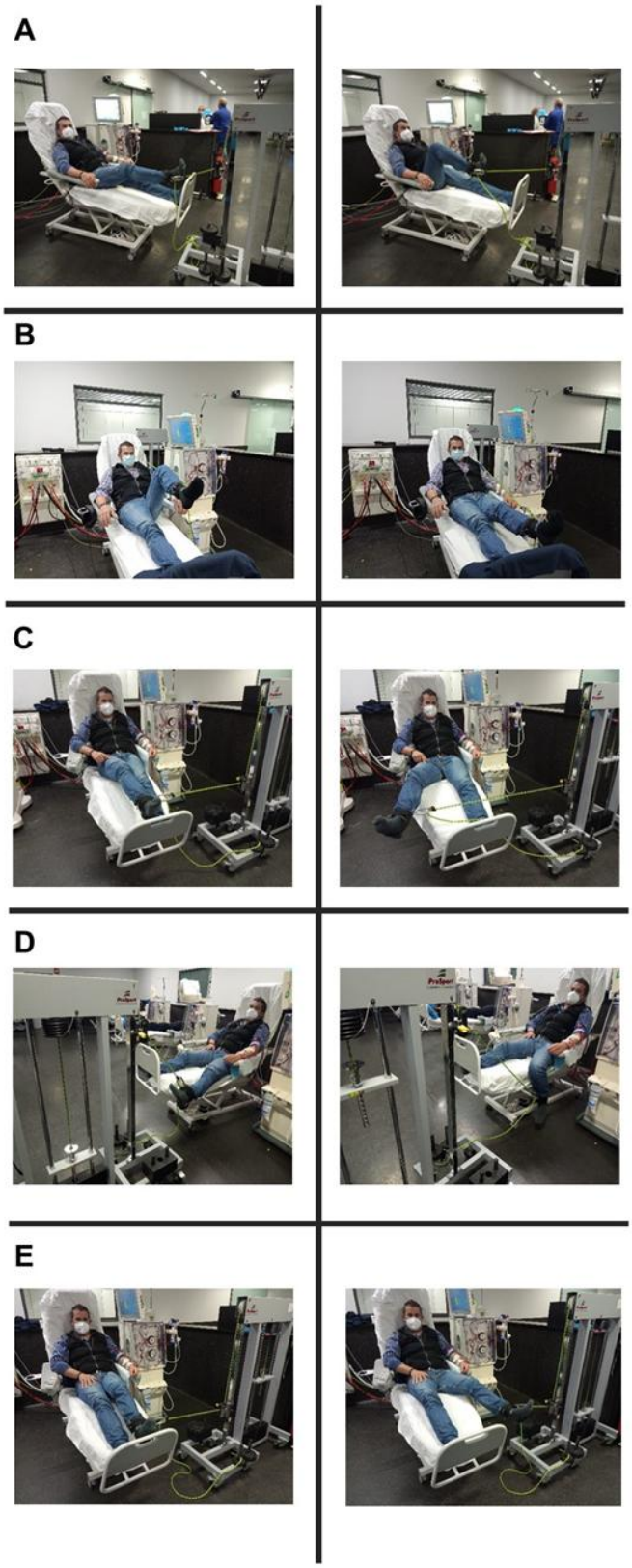

Figure 2.5. Intradialytic unilateral lower body exercises. A) Unilateral Hip Flexion; B) Unilateral Leg Press; C) Unilateral Hip Abduction; D) Unilateral Leg Curl; E) Unilateral Hip Adduction. Taken from Cardoso et al. (2023) [134].

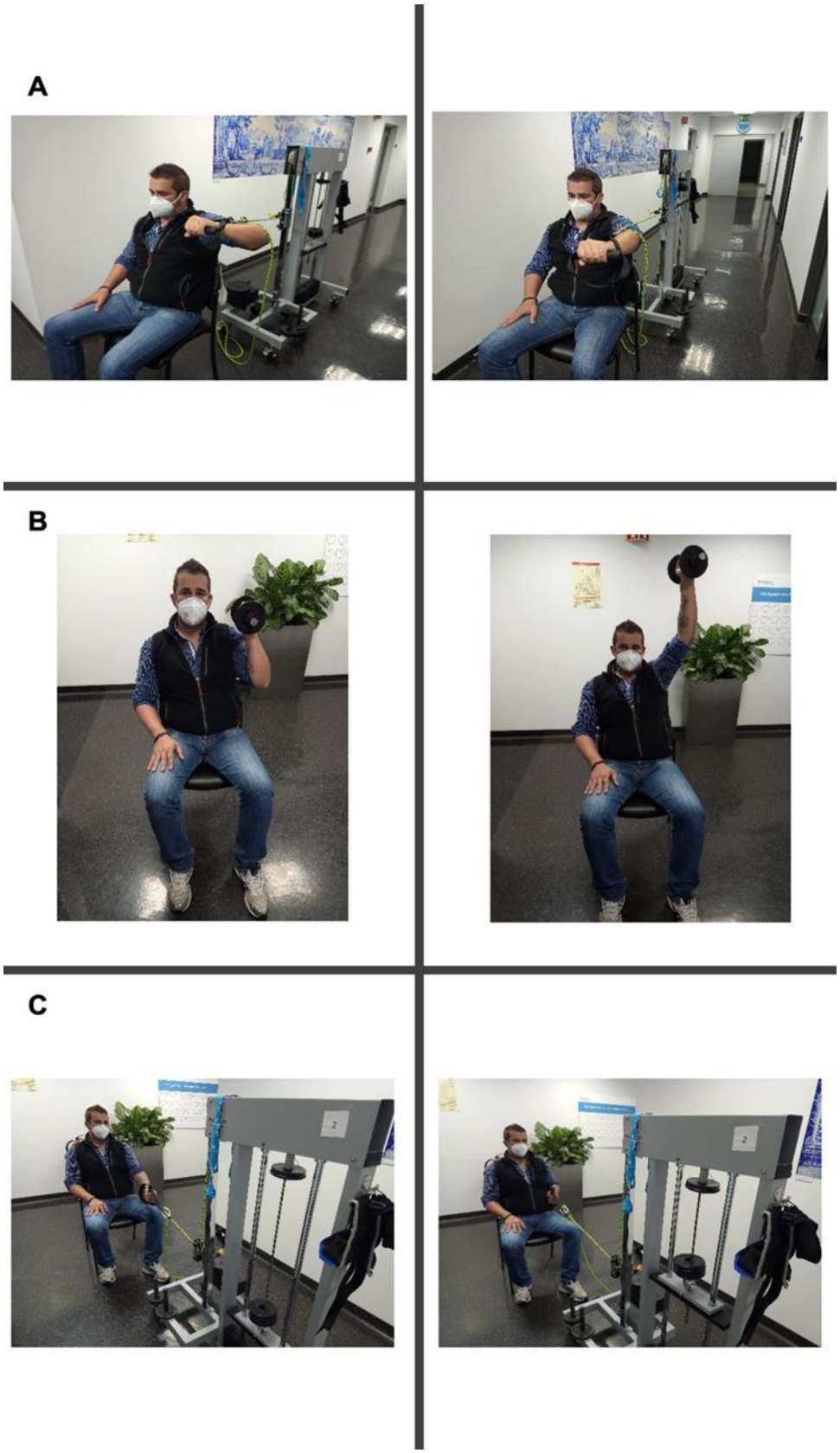

Figure 2.6. Unilateral exercises in a vascular access arm before HD treatment. A) Unilateral Chest Press; B) Unilateral Shoulder Press; C) Unilateral Rowing. Taken from Cardoso et al. (2023) [134].

2.4. Assessment of the outcomes

All outcomes' assessments were performed before the second and third weekly HD sessions. One week before the first time point outcomes assessments, all patients had a familiarization session with all tests. Outcomes of physical function, PA, body composition, muscle strength, and bone turnover biomarkers were assessed (fully described in the next pages).

2.4.1. Physical function

Physical Function was assessed through Incremental Shuttle Walk Test (ISWT) [135,136], Timed up and go (TUG) [137], Sit to Stand 60 test (STS-60) [138] and Short Physical Performance Battery (SPPB) [137].

- ISWT measures cardiorespiratory fitness and exercise capacity. Patients were asked to walk between two cones with 10 meters between each other. An external auditory beep regulated the patients' speed and at each minute walking speed increased by 0.2m/s. The test finished when patients achieved volitional exhaustion, or they no longer keep up with the required pace (if the participant failed to reach the cone/marker in the time allowed, being more than 0.5 meters away from the cone when the bleep sounds two times). Walking aid accessories were allowed. The total number of shuttles and distance walked (in meters) was calculated and recorded [135,136].
- TUG was performed to assess agility, dynamic balance, and risk of falls. This test consists in a time required to get up from a seated position, walk 3 meters, turn, and return to the seated position. The main goal is to complete the test as quickly and safely as possible. Walking aid accessories were allowed. Two repetitions were performed and the best time (in seconds) from the two trials was considered [137].
- The STS-60 was performed to measure muscle strength and endurance of lower limbs. Participants are required to sit in a 43-cm-high chair with arms crossed at the wrists and held against the chest. The sit- and stand-up movement begins after the start signal leading the participant to a maximum upright position and returning to the starting position.

Participants completed as many stand-ups as possible for 60 seconds. The chair (without arms) should be stabilized against the wall to prevent it from moving during the test. The score is calculated from the total number of stand-ups executed. Two trials were performed and the best number of repetitions on the trial was recorded [138].

- SPPB is composed by three tests that measure balance, endurance, and strength components. Each component is scored from 0 to 4 with a maximum total score of 12 points. To the test balance, patients needed to stand up in three different positions (side-by-side, semi-tandem and full tandem position) for 10 seconds. Then, a gait speed time over 4 m (s) was performed to test endurance. Patients were asked to walk at their usual pace over a 4-m course. Patients were allowed to use their walking aid to complete the test. Lastly, patients completed time to perform 5 chair rises (s) test. Patients were asked to rise and sit from a chair, 5 times, as quickly as possible, with hands folded across the chest. Two trials of each subtest were performed, and the best score obtained was considered [137].

2.4.2. Physical activity

All patients were instructed to wear a tri-axial accelerometer (Actigraph Corp, Pensacola, Fl, USA) on the right hip above the iliac crest with an elastic belt for 7 consecutive days removing only for night sleep and bathing. The intensity was captured at 50Hz. A non-wear period was defined as consecutive zero activity counts recorded for 60 minutes, and a valid day was considered when a minimum of 10h recording time was achieved. Raw data of the patients were excluded from analysis if they did not complete at least 3 days of wearable time or the minimum recording time described elsewhere [139]. Days of use and the average wearable time (h/day) were calculated for all patients and all data were normalised to wearable time of each patient. A custom MATLAB programme extracted the tri-axial accelerations that were filtered using a 4^{th} order Butterworth band-pass filter (0.2-15Hz) [140,141] and used to calculate the acceleration single vector magnitude (SVM) corrected for gravity by the

following equation [142], where a_x, a_y and a_z are the filtered accelerations in the three axis of motion:

$$SVMg = \sqrt{\left(a_x{}^2 + a_y{}^2 + a_z{}^2\right)} - 1$$

The SVM was used to estimate days of use, average wearable time, PA levels (counts per minute). Daily PA of all patients was classified into 5 categories: sedentary (<100 counts/min), light (100-2689 counts/min), moderate (2690 – 6166 counts/min), vigorous (6167 – 9642 counts/min), and very vigorous (>9642 counts/min) activity according to existing cut-points [143], and expressed as counts per minute (counts/min). Total activity time (hours), daily PA (min/day), percentage of PA, and average PA (counts/min) of each patient were also measured.

2.4.3. Body composition

The Fresenius medical care body composition monitor was used to determine body composition. This device uses the technique of bioimpedance spectroscopy to identify the resistances of extracellular and total body water. The clinically relevant output parameters were: overhydration (L), lean tissue mass (Kg), adipose tissue mass (Kg) and body mass index (BMI) (Kg/m^2).

2.4.4. Muscle strength

Isometric handgrip strength was assessed [138] by a handheld dynamometer (JAMAR®, Chicago, USA). Patients were seated with the elbow of their testing arm (non-fistulated arm) bent at approximately 90º and shoulder in a neutral position. After a signal, they squeezed the dynamometer as hard as possible for 3 seconds while exhaling. Two trials were performed, and the higher values were recorded in kilograms (Kg).

2.4.5. Bone turnover biomarkers

2.4.5.1. Blood sampling, handling, and analysis

Approximately 27ml of whole blood was drawn from the vascular access of each patient at each assessment (as previously described on subchapter 2.2.

Research design), into 9ml K3EDTA vacutainers (1.8 mg Ethylenediaminetetraacetic acid (EDTA) per each 1ml of blood) (Vacuette, Portugal, S.A.). Blood samples were collected in two different time points: 1) immediately before HD session and 2) at the end of the same HD session. All blood samples were collected before the second HD session of the week. A part of the collected blood sample was used to analyse haemoglobin and haematocrit (as described in next section 2.4.5.2), and the remaining was centrifuged at 1,500g for 10min in a refrigerated centrifuge (Medifriger-BLT, Barcelona Spain) at 4ºC to obtain the resulting plasma. Then, all plasma was aliquoted into 1ml cryovials and stored at -80º until analysis. Plasma was stored for further analysis of bone turnover biomarkers (RANKL, OPG, sclerostin, osteocalcin, TRAP-5b).

2.4.5.2. Haemoglobin and haematocrit

Haemoglobin and haematocrit were determined from each whole blood sample collected throughout the project. Haemoglobin was analysed in duplicate (HemoCue® Hb 201+ System, HemoCue AB, Ängelholm, Sweden). Additionally, the determination of haematocrit values was performed after centrifugation of whole blood (HCEN-202, Essex, United Kingdom), and the plasma volume of blood samples was determined. For acute exercise effects, the plasma volume from blood samples was determined and adjusted according to established equations [144]:

Plasma Volume (PV)_pre/post= Blood Volume (BV)_pre/post – red cell volume (RcV)_pre/post,

BVpre= BVpost (Haemoglobin_post/Haemoglobin_pre)

BVpost= 100

RcVpre= BVpre * Haematocrit_pre

RcVpost= BVpost * Haematocrit_post

ΔPV (%)= [(PVpre - PVpost)/ PVpost]*100

2.4.5.3. Enzyme-linked immunosorbent assays (ELISA)

a) RANKL

RANKL plasma concentration was assessed throughout commercially FREE soluble RANKL high sensitivity ELISA (Biomedica, Austria). On the same day of the analysis, samples were defrosted, vortexed (VV3, Pennsylvania, United States of America), and then used for the assay. Ninety-six precoated wells plates with OPG were used. Firstly, assay buffer, standards/controls and samples were added. All samples and standards were added in duplicate in each plate. After the incubation, the plate was washed to remove any unbounded particles and the biotinylated anti sRANKL antibody was added. Following a second incubation, streptavidin-HRPO was added, and a Tetramethylbenzidine (TMB) substrate was used. Then, the reaction was stopped by adding sodium hydroxide to each well. The plates were immediately read at 450nm using 4PL algorithm (MR-96-A, Shenzhen, China) and analysed on Graphpad (Graphpad Prism version 9.3.1, Graphpad Software, La Jolla, CA, USA). The intra- and the inter-assay coefficient variation (CV) were 5.6% and 8.1%, respectively.

b) Sclerostin

This biomarker was assessed by a commercially available ELISA kit (Biomedica Gruppe, Vienna, Austria). The analysis was similar to the previous biomarker; however, the plates were precoated with monoclonal mouse anti human sclerostin antibody. Additionally, before the first wash, a monoclonal mouse anti-human sclerostin antibody was added. The plates were read at an optical density of 450nm using four-parameter logistic equation (4PL algorithm) (MR-96-A, Shenzhen, China) and analysed on Graphpad (Graphpad Prism version 9.3.1, Graphpad Software, La Jolla, CA, USA). The intra- and the inter-assay CV were 6.8% and 9.1%, respectively.

c) Bone TRAP-5b assay

Plasma TRAP-5b concentrations were assessed through a commercially available ELISA kit (Bone TRAP; Immunodiagnostic Systems (IDS) Holdings Ltd, UK). All analyses were similar to the previous ELISAs described. However, microplates were coated with monoclonal anti-TRAP antibodies. The plates were read at an optical density of 405nm using linear method (MR-96-A,

Shenzhen, China) and analysed on Graphpad (Graphpad Prism version 9.3.1, Graphpad Software, La Jolla, CA, USA). The intra- and the inter-assay coefficient of variation (CV) were 3.2% and 5.8%, respectively.

2.4.5.4. DuoSet ELISAs

a) Osteoprotegrin

Plasma OPG concentrations were assessed through a commercially available Human Osteoprotegerin/TNFRSF11B DuoSet ELISA kit (R&D Systems, Inc, USA). First, a capture antibody was diluted (1ml of Phosphate buffered saline (PBS) on capture antibody), then reconstituted (61µl diluted capture antibody + 10939µl of PBS) and added into each well to bound with user-provided polystyrene microplate. The unbound capture antibody was removed after washes. Then, reagent diluent was added to the wells, and the plates were washed to remove any unbounded material. Following plate preparation, samples and standards were added in duplicate, and the analytes were bounded by the immobilized antibody, the remaining unbounded were removed by wash. The detection antibody was diluted with 1ml of reagent diluent, and reconstitution again (220µl normal goat serum + 61µl reconstituted detection antibody + 10719µl of reagent diluted) then added to each well. Following washes, Streptavidin-Horseradish Peroxidase was used to bound the detection antibody, and the unbounded materials were washed away. Finally, a substrate solution, TMB, was added to each well, the reaction was stopped with sodium hydroxide and the plates were immediately read at 450nm using 4PL algorithm (MR-96-A, Shenzhen, China) and analysed on Graphpad (Graphpad Prism version 9.3.1, Graphpad Software, La Jolla, CA, USA). All samples were diluted in 1:5 factor in reagent diluent (42µl of sample in 169µl of reagent diluent) and the correction read from the standard curve was multiplied by the dilution factor. The intra- and the inter-assay CV were 2.6% and 3.6%, respectively.

b) Osteocalcin

Plasma osteocalcin was measured by a commercially available Human Osteocalcin DuoSet ELISA kit (R&D Systems, Inc, USA). The analysis was

performed in an analogous way to the OPG kit. Contrastingly, all samples were diluted in 1:10 (21µl of sample and 189µl of reagent diluent) and the correction read from the standard curve was multiplied by the dilution factor. The plates were immediately read at 450nm using 4PL algorithm (MR-96-A, Shenzhen, China) and analysed on Graphpad (Graphpad Prism version 9.3.1, Graphpad Software, La Jolla, CA, USA). The intra- and the inter-assay CV were 3.2% and 4.8%, respectively.

2.5. Statistical analysis

The IBM SPSS Statistics version 29 (SPSS, Chicago, IL, USA) was used and all statistical procedures are detailed in each study in Chapter 4: Associations between bone biomarkers with physical health outcomes in haemodialysis patients: a cross-sectional study; Chapter 5: Effects of intradialytic resistance versus aerobic exercise training on bone biomarkers and physical health of haemodialysis patients: a randomized clinical trial and Chapter 6: Acute effects of resistance versus aerobic exercise on bone biomarkers in haemodialysis patients: a secondary analysis of a randomized clinical trial.

Chapter 3: Impact of physical activity and exercise on bone health in patients with chronic kidney disease: a systematic review of observational and experimental studies

3.1. Abstract

Introduction: Chronic Kidney Disease (CKD) patients frequently develop life-impairing bone mineral disorders. Despite the reported impact of exercise on bone health, systematic reviews of the evidence are lacking. This review examines the association of both physical activity (PA) and the effects of different exercise interventions with bone outcomes in CKD.

Methods: English-language publications in EBSCO, Web of Science and Scopus were searched up to May 2019, from which observational and experimental studies examining the relation between PA and the effect of regular exercise on bone-imaging or-outcomes in CKD stage 3–5 adults were included. All data were extracted and recorded using a spreadsheet by two review authors. The evidence quality was rated using the Cochrane risk of bias tool and a modified Newcastle-Ottawa scale.

Results: Six observational (4 cross-sectional, 2 longitudinal) and seven experimental (2 aerobic-, 5 resistance-exercise trials) studies were included, with an overall sample size of 367 and 215 patients, respectively. Judged risk of bias was low and unclear in most observational and experimental studies, respectively. PA was positively associated with bone mineral density at lumbar spine, femoral neck and total body, but not with bone biomarkers. Resistance exercise seems to improve bone mass at femoral neck and proximal femur, with improved bone formation and inhibited bone resorption observed, despite the inconsistency of results amongst different studies.

Conclusions: There is partial evidence supporting (i) a positive relation of PA and bone outcomes, and (ii) positive effects of resistance exercise on bone health in CKD. Prospective population studies and long-term RCT trials exploring different exercise modalities measuring bone-related parameters as endpoint are currently lacking.

Keywords: Physical activity, Exercise, Bone, CKD-MBD

3.2. Introduction

CKD is a worldwide health problem with an estimated global prevalence of 11–13% [1]. This prevalence is rising, driven by an aging population and the increasing incidence of obesity, hypertension, and diabetes [1]. In addition, most patients have an increased risk of comorbidities [25] and all-cause cardiovascular premature death [145]. As a result, CKD represents an enormous economic burden for healthcare systems worldwide with drastic personal health consequences [1].

Patients suffering from CKD frequently develop mineral and bone disorders (MBD) due to systemic alterations induced by the disease [3]. This syndrome has been associated with the spectrum of renal osteodystrophy [3], vascular calcifications, abnormalities in bone mineralisation and turnover [146], increased bone fractures [3], as well as increased morbidity and mortality, resulting in a diminished quality of life [147]. Thus, CKD-MBD encompasses a wide spectrum of clinical disorders such as alterations in mineral and bone metabolism [147], which are in turn associated with abnormalities in calcium, PTH, phosphate or vitamin D metabolism [26]. For instance, reduced levels of vitamin D and OC carboxylation, and elevated serum PTH and FGF-23 are key risk factors for bone disease [148]. PTH and FGF-23 are the main regulating hormones of bone integrity and mineral homeostasis [25]. Bone is a dynamic tissue which is constantly undergoing remodelling [43], a process that mediates the balance between bone formation and resorption to maintain bone health and skeleton integrity [5]. However, in CKD-MBD the rate of bone resorption exceeds the rate of bone formation, resulting in loss of bone quantity and quality, which consequently contributes to bone strength loss [5].

Different physical activities, including high-impact weight-bearing exercise, multi-directional weightbearing exercise, or resistance exercise have been pointed as potentially able to stimulate resident osteocytes to yield signalling molecules that regulate bone formation and bone resorption [116,149]. In addition, substantial evidence supports that PA and exercise interventions are effective in improving bone health across all ages [7,112]. Although different exercise interventions (varying on type, intensity, frequency, and duration) have been extensively explored in healthy and osteoporotic

populations [7,150], the impact of PA and exercise on bone health in CKD patients is less well-established. As there has been no definitive synthesis of these studies, the current systematic review makes a major contribution to research through the inclusion of observational and experimental studies, in order to explore the impact of different forms of mechanical loading on different imaging and biochemical bone outcomes in CKD.

Thus, the purpose of the present systematic review is to examine (1) the associations between PA and bone-related outcomes and (2) the effects of different exercise interventions on bone-related outcomes in CKD patients.

3.3. Methods

3.3.1. Eligibility criteria

The inclusion criteria for this systematic review were: (1) observational studies, or randomized controlled trials (RCTs), or non-randomized controlled trials (non-RCTs); (2) reported measures of PA or implemented an exercise intervention as the only intervention; (3) reported data on one or more of the following bone outcomes: bone density, geometry, microarchitecture, and biomarkers of bone turnover; (4) adult CKD patients (age ≥ 18 years old); and (5) CKD stage 3 to 5, including patients under dialysis or kidney transplant recipients. We did not include review articles, editorials, conference abstracts or animal-based trials. Studies published in non-English-language were also not included due to potential errors in the translation and interpretation of findings. Bone parameters were defined as areal BMD or BMC or T-score measured with DXA, bone macro- and micro-structure measured by 3D imaging techniques [quantitative computerized tomography (QCT) and magnetic resonance imaging (MRI)], and quantitative ultrasound measurements of bone density that included broadband ultrasound attenuation and the speed of sound. All skeletal sites were considered. Bone outcomes included any formation, resorption and regulators markers of bone turnover measured using any detection technique. Bone outcomes based on conventional radiography and bone biopsies were not included.

3.3.2. Search strategy and data source for studies identification

This systematic review is in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement guidelines (Attachment 2) [151]. A computer search of databases was conducted on EBSCO, Web of Science and Scopus up to May 2019. The search terms used were: “CKD”, “dialysis”, “renal function”, “glomerular filtration rate”, “hemodialysis”, “renal”, “bone”, “exercise”, “physical activity” (supplementary search strategy in Attachment 3). At this stage, there were no limits on the search, such as, language, animal and human-based study, or age. Hand searching on Google Scholar was also performed to identify possible missed studies in database search. The reference lists of all the included

studies have also been examined to identify any potential missed studies. Afterwards, all the duplicate data were identified and removed using a reference management software (EndNote®, version X7.8).

3.3.3. Data extraction

Data extraction was completed using a spreadsheet to record information on a range of characteristics of each study, including: first author and publication year, country, study design, sample size, type of population (stage of CKD), outcomes measured, description of PA assessment, description of the exercise intervention (for RCTs and non-RCTs) and main results for each outcome. Data were independently extracted by two review authors (DC and EAM) and in case of missing or unclear information, the authors of the included studies were contacted for further details.

3.3.4. Methodological quality

The risk of bias of the included studies was assessed using an adapted version of a modified Newcastle-Ottawa Scale (NOS) tool for observational studies [152] and the Cochrane Collaboration tool for the experimental studies [153]. The NOS includes the following five domains: methods for the selection of participants (selection bias), methods to control for confounding (performance bias), statistical methods (detection bias), methods of measuring outcome variables (information bias), and subject follow-up (attrition bias for longitudinal studies). Instead of using the scale 0 (for high risk of bias), 1 (for mostly no), 2 (for mostly yes) and 3 (for low risk of bias) as previously described [154], judgements were categorized as ‘low risk’ of bias, ‘moderate risk’ of bias, ‘high risk’ of bias or ‘unclear or unknown risk’ of bias following our adapted version (Attachment 4).

The Cochrane risk of bias tool [153] addresses the following six domains: selection bias (random sequence generation and allocation concealment), performance bias (blinding of participants and personnel), detection bias (blinding of outcome assessment), attrition bias (incomplete outcome data),

reporting bias (selective reporting) and other bias. For each entry, judgements were categorized as ‘low risk’ of bias, ‘high risk’ of bias, or ‘unclear risk’ of bias.

Two authors (DC and EAM) independently scored each of the included articles and discrepancies were resolved through discussion until consensus was met.

3.4. Results

3.4.1. Included studies

Figure 3.1 shows the flowchart of the search and selection process. A total of 2440 articles were identified by the search strategy. After removing duplicate records, the titles, keywords and abstracts of 654 articles were analysed and 18 relevant articles were identified for full text review. From those, 13 studies fulfilled the inclusion criteria and were included in our qualitative synthesis. Studies were classified based on inherent purpose and design features in observational (n = 6) or experimental (n = 7).

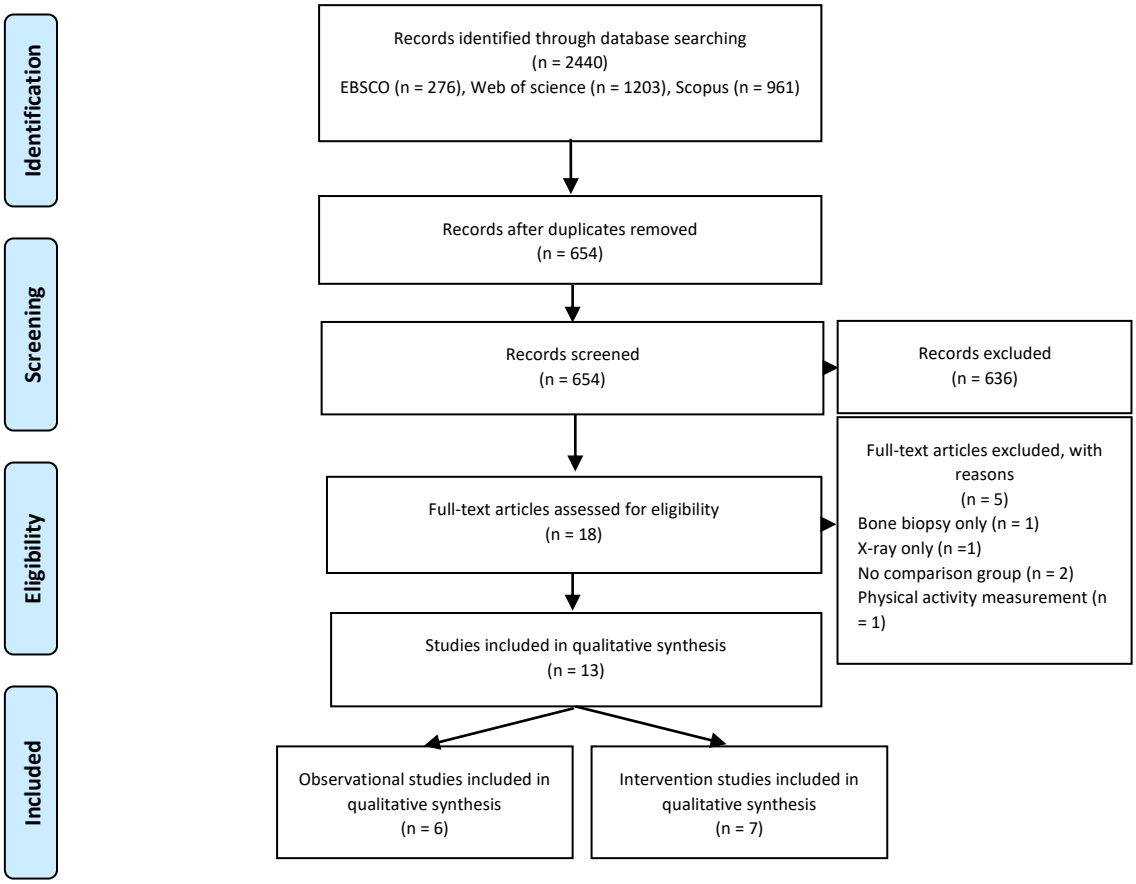

Figure 3.1. Flow diagram of studies.

3.4.2. Characteristics of the observational studies

The age range of our analytical sample in the observational studies was between 19 and 85 years old, mostly representing middle-aged adults and older adults. The characteristics of the included six observational studies [49,155–159] are presented in Table 3.1. Four studies had a cross-sectional design [49,155,158,159], and two had a longitudinal design [156,157] with an observational period of 12 and 24 months, respectively. Only one study had a multi-centre design [157].

Regarding patients' characteristics, four reports recruited patients under HD treatment [49,157–159] and two studies were performed on kidney transplant patients [155,156]. Sample size from individual studies ranged from 32 to 115 patients, and the overall sample size was 367 participants. Median age of participants was 56 years (based on the reported mean age), which varied from 19 to 85 years. The most common method to assess PA was the use of self-report questionnaires [49,155–157], while objective measures were only captured in two studies [158,159], using triaxial pedometry and triaxial accelerometery, respectively. Except for one cross-sectional study in HD patients [158], areal BMD (g/cm2) was measured trough DXA in all included observational studies. Only one study also used QCT, a 3D imaging technique, to assess total volumetric BMD (g/cm3) at the proximal femur and spine, and cortical or trabecular mass (g) and volume (cm3) at the proximal femur [157]. Most studies measured areal BMD at more than one skeletal site. Lumbar spine BMD was assessed in all studies, while proximal femur was assessed in two of these studies [49,155–157], total body BMD was measured in two studies [155,159] and one study also measured femoral neck BMD [49]. In addition to imaging-derived bone parameters, different biochemical markers of bone metabolism were measured in all observational studies included in this review.

Bone formation markers included alkaline phosphatase (ALP) [49,156,159], BALP [157,158], OC [156,159] and P1NP [157] and intact P1NP (iP1NP) [158]. Whereas one or more studies included data on bone resorption markers such as sclerostin [157], dickkopf-related protein 1 (DKK1) [157] and Trap-5b [157–159]. In addition, FGF-23, a local factor in bone remodelling that

stimulates bone formation and resorption, was also assessed in one study [157]. Finally, intact-PTH (iPTH) was reported in all studies.

Table 3.1. Characteristics of the observational studies.

Study (design)	Country	Sample size (male %); Population	BMD assessment techniques (anatomical sites)	Bone biomarkers	PA assessment method	Results BMD	Results Bone biomarkers
Dolgos et al. 2008 [155] (Cross-sectional)	Norway	n= 108 (68%) Kidney Transplant	DXA - Lunar (LS, proximal femur both sides, and total body)	iPTH	Self-report questionnaire Physical active vs. physical Inactive (defined as regular weight-bearing physical exercise performed at least twice a week for 30 minutes)	Association with total body BMD No association with LS and proximal femur BMD	No association with iPTH
Huang et al. 2009 [49] (Cross-sectional)	Taiwan	n= 35 HD	DXA - Hologic (LS and FN)	iPTH ALP	Self-report interview questionnaire Total weekly exercise time (min/week): regular exercise (yes or no), exercise type (impact or non-impact) and effective exercise time (min/week)	Positive association with all BMD outcomes	No association with all bone biomarkers
Morishita et al. 2014 [158] (Cross-sectional)	Japan	n= 32 (56%) HD	---	BALP iP1NP TRAP-5b iPTH	Device- Triaxial pedometer Vigorous and moderate PA volumes per week	---	No association with all bone biomarkers
Ota et al. 1997 [159] (Cross-sectional)	Japan	n= 32 (0%) HD	DXA - Lunar (Total body and LS)	iPTH OC ALP TRAP-5b	Device – Accelerometer Total energy expenditure per day (Kcal) for 7 days - mean energy expenditure per day	Positive association with total body BMD No association with LS BMD	No association with all bone biomarkers
Groth et al. 1995 [156] (Longitudinal - 2-year follow-up)	Germany	n= 115 (61%) Kidney Transplant	DXA- Lunar (LS)	ALP iPTH OC	Self-report questionnaire Estimated energy spending during sports	Positive association with bone gain ($r = 0.2$, $p<0.05$)	No association with all bone biomarkers
Malluche et al. 2017 [157] (Longitudinal - 1-year follow-up)	USA	n= 45 HD	DXA - Lunar and QCT (LS and proximal femur)	TRAP-5b; BALP P1NP; Sclerostin DKK1; FGF-23 iPTH	Self-report questionnaire Exercised 1+ days/week	No association with bone loss	No association with all bone biomarkers

3.4.3. Characteristics of the experimental studies

The age range of our analytical sample in the experimental studies was between 27 and 76 years old, mostly representing middle-aged adults and older adults. We identified five RCTs [160–164] and two non-RCTs [165,166] aiming to examine the effects of exercise on bone parameters, which are described in detail in Table 3.2. The sample size of the individual studies varied, ranging between 13 and 52 subjects, and the overall sample size was 215 patients. The median value of the mean age was 52 years and ranged between 27 to 76 years old. Participants were mostly HD patients; only one study included CKD stage 3–4 patients [161], and another study included subjects with history of kidney transplantation [160].

All outcomes were measured at baseline and at the end of each intervention period, corresponding to 8 weeks [163], 12 weeks [160,162,164], and 24 weeks [161,165,166].

All exercise sessions were supervised by a certified professional, except the home-based exercise trial that was weekly supervised [161]. The most common type of exercise training was resistance exercise, usually performed during dialysis (intradialytic exercise) three times per week [163–166]. Only one study performed resistance exercise for kidney transplant patients [160]. In addition, two studies performed aerobic exercise interventions, three times per week [161,162], and in one study [162] the aerobic exercise was performed during dialysis.

Except for two experimental studies with no imaging-derived bone parameters [161,163], all other five studies measured BMD through DXA devices [160,162,164–166]. Two studies reported T-score values [165] and BMC [164] from total body scans. All other studies reported DXA-derived outcomes from two or more skeletal sites. Areal BMD was reported for lumbar spine in three studies [160,162,165], for proximal femur in two studies [160,165], and for femoral neck in two studies [162,165]. One study reported only the T-score values for lumbar spine, proximal femur, femoral neck, and total body BMD [165]. In addition to imaging-derived bone parameters, different biochemical markers of bone metabolism were measured in five experimental

studies [161–163,165,166], including the following markers of bone formation: ALP [161], BALP [163], and OC [161,166]; while markers of bone resorption included sclerostin [161,163], Trap-5b [161], and OPG [166]. Other biomarkers of bone health were also reported, including osteopontin (OPN) [166], iPTH [162,163,166] and PTH [161,165].

Table 3.2. Characteristics of the experimental studies.

Study (design)	Country	Sample size (male %) Population	BMD assessment techniques (anatomical sites)	Bone biomarkers	Exercise intervention	Results BMD	Results Bone biomarkers
Eatemadololama et al. 2017 [160] (RCT)	Iran	n=24 CG=12 EG=12 Kidney Transplant	DXA - Hologic (proximal femur and LS)	---	Resistance exercise (10 min stretching exercises, 10 min walking, 10 min cycling, 20 min RE for UL, 20 min RE for LL; RE intensity 50% of 1RM increasing 5 to 10%; 2 days/week; 12 weeks)	*EG*: ↑ proximal femur = LS *CG*: ↓ proximal femur ↓ LS *EG vs CG*: no comparison	---
Gomes et al. 2017 [161] (RCT)	Brazil	n= 39 (71%) CG= 15 EG=24 CKD Stages 3-4	---	TRAP-5b PTH Sclerostin ALP OC	Aerobic exercise (40-60% of maximum VO_2; 30 min; 3 days/week; 24 weeks)	---	*EG*: ↑ ALP *CG*: = all biomarkers *EG vs CG*: ↑ ALP (favouring EG)
Liao et al. 2016 [162] (RCT)	Taiwan	n= 40 (43%) CG= 20 EG=20 HD	DXA (LS and FN)	iPTH	Intradialytic aerobic exercise (12-15 BPES; 30 min; 3 days/week; 12 weeks)	*EG*: = all bone sites *CG*: = all bone sites *EG vs CG*: ↑ FN BMD loss (favouring CG)	*EG*: = all biomarkers *CG*: = all biomarkers *EG vs CG*: no comparison
Marinho et al. 2016 [163] (RCT)	Brazil	n= 13 (46%) CG= 7 EG= 6 HD	---	iPTH BALP Sclerostin	Intradialytic resistance exercise (60%-70% of 3RM; 4 exercises; 3 days/week; 8 weeks)	---	*EG*: ↑ BALP *CG*: = all biomarkers *EG vs CG*: no comparison
Rosa et al. 2018 [164] (RCT)	Brazil	n= 52 (67%) CG= 24 EG= 28 HD	DXA - Hologic (Total body - BMC)	---	Intradialytic resistance exercise (60% of 1RM; 40-50min; 3 days/week; 12 weeks)	*EG*: = total body BMC *CG*: = total body BMC *EG vs CG*: ↑ BMC (favouring EG) Effect size= 0.65	---
Marinho et al. 2017 [166] (Non-RCT)	Brazil	n= 26 (65%) CG=12 EG= 14 HD	---	OC OPN OPG iPTH	Intradialytic resistance exercise (60%-70% of 1RM; 4 exercises; 3 sets; 10 repetitions; 3 days/week; 24 weeks)	---	*EG*: ↑ OPG *CG*: = all biomarkers *EG vs CG*: no comparison
Marinho et al. 2016 [165] (Non-RCT)	Brazil	n= 21 (67%) CG=11 EG= 10 HD	DXA - Lunar (FN, LS, proximal femur and total body)	PTH	Intradialytic resistance exercise (60%-70% of 1RM; 4 exercises; 3 sets; 10 repetitions; 3 days/week; 24 weeks)	*EG*: ↑ femoral neck *CG*: = all bone sites *EG vs CG*: no comparison	*EG*: = PTH *CG*: no comparison *EG vs CG*: no comparison

3.4.4. Risk of bias in the observational studies

In total, six observational studies were examined in the present review. From these, overall assessment showed that one study had low [155] and another had moderate [157] risk of bias, with the remaining revealing high risk of bias [49,156,158,159], as seen in Figure 3.2. Furthermore, all studies examined bone biomarkers and BMD as the main outcomes, except for Morishita et al., 2014 [158], and the summary assessment for outcome replicates the overall summary assessment for all observational studies. Examining each domain separately, high risk is attributed to bias in Selection and bias in Performance only. All other domains were rendered as low risk of bias.

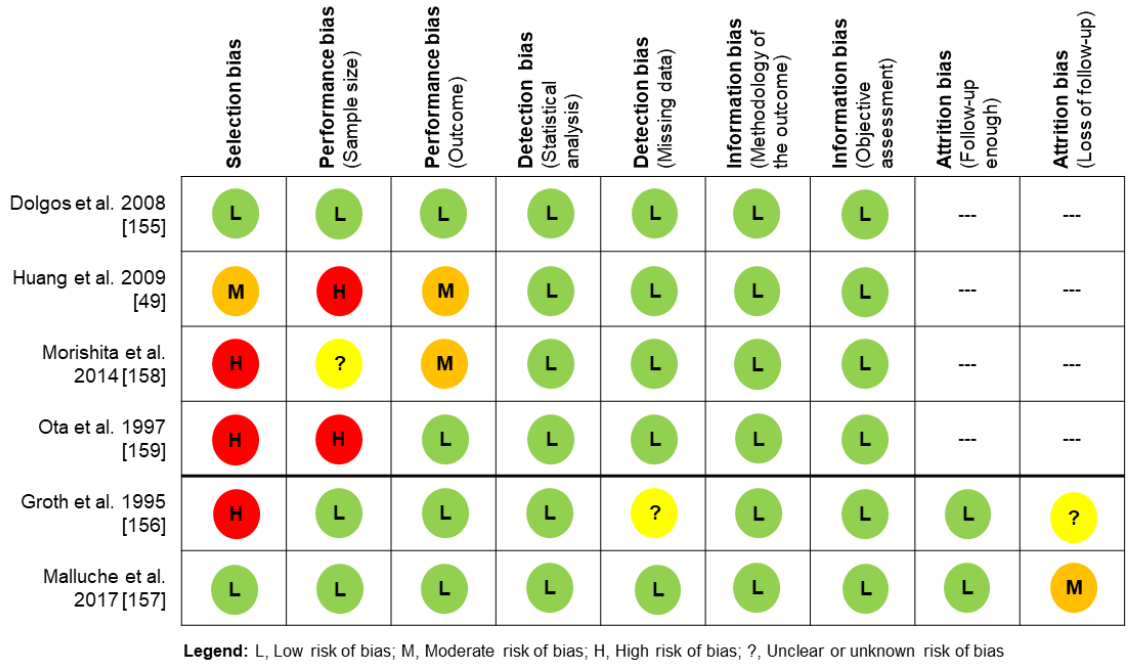

Figure 3.2. Risk of bias summary of observational studies.

3.4.5. Risk of bias in the experimental studies

This present review has examined seven experimental studies. Determination of the summary of overall risk of bias in each study shows that from these, three studies were judged to have high risk of bias [164–166],

whereas the remaining four studies [160–163] are classified as unclear risk of bias (Figure 3.3.). Additionally, when examining the risk of bias for outcome, risk of bias was unclear [160,162] and high [164,165] in the four studies that examined BMD. Bone biomarkers have been examined in five of the seven experimental studies, with overall unclear [161–163] and high [165,166] risk of bias observed. On the other hand, when examining each domain individually (i.e., risk of bias across studies), Reporting and Attrition bias were judged as low risk, whereas all other domains may be interpreted as having unclear risk of bias.

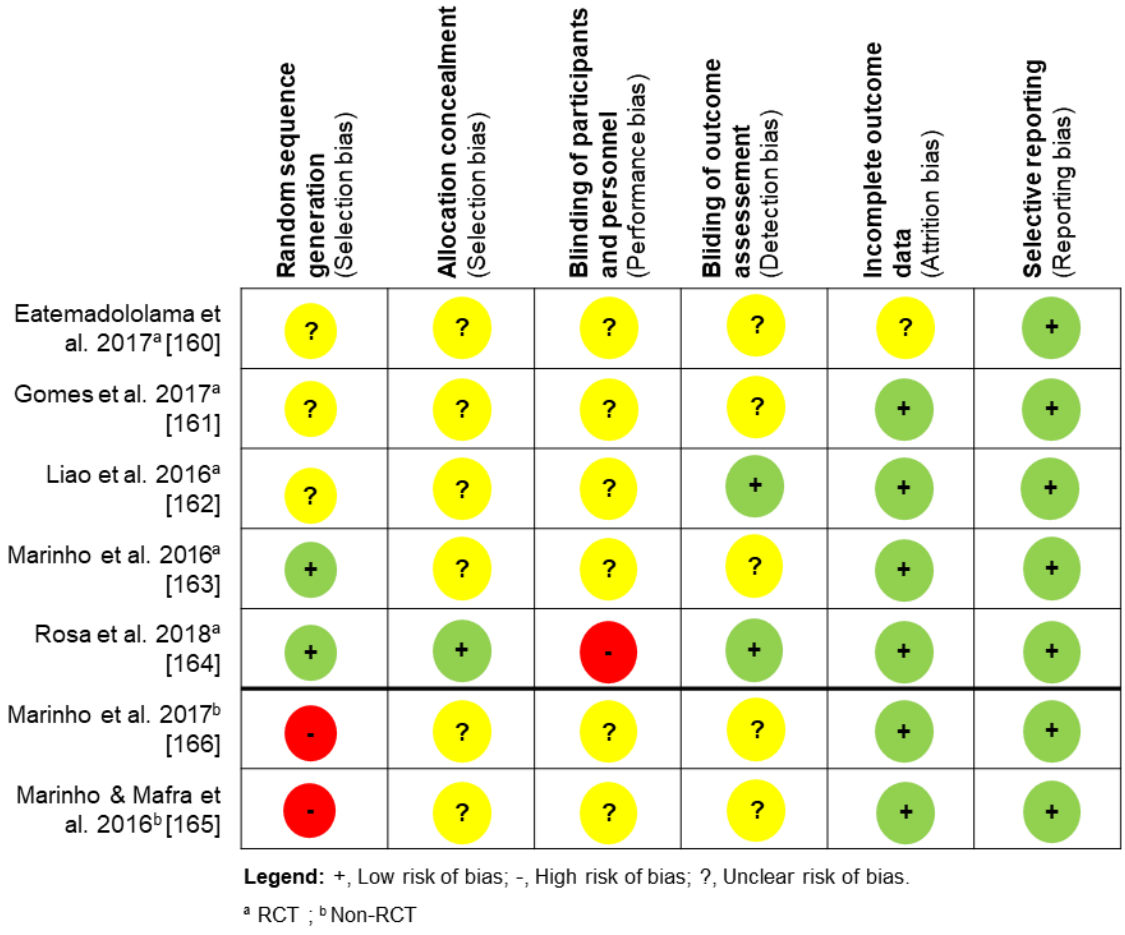

Figure 3.3. Risk of bias summary of experimental studies.

3.4.6. Association between physical activity and bone outcomes (observational studies)

Based on cross-sectional studies results, higher PA was associated with higher DXA-derived areal BMD (g/cm2) measured at different skeletal sites. Associations were more consistent for total body [155,159] and femoral neck [49], but only three studies explored these outcomes. Only one of the three cross-sectional studies that measured lumbar spine BMD in HD patients found a significant association with exercise duration (min/week) [49].

Two longitudinal studies reported the association between PA and bone mass changes, and findings were inconsistent. Only one study explored this association with proximal femur bone loss (areal BMD, cortical and trabecular volume and mass) and found no evidence of association with PA [157]. Similarly, to cross-sectional data, estimates of association between PA and lumbar spine bone loss pointed for inconsistent results. A positive correlation between estimated energy expenditure during sports with bone gain ($r = 0.2$, $P < 0.05$) was reported in kidney transplant patients [156], while a lack of association was reported with two-year spinal BMD loss measured by QCT or DXA [157] in HD patients. Finally, the results for biochemical markers of bone metabolism were consistent, with all cross-sectional and longitudinal studies reporting no significant associations. In summary, cross-sectional data shows positive associations between PA and BMD at femoral neck, lumbar spine and total body. Evidence from longitudinal studies was conflicting, with only one study supporting a positive association between PA and lumbar spine BMD gain. Based on all included observational studies, PA is not related with bone metabolism biomarkers in CKD patients.

3.4.7. Exercise-related effects on bone outcomes (experimental studies)

Only one study explored the effect of aerobic exercise (intradialytic) on BMD and found no significant differences at both lumbar spine and femoral neck areal BMD in the exercise and control groups after 12 weeks [162]. Of note, this study reported a significant bone loss at the femoral neck in the control group compared to the exercise group. Data from trials evaluating the effects of resistance exercise protocols reported inconsistent results on areal

BMD at different skeletal sites. Taken together, results suggested an increase – particularly at the femoral neck and proximal femur [160,165] – or no significant changes in areal BMD after resistance exercise training, mostly at lumbar spine and total body [160,164,165].

Based on the results of the five studies reporting data on biochemical markers of bone metabolism, only three studies found a significant effect of exercise in a limited number of bone biomarkers linked to bone formation (ALP and BALP) and bone resorption (OPG) [161,163,166]. The significant increase in ALP levels were observed only in the exercise groups after 24 weeks of aerobic exercise in CKD stages 3–4 patients [161] and in BALP after 8 weeks of resistance exercise in HD patients [163]. In addition, 24 weeks of intradialytic resistance exercise significantly improved OPG levels, while no changes were observed in the control group [161]. Levels of OC, Trap-5b and OPN were consistently unchanged following any type of exercise intervention [161,163,166].

3.5. Discussion

3.5.1. Summary of the main evidence

The current systematic review showed that PA may be associated with BMD in HD and kidney transplant patients. However, these findings are mostly supported by cross-sectional data showing positive associations between PA and BMD, despite evidence from longitudinal studies was contradictory (i.e., the analysis of observational studies proposes that PA is not related with bone metabolism biomarkers in CKD patients). Evidence from the experimental studies highlights exercise interventions as beneficial to improve bone health in CKD stages 3–4, HD and kidney transplant patients improve bone health in CKD stages 3–4, HD, and kidney transplant patients, with resistance exercise training drawing more solid conclusions than aerobic exercise in its potential effectiveness in improving BMD and bone markers in HD and kidney transplant patients.

3.5.2. Overall completeness and applicability of the evidence

The overall patients included in this review were recruited from seven different countries. Despite such variety, ethnicity was poorly reported, and most patients were male (range between 43 and 71%) The individual studies included in the current review are characterized by small sample sizes and mostly completed in HD patients, thus the summarized data may not apply to a broader adult population with CKD. Additionally, the methods used to assess PA and the exercise interventions varied substantially. Despite such lack of substantial studies, exercise is a non-pharmacological strategy widely recognized as a vital mechanical stimulus for the development and maintenance of optimal bone strength throughout life and the main results of this review point towards a positive effect of exercise on bone health in CKD patients, strengthening its usefulness and applicability within a clinical setting.

3.5.3. Quality of the evidence

This systematic review included 13 studies, six observational (n = 367) and seven experimental (n = 215) studies. However, some of the included studies have methodological limitations that may limit their internal validity. PA was mainly assessed by self-report questionnaires and was poorly described. Regarding our main outcomes, most bone density data was measured with DXA scans, which do not distinguish between trabecular and cortical bone compartments and provide no measure of bone geometry. This may be interpreted as a limitation, as decreased bone strength in CKD patients is associated with the loss of both bone quantity (BMD) and quality (such as the bone microarchitecture) [5]. Furthermore, it has been proposed that DXA may erroneously attribute low BMD values in individuals with low volumetric density, due to a less accurate detection of bone edges, therefore underestimating BMD [167]. In addition, three studies had follow-up lengths of less than 16 weeks. As bone formation biologically takes around 4 months (approximately 16 weeks) to occur [10], interventions completed in shorter periods may not reliably detect skeletal changes with this imaging tool. Additionally, the experimental studies scrutinised in this review examined the effects of different types of exercise, durations, and intensities, and had an unclear risk of bias for most key domains.

As an example, two of the experimental studies presented, despite a similar design (i.e., 12-week, intradialytic exercise program), used different exercise modes (one aerobic [162], the other resistance [164]) and intensities, which may explain the different changes in BMD outcomes and the observed distinct risk of bias (unclear and high, respectively). Therefore, results should be interpreted with caution. Taken together, these methodological limitations impose some constraints to the quality of evidence summarized in the present review.

3.5.4. Potential bias in the review process

A comprehensive search of journals in several databases was conducted and all published trials were identified. Different study designs were included in the review (i.e., longitudinal, cross-sectional, RCT and non-RCT), and all stages of the CKD as well as kidney transplant patients were considered. Additionally, the methodological quality of each study was corroborated by another reviewer.

Some authors were contacted to clarify specific details required during data extraction and to ascertain if any newer data were available since publication. However, there is some potential bias associated in this review. Unpublished trials were not included, as we did not conduct comprehensive searches of conference databases, and although we did not limit the searches to a particular language, only English language studies were included. We are unaware of any other potential biases in the review process.

3.5.5. Agreements and disagreements with other studies or reviews

The link between PA and exercise with bone health has been extensively studied and summarized in several reviews [9,10,112,150]. However, there are no previous reviews exploring this topic in CKD patients. Our systematic review demonstrated that only 3 studies in a total of 6 included in the review, suggests a positive association between PA and BMD at femoral neck and lumbar spine in HD [49,159] and total body also in HD [159] and kidney transplant patients [155]. In fact, these findings are in agreement with other reviews in young adults [168] and post-menopausal women [169], suggesting that similar positive

effects would also be expected in CKD patients. However, the lack of significant associations described in the current review, may be explained by the possible limited reliability of PA questionnaires to assess bone-specific loading exercise rather their intensity [170–172]. Previous studies support a relationship between PA and some bone markers such as BALP, OC in healthy subjects [173] and OPG in breast cancer [174] and post-menopausal [175] women, which is not corroborated by our present review in CKD patients.

Exercise trials in CKD patients were mostly based on resistance training protocols. This type of exercise has been shown to have a significant osteogenic effect [116]. Our review support that resistance exercise may be more effective in improving bone health outcomes than aerobic exercise, which is in agreement with the main literature in older adults and healthy adults [112,176,177]. Intradialytic resistance exercise training revealed to be effective in improving BMD at femoral neck and total body BMC in HD patients [164,165], while resistance exercise performed by kidney transplant patients only improved proximal femur BMD [160]. Several systematic reviews in non-CKD subjects reported that high impact, resistance exercise or combined resistance with high impact exercise induced significant improvements in BMD at femoral neck of premenopausal women and older adults [7,9,177], which is in line with some of the results of this review. A positive effect of resistance exercise on the regulation of the bone formation and resorption biomarkers in healthy subjects has been described [178]. Our findings also support that resistance exercise may elicit positive changes in bone metabolism in CKD patients, particularly in OPG [166], ALP [161] and BALP [163]. Of note, the impact of exercise in bone metabolism was not significant in all measured biomarkers (PTH, iPTH, TRAP5b, sclerostin, OC, OPN), which may be explained by differences in the detection of some biomarkers, measurement techniques, and exercise characteristics (type, intensity, duration, and frequency).

3.6. Conclusions

3.6.1. Implications for practice

This is the first systematic review on observational and experimental studies to analyse the association of PA and exercise with bone outcomes and health in CKD patients. The main goal of the current review was to better inform about the association of PA with bone health and the exercise-related effects on bone health outcomes in CKD patients, and consequently to help improving exercise prescription recommendations. Although the evidence summarized in this review on PA and bone health is limited, clinicians and exercise physiologists should advise CKD patients to increase their PA levels as it may be related with higher BMD, apart from other physiological and psychological benefits that may be derived from increasing PA. Currently, CKD patients are advised to perform resistance exercise [179,180] even though the exercise guidelines vary depending on the referenced organisation. However, most trials included in our review poorly described the resistance exercise protocols in terms of the exercise characteristics (i.e., mode, intensity, duration, and frequency), and progression. The overall effects reported in this review pointed to an increase of BMD at femoral neck and proximal femur, which may prevent and/or decrease the risk of hip fractures. In addition, based on the current evidence, resistance exercise may increase OPG levels, which may be an indicator of better bone mass and strength. OPG protects bone from excessive resorption [181] and was recently associated with bone fractures in CKD patients [182]. Currently, there are no support for a positive effect of aerobic exercise in OPG levels [183]. This review included a limited number of resistance exercise trials; therefore, the summarized data is insufficient to support any recommendation on resistance exercise protocols as a more efficient intervention to improve bone health in CKD patients. Aerobic exercise is also broadly recommended for CKD patients [180], as it may be associated with better exercise capacity and physical functioning [184,185]. Based on evidence from previous reviews in healthy adults, aerobic exercise of moderate intensity and low impact (such as walking) has limited effects on bone parameters [186–188]. Similar results should be expected in CKD patients.

3.6.2. Implications for future research

This systematic review demonstrated that studies exploring this topic are currently lacking. Future studies should implement exercise interventions with a minimum duration of 16 weeks, using more sophisticated imaging techniques such as QCT or MRI, and including a set of key bone metabolism biomarkers of bone formation, resorption and CKD-MBD markers, as they quickly reveal changes in bone turnover. Useful bone biomarkers include: Type I Collagen Cross-Linked C-Telopeptide, P1NP ALP, BALP, OC, Trap-5b, Cathepsin K, sclerostin, DKK1, OPG, receptor activator of nuclear factor kappa-B ligand (RANKL), RANKL/OPG ratio, among enzymes and nonenzymatic peptides derived from the cellular and noncellular compartments of bone. Unfortunately, our summary of results showed inconsistent findings that were mostly based on small trials; thus, studies with appropriate statistical power are needed. In addition, most of the studies included in this review failed to describe the methods used to assess daily PA levels and the details of the exercise protocols and equipment. For instance, the use of elastic bands makes harder to define intensity and training progression.

More RCTs exploring resistance exercise, aerobic, and combined exercise interventions exploring different intensities, durations, and in all spectrum of CKD disease, including pre-dialysis patients are clearly needed. Finally, the acute effects of exercise on bone health outcomes (mass quantity and quality, strength, and biomarkers) should be investigated.

Chapter 4: Associations between bone biomarkers with physical health outcomes in haemodialysis patients: a cross-sectional study

4.1. Abstract

Introduction: HD patients commonly have bone comorbidities and sedentary behaviour, which may impact the patients' physical health and aggravate bone health-related outcomes. However, there is a scarcity of evidence reporting the associations between bone biomarkers and physical health outcomes. Thus, this study aims to describe the bone biomarkers status and the relationship between them, as well as to investigate their association with physical health of HD patients.

Methods: This is a cross-sectional study that included eighty-three HD patients (65.87±14.23 years old). Physical health was assessed trough the ISWT, TUG, STS, Gait Speed, Balance test, HGS, and daily PA assessed through an accelerometer. Additionally, blood samples were collected to analyse markers of bone resorption (TRAP-5b), bone formation (OC) and regulators of bone turnover (OPG), (SOSt) and (RANKL). The Pearson correlation test and linear regression analysis was used to describe the relation of bone biomarkers, as well as associate each one with the physical health outcomes.

Results: RANKL and OPG, both regulator of bone turnover, had a weak and negative association (r= -0.325; p=0.020; n=58). Additionally, SOSt, a regulator of bone turnover, was moderate and negatively associated with OC (r= -0.495; p<0.001; n=51), a bone formation marker. Regarding the association between bone resorption and physical health outcomes, TRAP-5b had a weak and negative correlation with ISWT (r= -0.252; p=0.046; n=63) and HGS (r= -0.259; p=0.046; n=63). OPG had a weak and negative correlation with ISWT (r= -0.372; p=0.004; n=59), STS-60 (r= -0.259; p=0.048; n=59), Gait speed (r= -0.355; p=0.006; n=59), Balance test (r= -0.289; p=0.027; n=59), HGS (r= -0.354; p=0.007; n=56) and moderate activity time (r= -0.302; p=0.037; n=48), while had a moderate and positive correlation with TUG (r= 0.452; p<0.001; n=59). OC had a moderate and positive correlation with light activity time (r= 0.425; p=0.003; n=48). Also, linear regression, adjusted for age, HD vintage and Charlson Comorbidity Index age-adjusted, predicts that increases in ISWT (p=0.017) and HGS (p=0.019) can explain 11% of the variability observed for reduction in TRAP-5b. Additionally, increases of ISWT (p=0.001), STS-60 (p=0.020), gait speed (p=0.003), balance test (p=0.015), HGS (p=0.005) and

light activity time (p=0.005) may explain 16.5%, 9.1%, 14.7, 12.2% and 38%, respectively, of the variability observed for OPG, while increases of TUG (p=<0.001) may explain 20% of the variability observed for OPG, all in the non-adjusted model. Finally, increases on light activity time may explain a reduction of OC of 54% in adjusted model.

Conclusion: There is an inverse association between bone turnover regulators (RANKL and OPG), as well as between OC induced bone formation and SOSt induced bone resorption. Furthermore, an increase in aerobic capacity and muscle strength may be associated with a reduction in bone resorption (TRAP-5b), while increases in light PA may represent bone formation. Furthermore, improvements in physical health seems to be associated with lower OPG levels.

Keywords: Chronic kidney disease, Haemodialysis, Bone Density, Physical Activity, Physical Functional Performance.

4.2. Introduction

CKD patients commonly have low levels of PA that tend to decline with disease progression getting worse in dialysis [13]. Additionally, dialysis patients frequently experience reduced physical function which is also aggravated throughout dialysis vintage [12]. It is well known that poor physical function is manifested by muscle weakness, poor aerobic capacity and poor balance associated with an increased risk of mortality on CKD patients [189,190]. Furthermore, those patients commonly have bone disorders which are aggravated with the disease progression [191].

HD patients commonly develop low bone turnover which is characterized by a decrease in bone resorption and formation, which are explained by the suppression of bone formation through the osteoblasts' apoptosis [5]. It seems that bone and skeletal muscle are negatively affected by the disease and the lack of PA contributing to reduce physical function of HD patients [13]. According to the evidence, bone and muscle are closely connected through the interaction of myokines and osteokines [14]. So, a positive effect in any of this tissue it will be benefit for both structures.

To the best of our knowledge, there is a lack of evidence about the association between bone biomarkers and physical health including PA and physical function in HD patients. Thus, the present study aims to characterize HD patients in terms of their bone biomarkers status, to investigate the relationship between them, as well as their association with the physical health outcomes (physical function and PA).

4.3. Methods

4.3.1. Participants and study design

This is a cross-sectional study is in accordance with the guidelines for reporting observational studies [The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)] (checklist can be found in attachments: Attachment 6). This study encompasses a secondary analysis from the iPRT project (fully described in General Methods). Eighty-eight HD patients were recruited according to the inclusion and exclusion criteria described in General Methods, section 2.1, page 44. However, only 83 were assessed.

4.3.2. Outcomes measures

a) Physical function

Physical function was assessed through HGS [138], STS-60 [138], STS-5 [137], Balance [137], Gait Speed [137], ISWT [135] and TUG [137] tests, as detailed in chapter 2.4.1, pages 41 and 42.

b) Physical activity

Daily PA (min/day) of all patients was monitored through accelerometry, as described in General Methods, section 2.4.2, pages 52 and 53.

c) Bone biomarkers

Blood samples of each patient were collected immediately before HD treatment. All blood samples were collected and analysed according to described in General Methods, section 2.4.5.1, pages 54. Markers of bone resorption: TRAP-5b (Bone TRAP; Immunodiagnostic Systems (IDS) Holdings Ltd, UK); bone formation: OC (Human Osteocalcin DuoSet ELISA kit, R&D Systems, Inc, USA) and regulators of bone turnover: OPG (Human Osteoprotegerin/TNFRSF11B DuoSet ELISA kit (R&D Systems, Inc, USA), SOSt (Biomedica Gruppe, Vienna, Austria) and RANKL (Biomedica, Austria) were assessed through a commercially available ELISA kits and DuoSets.

4.3.3. Statistical analysis

Normality was verified through the Kolmogorov-Smirnov test. Data are expressed as median and interquartile range. Bone biomarkers and physical

health outcomes were transformed using logarithmic (LOG10) transformation for parametrical statistical analysis, but the original data were presented. The Pearson coefficient test was performed to verify the association between bone turnover biomarkers and physical health outcomes. Additionally, linear regression analysis was carried out using the real values to verify how physical health outcomes are associated with the variance of bone turnover biomarkers. Statistical significance was set as $p \leq 0.050$. All data were analysed using IBM SPSS (IBM SPSS Statistics, version 29.0).

4.4. Results

Eighty-three patients were included is this study, as described on figure 4.1. Patients were aged 65.87±14.23 years old with approximately 40.93±51.61 months of dialysis vintage (Table 4.1.). All patients' characteristics are fully described in table 4.1.

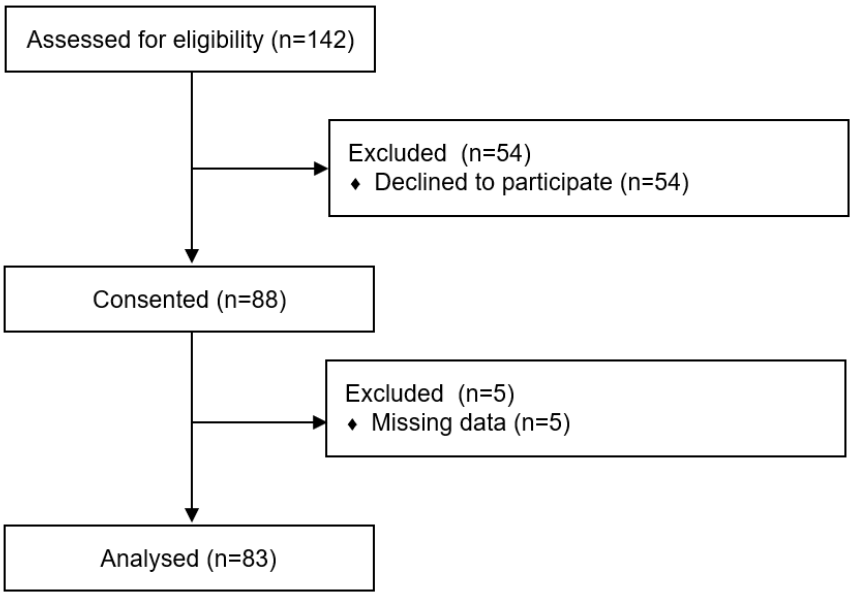

Figure 4.1. Study flow chart

Table 4. 1. Patients' characteristics.

Outcomes	n=83
Age (years)	65.87±14.23
Dialysis Vintage (months)	40.93±51.61
Male, n (%)	55 (66.3)
BMI (Kg/m^2)	26.13±4.62
Dry weight (Kg)	69.49±13.48
CCI	3.92±2.27
Age-adjusted CCI	6.23±3.01
Dialysis Time (min)	243.36±8.32
Albumin (g/dL)	3.96±0.23
iPTH (ng/dL)	308.39±207.46
Total Calcium (mg/dL)	8.87±0.69
Phosphorus (mg/dL) ¥	4.41±0.96
Phosphorus-Calcium Product (mg/dL^2) ¥	39.17±8.87
Magnesium (mg/dL)	2.41±0.43
Haematocrit (%)	34.21±4.34
Haemoglobin (g/dL)	10.86±1.28
Kt/V ¥	1.78±0.30

Note: BMI, Body mass index; CCI, Charlson Comorbidity Index Score; iPTH, Parathyroid hormone; Kt/V, Dialysis efficiency.
¥ parametrical data.

Table 4.2, 4.3, and 4.4 shows the values of bone biomarkers, physical health, and PA levels outcomes, respectively.

Table 4.2. Bone biomarkers.

Outcomes	
TRAP-5b (U/L) [a]	3.33±1.29
SOSt (pmol/l) [b]	126.19±55.60
RANKL (pg/ml) [d]	143.82±177.76
OPG (pg/ml) [c]	6,045±2,518
OC (pg/ml) [e]	72,299±27,943

Note: data in mean and standard deviation. TRAP, Tartrate-resistant acid phosphatase 5b; SOSt, Sclerostin; RANKL, receptor activator of nuclear factor kappa beta; OPG, Osteoprotegerin; OC, Osteocalcin.
Sample size: [a]n=65; [b]n=62; [c]n=59; [d]n=55; [e]n=52.

Table 4.3. Physical health outcomes.

Outcomes	
ISWT (m) [a]	324.79±177.41
TUG (s) [c]	8.08±3.58
STS-60 (times) [d]	24.03±8.50
Gait speed (m/s) [b]	1.05±0.24
HGS (Kg) [c]	28.0±11.45
STS-5 (s) [e]	11.19±4.76
Balance test (score) [b]	3.59±0.77

Note: data in mean and standard deviation. ISWT, Incremental shuttle walk test; TUG, Time up and Go; STS-60, Sit-to-stand 60 seconds; HGS, Handgrip strength; STS-5, sit-to-stand 5 repetitions.
Sample size: [a]n=83; [b]n=82; [c]n=81; [d]n=80; [e]n=79.

Table 4.4. Physical activity levels.

Outcomes	**n=76**
Sedentary time (min/day)	672.78±169.17
Light activity time (min/day)	153±98.94
Moderate activity time (min/day)	6.86±10.05
Vigorous activity time (min/day)	0.14±0.72
Very vigorous activity time (min/day)	0.0±0.0

Note: data in mean and standard deviation.

4.4.1. Associations between bone biomarkers

RANKL and OPG, both bone turnover biomarkers, had a weak and negative association (r= -0.325; p=0.020; n=58) (Figure 4.2.). Additionally, SOSt, a regulator of bone turnover, was moderate and negatively associated with OC (r= -0.495; p<0.001; n=51) (Figure 4.3.), a bone formation biomarker. Other bone biomarkers were not associated (p>0.050).

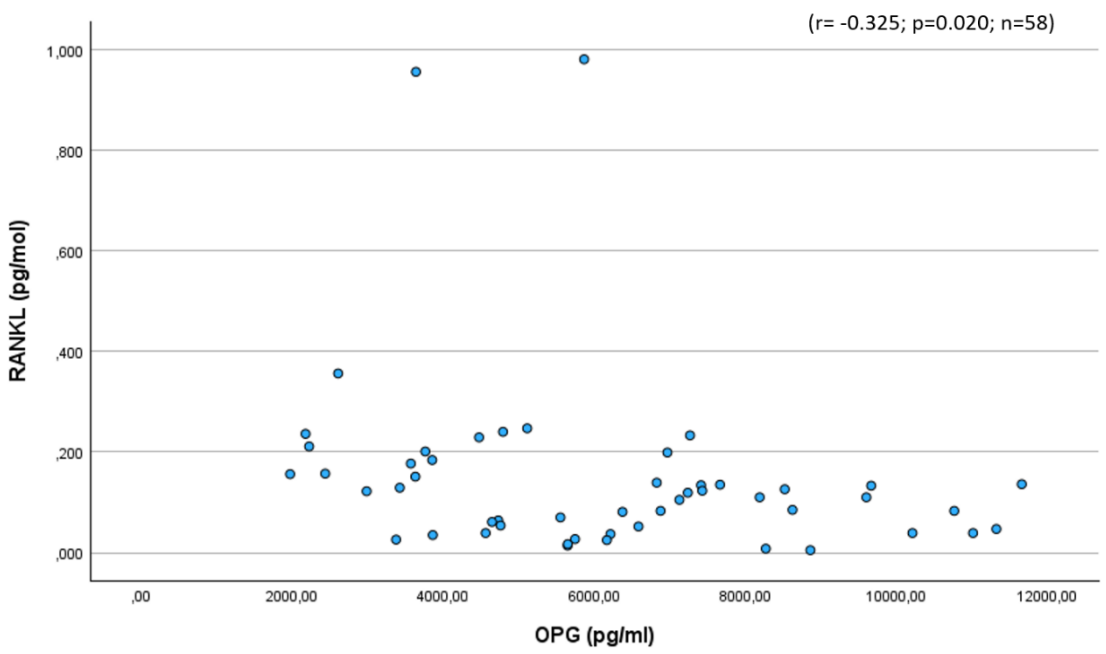

Figure 4.2. Association between RANKL and OPG biomarkers. RANKL, receptor activator of nuclear factor-κB-ligand; OPG, Osteoprotegerin.

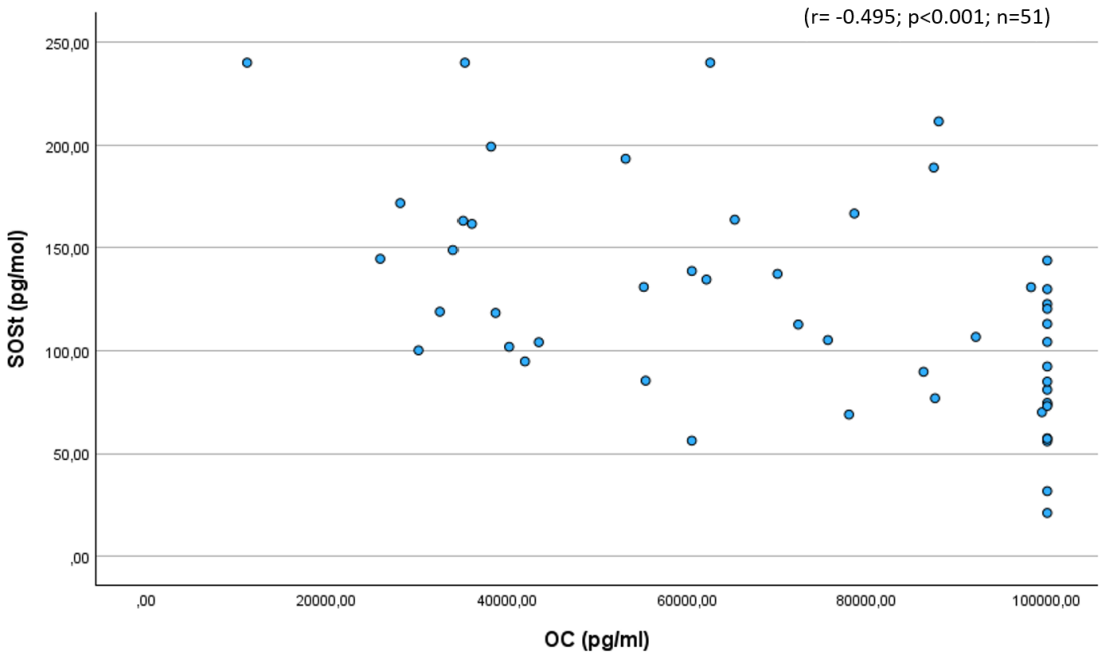

Figure 4.3. Association between SOSt and OC biomarkers. SOSt, Sclerostin; OC, Osteocalcin.

4.4.2. Associations between bone biomarkers and physical health outcomes

Table 4.5. and 4.6. describe the associations between physical health outcomes and PA with bone turnover biomarkers, respectively. TRAP-5b had a weak and negative correlation with ISWT (r= -0.252; p=0.046; n=63) and HGS (r= -0.259; p=0.046; n=63). OPG had a weak and negative correlation with ISWT (r= -0.372; p=0.004; n=59), STS-60 (r= -0.259; p=0.048; n=59), Balance test (r= -0.289; p=0.027; n=59), HGS (r= -0.354; p=0.007; n=56) and moderate with physical activity time (r= -0.302; p=0.037; n=48), while had a moderate and positive correlation with TUG (r= 0.452; p<0.001; n=59), Gait speed (r= 0.355; p=0.006; n=59), and light physical activity time (r=-0.376; p=0.005; n=55). Finally, OC had a moderate and positive correlation with light physical activity time (r= 0.425; p=0.003; n=48). SOSt and RANKL were not associated with the physical health outcomes.

4.4.3. Physical function as variance of bone biomarkers

Increases in ISWT may represent a significant reduction of -0.003 (CI 95% -0.005 to -0.001; p=0.017) U/L in TRAP-5b in the adjusted model. This model explains 11% (R^2=0.111) of the observed variability. Additionally, in the non-adjusted model, the reduction may be -0.002 (CI 95% -0.004 to 0.000; p=0.031) U/L and this model explains 7.4% (R^2=0.074) of the observed variability.

Increases in HGS may represent a significant reduction of -0.040 (CI 95% -0.074 to -0.007; p=0.019) U/L in TRAP-5b in the adjusted model. This model explains 11.1% (R^2=0.111) of the observed variability. Additionally, in the non-adjusted model, the reduction may be -0.030 (CI 95% -0.057 to -0.002; p=0.033) U/L and this model explains 7.6% (R^2=0.076) of the observed variability.

Increases in ISWT may represent a significant reduction of -5.66 (CI 95% -9.04 to -2.28; p=0.001) pg/mL in OPG in the non-adjusted model. This model explains 16.5% (R^2=0.165) of the observed variability. On the other hand, the

adjusted model does not represent a significant change in OPG (-0.65; CI 95% -4.42 to 3.12; p=0.732).

Increases in TUG¸ i.e. a worsening of its performance, may represent a significant increase of 358.71 (CI 95%167.79 to 549.63; p=<0.001) pg/mL in OPG in the non-adjusted model. This model explains 20% (R^2=0.199) of the observed variability. On the other hand, the adjusted model does not represent a significant change in OPG (130.92; CI 95% -67.52 to 329.36; p=0.191).

Increases in STS-60 may represent a significant reduction of -97.54 (CI 95% -179.20 to -15.89; p=0.020) pg/mL in OPG in the non-adjusted model. This model explains 9.1% (R^2=0.091) of the observed variability. On the other hand, the adjusted model does not represent a significant change in OPG (-21.29; CI 95% -99.32 to 56.75; p=0.587).

Increases in gait speed may represent a significant reduction of -4,058 (CI 95% -6,651 to -1,464; p=0.003) pg/mL in OPG in the non-adjusted model. This model explains 14.7% (R^2=0.147) of the observed variability. On the other hand, the adjusted model does not represent a significant change in OPG (-446.81; CI 95% -3,222 to 2,328; p=0.748).

Increases in balance test may represent a significant reduction of -1,155 (CI 95% -2,081 to -230.12; p=0.015) pg/mL in OPG in the non-adjusted model. This model explains 9.9% (R^2=0.099) of the observed variability. On the other hand, the adjusted model does not represent a significant change in OPG (-293.73; CI 95% -1,153 to 565; p=0.496).

Increases in HGS may represent a significant reduction of -77.45 (CI -95% 130.18 to -24.72; p=0.005) pg/mL in OPG in the non-adjusted model. This model explains 12.2% (R^2=0.122) of the observed variability. On the other hand, the adjusted model does not represent a significant change in OPG (-3.79; CI 95% -56.70 to 49.11; p=0.886).

Increases in light activity time may represent a significant reduction of -0.19 (CI 95% -0.33 to -0.06; p=0.005) pg/mL in OPG in the non-adjusted model. This model can explain 38% (R^2=0.376) of the observed variability. On the other

hand, the adjusted model does not represent a significant change in OPG (-0.4; CI 95% -0.15 to 0.08; p=0.513).

Increases in light activity time may represent a significant increase of 0.21 (CI 95% -0.04 to 0.39; p=0.017) pg/mL in OC in the adjusted model. This model explains 54% (R^2=0.541) of the observed variability. Additionally, in the non-adjusted model, the increase may be 0.24 (CI 95% 0.09 to 0.39; p=0.003) pg/mL and this model explains 43% of the observed variability.

There have been no other analyses that show that physical health may predict bone biomarkers (p>0.050).

Table 4.5. Correlation between bone biomarkers and physical function.

Outcomes	TRAP-5b (U/L)		SOSt (pmol/L)		RANKL (pmol/L)		OPG (pg/mL)[¥]		OC (pg/mL)	
	r	p	r	p	r	p	r	p	r	p
ISWT (m)	-0.252	**0.046** [a]	-0.011	0.932 [b]	-0.056	0.686 [h]	-0.372	**0.004** [e]	0.130	0.359 [j]
TUG (s)	0.078	0.543 [a]	0.073	0.571 [b]	-0.087	0.528 [h]	0.452	**<0.001** [e]	-0.132	0.350 [j]
STS-60 (times)	-0.136	0.287 [a]	0.029	0.822 [b]	-0.074	0.590 [h]	-0.259	**0.048** [e]	0.114	0.423 [j]
Gait Speed (m/s)	0.056	0.661 [a]	0.065	0.618 [b]	-0.040	0.772 [h]	0.355	**0.006** [e]	-0.202	0.150 [j]
HGS (Kg)	-0.259	**0.046** [a]	0.016	0.904 [e]	-0.156	0.269 [j]	-0.354	**0.007** [g]	0.084	0.556 [k]
STS-5 (s)	0.177	0.169 [d]	0.048	0.711 [c]	0.053	0.702 [i]	0.235	0.073 [e]	-0.247	0.078 [j]
Balance test (score)	0.028	0.826 [a]	-0.042	0.748 [b]	0.035	0.801 [h]	-0.289	**0.027** [e]	-0.077	0.586 [j]

Note: TRAP, Tartrate-resistant acid phosphatase 5b; SOSt, Sclerostin; RANKL, receptor activator of nuclear factor kappa beta; OPG, Osteoprotegerin; OC, Osteocalcin; ISWT, Incremental shuttle walk test; TUG, Time up and Go; STS-60, Sit-to-stand 60 seconds; HGS, Handgrip strength; STS-5, sit-to-stand 5 repetitions.

Sample size: [a]n=63; [b]n=62; [c]n=61; [d]n=60; [e]n=59; [f]n=58; [g]n=56; [h]n=55; [i]n=54; [j]n=52; [k]n=51; [l]n=48; [m]n=46; [n]=41, [o]n=11; [p]n=10, [q]n=8.

Table 4.6. Correlation between bone biomarkers and physical activity level.

Outcomes	TRAP-5b (U/L)		SOSt (pmol/L)		RANKL (pmol/L)		OPG (pg/mL)[¥]		OC (pg/mL)	
	r	p	r	p	r	p	r	p	r	p
Sedentary time (min/day)	0.103	0.439 [a]	-0.052	0.699 [b]	0.074	0.600 [d]	0.113	0.409 [c]	0.018	0.904 [f]
Light activity time (min/day)	-0.174	0.188 [a]	-0.206	0.120 [b]	-0.116	0.414 [d]	-0.376	**0.005** [c]	0.425	**0.003** [f]
Moderate activity time (min/day)	-0.124	0.381 [d]	-0.049	0.730 [e]	0.056	0.713 [g]	-0.302	**0.037** [f]	0.092	0.569 [h]
Vigorous activity time (min/day)	-0.020	0.953 [i]	0.008	0.981 [i]	-0.010	0.981 [k]	-0.590	0.073 [j]	-0.562	0.091 [j]

Note: TRAP, Tartrate-resistant acid phosphatase 5b; SOSt, Sclerostin; RANKL, receptor activator of nuclear factor kappa beta; OPG, Osteoprotegerin; OC, Osteocalcin; ISWT, Incremental shuttle walk test; TUG, Time up and Go; STS-60, Sit-to-stand 60 seconds; HGS, Handgrip strength; STS-5, sit-to-stand 5 repetitions.

Samples size: [a]n=59; [b]n=58; [c]n=55; [d]n=52; [e]n=51; [f]n=48; [g]n=46; [h]=41, [i]n=11; [j]n=10, [k]n=8.

4.5. Discussion

This cross-sectional study aimed to describe bone biomarkers status, especially those related to bone resorption, bone formation and bone turnover, and the relationship between them, as well as to investigate their association between physical health outcomes, such as physical performance and PA in HD patients. To our knowledge, this is the first study describing the relationship between these outcomes in HD patients.

RANKL seems to have a negative and weak association with OPG. It is recognised that OPG acts by inhibiting RANKL, a biomarker that stimulates osteoclastogenesis. According to the evidence, when RANKL expression is up-regulated, OPG expression is down-regulated, which is an indicative of an increase in osteoclastogenesis [192,193]. OPG is a protein secreted from osteoblasts that links with RANKL preventing bone resorption [85]. An OPG overexpression may induce an increase in bone density, while OPG downregulation may promote an increase in bone turnover with a decrease in bone mass [85], thus the balance in this biomarker levels contribute to maintain bone health. Thus, our results seem to reflect a balance between bone formation and resorption. This is also confirmed by the inverse relationship between SOSt and OC observed in our study, although this correlation was considered moderate. OC regulates bone mineralization while SOSt is known to be a negative regulator of bone formation, and this last biomarker seems to be inversely correlated with bone formation rate in dialysis patients [96,194]. This may explain the negative association between those biomarkers observed in our study, contributing to bone health in these patients. According to recent data, despite the negative associations of SOSt with bone turnover, an increased in SOSt levels is also associated with a decrease in kidney function [195] .

Regarding the bone resorption indicated by TRAP-5b levels, its reduction may be influenced by improvements in aerobic capacity and muscle strength, although this correlation was considered weak. Improvements in overall physical function may also represent an increase in osteoclastogenesis by reducing OPG and, consequently, this reduction may represent an increase in RANKL, as mentioned above. Moreover, increasing PA, even at light intensities, may represent improvements in OC-induced bone formation.

Physical function may also play an important role in bone health-related biomarkers, since there is a crosstalk between muscle and bone [14,196]. It is well known that CKD patients, especially those on dialysis, have low levels of physical function when comparing to healthy subjects [12]. Our study is in line with muscle-bone crosstalk, as it was verified that high physical function is related to low bone resorption due to changes in TRAP-5b levels and is also associated with low bone formation due to changes in OPG levels. Both findings, although significant, present a low explanation of variance. On the other hand, our results may suggest a potential effect of PA on bone turnover in those patients due to the increase in OC levels, with a good explanation of variance. Exercise has an important impact on bone and muscle health, through the release of some myokines that will induce benefit effects in those structures [14],and it has been implemented as a strategy to improve overall physical function in those patients [110,197]. Alongside, exercise has been well documented across the lifespan [112] including in some diseases i.e. in osteoporotic patients [198,199].

CKD patients including those on dialysis use to develop abnormalities in bone turnover where the rate of bone resorption exceeds the rate of bone formation, resulting in bone loss [5]. Lack of PA may induce loss of bone among these patients [5]. According to our results, HD patients spent most of time in sedentary behaviour and these findings are in line with previous studies [101,200]. In addition, it was observed that HD patients are far from the current PA guidelines recommendations [102]. It is recommended that CKD patients perform at least 150 minutes of moderate PA or 75 minutes of vigorous activity or a combination of both per week for health benefits [102]. However, in our study HD patients only performed around 7min/week of moderate PA.

The lowest PA levels may be explained by the severity of the disease and the high time spent on HD treatments. According to the literature, these patients have lower levels of PA in HD treatment days comparing to non-HD days (107), which corresponds to 3 times of treatment per week, at least. Our results revelled controversial data, since increased levels of PA may lead to lower levels of OPG. On the contrary, increased levels in PA, even light, may lead to higher levels of OC. Despite significant, those associations were

considered weak. A systematic review showed that despite PA was not related with any bone biomarker, a positive association between PA and BMD was observed, which, in a way, corroborates our results in relation to OC.

Some efforts have been made to increase overall PA in HD patients [197] and consequently improve their physical function and bone health in HD patients [11,12]. However, the few exercise interventions in the literature, have low volume and intensity [111] which may be insufficient to promote osteogenic effects as well as to increase physical function and strength in those patients.

Despite these results some limitations have been recognized. Poor correlations between outcomes could impact the results, however the variance models confirm this associations even weak. Thus, the results should be interpreted carefully. In addition, some patients missed some timepoints, which decreased sample size in some outcomes and analysis.

4.6. Conclusion

Our data suggest an inverse association between bone turnover regulators (RANKL and OPG), as well as between OC induced bone formation and SOSt induced bone resorption. Furthermore, an increase in aerobic capacity and muscle strength may be associated with a reduction in bone resorption (TRAP-5b), while increases in PA levels, even light, may represent an increase in OC.

Finally, some results were controversial, showing that improvements in physical health seem to be associated with lower OPG induced bone turnover and vice-versa. More studies with this population using a large sample size are needed to verify the association between physical function and activity with bone biomarkers.

Chapter 5: Effects of intradialytic resistance versus aerobic exercise training on bone biomarkers and physical health of haemodialysis patients: a randomized clinical trial

5.1. Abstract

Introduction: It is well known that resistance exercise can promote osteogenic effects in general populations and the same may be expected to occur in HD patients. Bone biomarkers revealed changes in bone turnover and evidence supporting the exercise effects on biomarkers in those patients are scarce (12). Thus, this study aims to verify the effects of 12 weeks of iPRT compared with iAET in HD patients on bone biomarkers and physical health outcomes.

Methods: Eighty-eight patients, recruited from Nephrocare Clinics, were under 6-week run-in control period, without changes in their daily routine. After this period, patients were randomly divided into iAET (cycle ergometer at 50-70 rpm for 30 minutes at a rating of perceived exertion between 12-14) or iPRT (10 exercises, 2 sets of 10-12 repetitions for upper and lower limbs performed at 5-eRM, using free weight-dumbbells and weight exercise machine). Plasma biomarkers of bone formation: OC; bone resorption: TRAP-5b; and regulators of bone turnover: OPG, RANKL and SOSt were measured through commercially available Enzyme-Linked Immunosorbent Assay kits and DuoSets. Physical health was evaluated through the ISWT, TUG, STS-60, Gait Speed, HGS, STS-5, balance test and daily PA levels.

Results: Six-week run-in control period decreased OC levels (p=0.026) and light (%/week, p=0.009; and min/day, p=0.020) and moderate (min/day, p=0.039) physical activity time. While increasing TUG time (p=0.014) and sedentary behaviour (%/week, p=0.010). The comparison between iAET and iPRT before and after 12 weeks of intervention only demonstrated a significant difference in ISWT (iAET ∆12.88±46.71 versus iPRT ∆38.04±48.99 m; p=0.022). While the intragroup analysis demonstrated that iAET increased STS-60 (22.05±8.65 to 25.77±8.92 reps; p=0.033) and TRAP-5b (3.36±1.37 to 44.48±1.52 U/L; p <0.001). A statistical significance was also observed in physical function outcomes when comparing differences observed in the run-in control period with differences observed in the exercise intervention period. The time spent on TUG (p=0.002) and STS-5 (p<0.001) decreased after the intervention, and HGS (p=0.006) and STS-60 (p=0.001) increased.

Conclusion: Non-exercise decreased bone formation and physical health. Moreover, 12 weeks of exercise regardless of modality, seems to improve overall physical function and muscle strength in HD patients. However, 12 weeks of iAET seems to promote bone resorption. Therefore, 12 weeks of exercise seems to not be enough to promote an osteogenic effect in those patients.

Keywords: Chronic kidney disease, Resistance exercise, Aerobic exercise, Bone biomarker, Physical function.

5.2. Introduction

HD patients commonly experience bone disorders which is exacerbated throughout dialysis vintage compromising their quality of life [148,201]. HD patients are known to have an imbalance in bone turnover, where bone resorption usually exceeds bone formation resulting in loss of bone strength [5]. It is well known that bone biomarkers are good predictors of bone turnover in CKD patients [56]. Those biomarkers reveal changes more quickly than other clinical methods and reflect the skeleton as whole [56]. Several bone biomarkers have been used in CKD setting, however markers of bone formation such as OC; bone resorption like TRAP-5b as well as regulators of bone turnover OPG and SOSt have been suggested to assess bone turnover in CKD patients [56,60,62].

In CKD patients, those biomarkers tend to be elevated [60,77,83], which can impact bone health inducing an unbalance between formation and resorption leading to bone loss in those patients [5,14]. Additionally, HD patients commonly have low levels of PA that tend to decline with disease progression which may aggravate bone health status [13]. It is widely recognised that greater levels of PA reduce mortality [108], have a positive impact on clinical outcomes [202] as well as in the prevention of bone loss [11] among those patients. Exercise has been pointing as a non-pharmacological therapy that used to ameliorate bone health-related markers in healthy subjects [112] as well as in osteoporotic [203] and in elderly [7]. Mechanical strain, induced throughout exercise, has a positive impact on the regulation of bone formation [204]. Osteocytes (bone cells) transform mechanical stimuli to intracellular signals, which allow to regulate bone turnover promoting bone health [204].

However, there is a lack of evidence related to those effects on HD patients [11]. Additionally, the few studies that describe the benefits of exercise on bone biomarkers in CKD patients, report exercise programmes with low volume and intensity as well as lack of load progression which may be insufficient to stimulate bone formation and promote bone turnover [111]. To the best of our knowledge, there is also a lack of studies comparing the effects of both types of training: aerobic and resistance exercise on bone biomarkers [11].

Thus, this study aims to investigate the effects of 12 weeks of both iAET and iPRT on bone biomarkers and physical health outcomes in HD patients.

5.3. Methods

5.3.1. Study design and participants

This study was a RCT from iPRT study (fully described on General Methods) and is in accordance with CONSORT guidelines for reporting randomised trials (Attachment 7). Eighty-eight HD patients were recruited from January 2019 to September 2021. After a 6-week run-in period, seventy-six patients were randomly divided into iAET (n=38) or iPRT (n= 38) groups for 12 weeks of exercise intervention. Inclusion and exclusion criteria are detailed in section 2.1. page 44 and 45.

5.3.2. Exercise intervention

Intradialytic exercise sessions were performed 3 times per week for 12 weeks. Resistance exercise training consisted of 10 exercises (2 sets of 12 repetitions) for upper and lower limbs, performed at 5-eRM, using a weight machine and free-weight dumbbells. The vascular access limb was exercised immediately before each HD treatment. Training volume was adjusted at every 4 weeks according to 5-eRM using established equations. Aerobic exercise training was performed on a cycle ergometer at 50-70 rpm for 30 minutes at an RPE between 12-14, with 10 min of increments at every 4 weeks. Exercise training protocol is fully described in Section 2: General Methods.

5.3.3. Outcome measures

Blood samples were collected before the second weekly HD session at each time point. All analytical procedures are fully described in section 2: general methods. Albumin, iPTH, total calcium, phosphorus, phosphorus-calcium product, and magnesium were measured through routine clinical laboratory. Plasma levels of RANKL (Biomedica Gruppe, Vienna, Austria), Sclerostin (Biomedica Gruppe, Vienna, Austria), TRAP-5b (IDS, UK), OPG

(R&D Systems, USA) and Osteocalcin (R&D Systems, USA) were assessed in duplicate through ELISA kits.

5.3.4. Statistical analysis

To determine the number of patients required, a statistical power (G*Power 3) based on a study examining the effects of intradialytic resistance training, which reported a significant increase on muscle strength against a sham intervention group [205] was computed. Sample size calculation revealed a need for 74 subjects (37 in each group) to detect a significant interaction effect (time X treatment) with a power of 80% and $\alpha=0.05$. A target of 88 patients (44 for each group) was identified to accommodate an expected dropout rate around 20%.

Normality was tested through Shapiro-Wilk test. Data were described as mean ± SD. Bone biomarkers and physical function data were transformed using logarithmic (LOG10) transformation for parametrical statistical analysis, but the original data were presented. The baseline patients' characteristics differences between groups were assessed through independent T-test or Mann-Whitney. To verify the differences between bone biomarkers, physical function and PA level in the baseline versus pre-exercise period (i.e., 6-week control) was applied the Paired T-test. The difference between groups was analysed by an Analysis of Covariance (ANCOVA) followed by Bonferroni posthoc test where the dependent variables were the difference between the change before and after each exercise intervention, i.e., the delta value, and the covariate included baseline value. The linear mixed models followed by Bonferroni posthoc were used in both groups for the intragroup comparisons. Statistical significance was set as $p \leq 0.05$.

All data were analysed using IBM SPSS (IBM SPSS Statistics, version 29.0).

5.4. Results

5.4.1. Patients' baseline characteristics and recruitment

The study flow chart is depicted in Figure 5.1. There were 142 eligible patients from medical screening, however only 88 patients consented to participate in the study. Twelve patients were excluded during the 6-week control period mainly due to voluntary withdrawal (50%) and clinical complications. Seventy-six patients were randomly divided into iAET or iPRT (as described in section 2: general methods); however, only 36 and 29 patients completed iAET (95% retention), and iPRT (75% retention) programme, respectively. No adverse events were observed during all interventions.

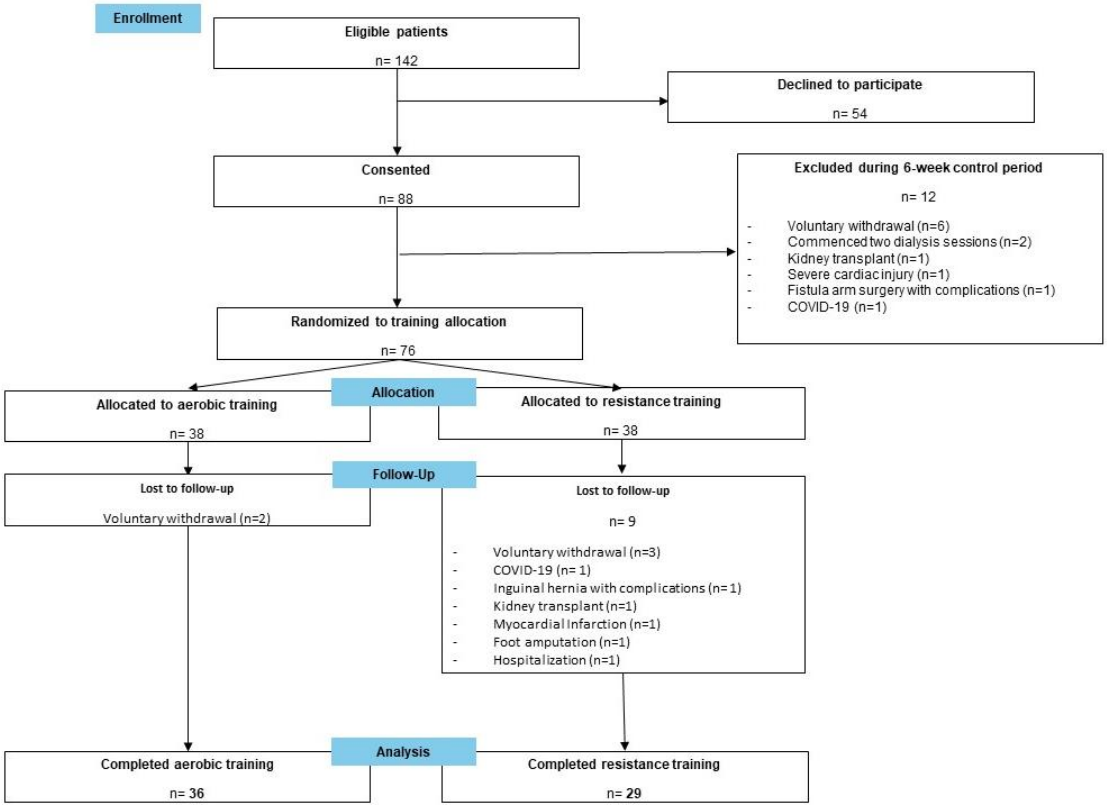

Figure 5.1. Study flow chart.

As described in Table 5.1., there are no significant differences between groups.

Table 5. 1. Baseline patients' characteristics.

Variable	Baseline (n= 88)	iAET (n= 38)	iPRT (n=38)	p-value*
Age (years)	65.99±14.10	66.55±14.45	65.18±13.94	0.409
Dialysis Vintage (months)	41.72±51.71	39.71±61.93	35.39±31.05	0.240
Male, n (%)	60 (62.5%)	25 (65.8)	28 (73.7)	0.454
BMI (kg/m^2)¥	26.04±4.63	21.18±4.71	25.78±4.16	0.173
CCI	4.0±2.29	3.97±2.01	3.97±2.61	0.698
Age-adjusted CCI	6.32±3.04	6.37±2.88	6.18±3.13	0.619
Dry Weight (Kg)¥	69.81±13.89	72.23±12.62	69.73±14.45	0.425
Dilaysis Time (min)	243.52±8.14	243.82±7.32	244.97±6.96	0.521
Albumin (g/dL)	3.96±0.23	3.95±0.22	3.98±0.24	0.622
iPTH (ng/dL)	307.39±201.62	280.46±222.94	325.59±166.60	0.060
Total Calcium (mg/dL)	8.97±0.41	8.69±0.89	9.0±0.40	**0.050**
Phosphorus (mg/dL)¥	4.43±0.95	4.50±0.95	4.34±0.84	0.438
Phosphorus-Calcium Product (mg/dL^2)¥	39.40±8.85	39.67±8.41	39.03±7.92	0.736
Magnesium (mg/dL)	2.40±0.42	2.36±0.38	2.44±0.43	0.584
Kt/V¥	1.78±0.29	1.76±0.31	1.78±0.28	0.731
Haematocrit (%)	34.22±4.25	34.75±4.81	33.54±3.65	0.430
Haemoglobin (g/dL)	10.87±1.26	10.91±1.20	10.83±1.33	0.946

Note: BMI, Body mass index; CCI: Charlson Comorbidity Index Score; iPTH, Parathyroid hormone; Kt/V, Dialysis efficiency.
Data described as mean ± standard deviation.
¥parametrical data.

5.4.2. Control period

Bone biomarkers. Apart from a decrease in OC levels (p=0.026) after control period (pre-exercise timepoint), no changes were observed in other variable (Table 5.2.).

Table 5. 2. Bone biomarkers comparisons between baseline and pre-exercise period.

Variable	Baseline	Pre-exercise	p-value
TRAP-5b (U/L)	3.33±1.29 [a]	3.54±1.46 [a]	0.248 [a]
SOSt (pmol/L)	126.19±55.60 [c]	125.21±56.16 [b]	0.505 [d]
RANKL (pg/mL)	143.82±177.76 [h]	150.18±148.88 [l]	0.626 [m]
OPG (pg/mL)	6,045±2,518 [f]	6,223±2,791 [e]	0.868 [g]
OC (pg/mL)	72,299±27,943 [i]	64,216±29,435 [m]	**0.026 [n]**
RANKL/OPG ratio	0.03±0.05 [j]	0.03±0.03 [n]	0.443 [o]

Note: TRAP, Tartrate-resistant acid phosphatase 5b; SOSt, Sclerostin; RANKL, receptor activator of nuclear factor kappa beta; OPG, Osteoprotegerin; OC, Osteocalcin.
Data described as mean ± standard deviation.
Sample size:[a]n=65; [b]n=63; [c]n=62; [d]n=61; [e]n=60; [f]n= 59; [g]n=58; [h]n=55; [i]n=52; [j]n=51; [l]n=50; [m]n= 49; [n]n=47; [o]n=46.

Physical function. After 6-week control period, an increase in time walked on TUG test was observed (p=0.014) (Table 5.3.).

Table 5. 3. Physical function comparisons between baseline and pre-exercise period.

Variable	Baseline	Pre-exercise	p-value
ISWT (m)	324.79±177.41 [a]	320.99±185.18 [d]	0.211 [f]
TUG (s)	8.08±3.58 [c]	8.58±4,17 [f]	**0.014 [h]**
STS-60 (reps)	24.03±8.50 [d]	23.49±8.47[h]	0.666 [i]
Gait speed (m/s)	1.05±0.24 [b]	1.06±0.26 [e]	0.245 [g]
HGS (KgF)	28.0±11.45 [c]	27.48±11.77 [c]	0.063 [g]
STS-5 (s)	11.19±4.76 [e]	11.15±3.70 [h]	0.619 [j]
Balance test (score)	3.59±0.77 [b]	3.43±0.97 [e]	0.217 [h]

Note: ISWT, Incremental shuttle walk test; TUG, Time up and Go; STS-60, Sit-to-stand 60 seconds; HGS, Handgrip strength; STS-5, sit-to-stand 5 repetitions.
Sample size: [a]n=83; [b]n=82; [c]n=81; [d]n=80; [e]n=79. [f]n=78; [g]n=77; [h]n=76; [i]n=74; [j]n=73.

Physical activity. The percentage of time spent in sedentary behaviour for a week increased (p=0.010) after control period, while sedentary time measured in counts per minute decreased (p=0.047). In parallel, light activity time demonstrated a significant decrease in both percentage per week (p=0.009), minutes per day (p=0.020) and hour (p=0.021). Moreover, moderate activity time in minutes per day also exhibited a significant decrease (p=0.039).

Table 5. 4. Physical activity comparison between baseline and pre-exercise period.

Outcomes	Baseline	Pre-exercise	p-value
Wearable time (h/day)	12.95±2.11 [b]	13.63±2.45 [a]	0.050 [d]
Sedentary time (%/week)	80.77±11.55 [b]	83.37±11.93 [c]	**0.010** [e]
Sedentary time (min/day)	672.78±169.17 [b]	696.20±181.38 [c]	0.379 [e]
Sedentary time (hour)	78.49±19.74 [b]	81.23±21.16 [c]	0.377 [e]
Sedentary (counts/min)	7.50±2.89 [b]	6.92±3.33 [c]	**0.047** [e]
Light activity time (%/week)	18.39±11.0 [b]	15.83±11.18 [c]	**0.009** [e]
Light activity time (min/day)	153±98.94 [b]	134.40±101.49 [c]	**0.020** [e]
Light activity time (hour)	17.95±11.55 [b]	15.59±11.84 [c]	**0.021** [e]
Light activity (counts/min)	478.72±134.42 [b]	467.03±134.06 [c]	0.366 [e]
Moderate activity time (%/week)	0.82±1.21 [b]	0.79±1.34 [c]	0.104 [h]
Moderate activity time (min/day)	6.86±10.05 [b]	6.54±11.21 [c]	**0.039** [h]
Moderate activity time (hour)	0.81±1.17 [b]	0.77±1.31 [c]	0.070 [i]
Moderate activity (counts/min)	3,398±468.64 [f]	3,407±555.29 [g]	0.722 [h]
Vigorous activity time (%/week)	0.02±0.09 [b]	0.02±0.09 [c]	0.662 [m]
Vigorous activity time (min/day)	0.14±0.72 [b]	0.18±0.68 [c]	0.590 [m]
Vigorous activity time (hour)	0.01±0.08 [b]	0.02±0.09 [c]	0.851 [n]
Vigorous activity (counts/min)	6,748±369.48 [j]	6,628±460.13 [l]	0.817 [m]

Note: Data is described as mean ± standard deviation.
Sample size: [a]n=81; [b]n=76; [c]n=75; [d]n=73; [e]n=70; [f]n=62; [g]n=58; [h]n=52; [i]n=43; [j]n=12; [l]n=10; [m]n=8; [n]n=4.

5.4.3. Exercise intervention

Training adherence is described on Table 5.5. No significant differences were observed between groups (p=0.780).

Table 5. 5. Training adherence.

Variable	iAET (n=36)	iPRT (n=29)	p-value
Training Adherence (%)	92.0 (83.0 – 97.0)	92.0 (81.0 – 97.0)	0.780
Pre-HD Training Adherence (%)	-	94.0 (84.5 – 98.5)	-
Intradialytic Time Week 4, min	40.0 (40.0 – 40.0)		
Intradialytic Time Weeks 8, min	50.0 (50.0 – 50.0)	-	-
p-value	<0.001		
Intradialytic Time Week 8, min	40 ± 2		
Intradialytic Time Week 12, min	48 ± 5	-	-
p-value	<0.001		
Intradialytic Total Workload Week 0-4		586 ± 254	
Intradialytic Total Workload Week 4-8	-	781 ± 306	-
p-value		<0.001	
Intradialytic Total Workload Week 4-8		781 ± 306	
Intradialytic Total Workload Week 8-12	-	909 ± 366	-
p-value		<0.001	
Pre-HD Total Workload Week 0-4		502 ± 259	
Pre-HD Total Workload Week 4-8	-	640 ± 313	-
p-value		<0.001	
Pre-HD Total Workload Week 4-8		640 ± 313	
Pre-HD Total Workload Week 8-12	-	780 ± 380	-
p-value		<0.001	

Note: Data described as median (interquartile range) and mean ± standard deviation

Bone biomarkers. Changes in bone biomarkers during the 12 weeks of exercise intervention in both groups are present in Table 5.6. No significant changes were observed in any variable when comparing between and intragroup. However, TRAP-5b increased significantly after 12 weeks of iAET ($p<0.001$).

Table 5. 6. Effects of 12-week exercise training on bone biomarkers.

Variable	iAET	iPRT	p-value
TRAP-5b, U/L			
Pre-exercise	3.36±1.37 [a]	3.77±1.57 [e]	0.273 †
Post-exercise	4.48±1.52 [a]	4.46±1.80 [e]	
Change	1.12±1.33	0.69±0.87	
p-value intragroup	**<0.001**	0.069	
SOSt, pmol/L			
Pre-exercise	120.67±57.09 [a]	131.26±55.38 [g]	0.418 ‡
Post-exercise	127.06±56.80 [a]	129.93±56.15 [f]	
Change	6.38±32.43	3.20±19.48	
p-value intragroup	0.524	0.936	
RANKL, pg/mL			
Pre-exercise	159.04±182.15 [f]	138.91±93.94 [i]	0.980 *
Post-exercise	130.70±162.19 [d]	123.86±71.42 [j]	
Change	-23.74±92.59	-18.33±72.27	
p-value intragroup	0.227	0.633	
OPG, pg/mL			
Pre-exercise	6,613±2,927 [b]	5,747±2,362 [g]	0.472 #
Post-exercise	6,757±2,776 [b]	5,713±2,249 [f]	
Change	143.83±1,528	43.71±1,521	
p-value intragroup	0.745	0.951	
OC, pg/mL			
Pre-exercise	61,262±29,779 [c]	69,304±28,950 [m]	0.419 ¶
Post-exercise	60,475±32,721 [b]	60,403±26.942 [f]	
Change	-3,337±27,506	-4,908±16,365	
p-value intragroup	0.393	0.584	
RANKL/OPG ratio			
Pre-exercise	0.03±0.04 [h]	0.03±0.03 [j]	0.800 ¶
Post-exercise	0.03±0.04 [g]	0.03±0.02 [l]	
Change	-0.00±0.02	-0.01±0.02	
p-value intragroup	0.313	0.551	

Note: TRAP, Tartrate-resistant acid phosphatase 5b; SOSt, Sclerostin; RANKL, receptor activator of nuclear factor kappa beta; OPG, Osteoprotegerin; OC, Osteocalcin.
Data described as mean ± standard deviation.
Sample size for intragroup analysis: [a]n=36; [b]n=33; [c]n=31; [d]n=30; [e]n=29; [f]n=28; [g]n=27; [h]n=26; [i]n=22; [j]n=21; [l]n=20; [m]n=18.
Sample size for intergroups analysis: †n=36 aerobic and n=29 resistance; ‡n=36 aerobic and n=26 resistance; *n=27 aerobic and n=21 resistance; #n=33 aerobic and n=27 resistance; ¶n=31 aerobic and n=17 resistance; ¶¶n=25 aerobic and n=20 resistance.

Physical function. As described in Table 5.7., the comparison between iAET and iPRT before and after 12 weeks of exercise intervention demonstrated a significant difference in ISWT (iAET ∆12.88±46.71 *versus* iPRT ∆38.04±48.99; p=0.022). Additionally, the intragroup analysis showed a significant increase in STS-60 (p=0.033) on iAET.

Table 5. 7. Effects of 12-week exercise training on physical function outcomes.

Variable	iAET	iPRT	p-value
ISWT (m)			
Pre-exercise	316.68±193.02 [b]	343.50±179.74 [a]	**0.022†**
Post-exercise	339.72±199.44 [d]	406.50±181.98 [f]	
Change	12.88±46.71 †	38.04±48.99 †	
p-value intragroup	0.708	0.164	
TUG (s)			
Pre-exercise	8.73±3.84 [b]	8.03±3.32 [a]	0.134†
Post-exercise	8.25±3.60 [d]	6.74±1.63 [f]	
Change	-0.49±1.39 †	-0.73±1.46 †	
p-value intragroup	0.493	0.110	
STS-60 (times)			
Pre-exercise	22.05±8.65 [b]	25.0±8.25 [b]	0.697†
Post-exercise	25.77±8.92 [d]	28.48±8.18 [f]	
Change	3.23±3.23 †	2.41±3.64 †	
p-value intragroup	**0.033**	0.102	
Gait speed (m/s)			
Pre-exercise	1.04±0.26 [b]	1.11±0.24 [a]	0.102†
Post-exercise	1.06±0.27 [d]	1.19±0.18 [f]	
Change	-0.03±0.81 †	-0.20±0.54 †	
p-value intragroup	0.804	0.152	
HGS (kg)			
Pre-exercise	26.36 ± 11.21 [c]	29.63 ± 12.65 [a]	0.936‡
Post-exercise	27.91 ± 11.51 [e]	31.41 ± 13.88 [f]	
Change	0.0 (-1.0 to 4.0) ‡	1.0 (-1.0 to 4.50) ‡	
p-value intragroup	0.569	0.634	
STS-5 (s)			
Pre-exercise	11.48±4.17 [b]	10.94±3.26 [b]	0.779†
Post-exercise	9.97±3.57 [d]	9.04±2.68 [f]	
Change	-1.53±2.07 †	-1.42±1.56 †	
p-value intragroup	0.547	0.263	
Balance test			
Pre-exercise	3.41±0.90 [b]	3.50±0.92 [a]	0.535†
Post-exercise	3.57±0.85 [d]	3.62±0.86 [f]	
Change	0.14±0.69 †	-0.03±0.73 †	
p-value intragroup	0.522	0.611	

Note: ISWT, Incremental shuttle walk test; TUG, Time up and Go; STS-60, Sit-to-stand 60 seconds; HGS, Handgrip strength; STS-5, sit-to-stand 5 repetitions.
Data described as mean ± standard deviation.
Sample size for intragroup analysis: [a]n=38; [b]n=37; [c]n=36; [d]n=35; [e]n=33; [f]n=29.
Sample size for intergroup analysis: †n=35 aerobic and n=29 resistance; ‡n=33 aerobic and n=29 resistance.

Physical activity. PA data are demonstrating on Table 5.8. There were no differences in the comparisons before and after the 12 weeks of exercise in both protocols (p>0.050 in all), except on sedentary time in counts/min (iAET ∆-0.03±2.11 versus iPRT ∆1.01±2.41; p=0.041). In addition, iAET group decreased the time spent in vigorous activity after 12 weeks of this exercise intervention (∆-0.09±0.48, p=0.048).

Table 5.8. Effects of 12-week exercise interventions on physical activity level.

Outcomes	iAET	iPRT	p-value
Wearable time (h/day)			
Pre-exercise	13.89±2.22 [a]	13.62±2.50 [a]	
Post-exercise	13.61±2.34 [c]	12.82±2.42 [f]	0.294 [†]
Change	-0.38±1.90	-0.52±3.26	
p-value intra-group	0.550	0.151	
Sedentary time (%/week)			
Pre-exercise	83.56±13.07 [d]	82.02±11.16 [b]	
Post-exercise	82.22±12.55 [e]	79.79±12.27 [g]	0.185[‡]
Change	0.19±3.30	-1.79±7.11	
p-value intra-group	0.708	0.464	
Sedentary time (min/day)			
Pre-exercise	738.53±207.45 [d]	667.98±156.32 [b]	
Post-exercise	714.92±184.52[e]	617.11±160.53 [g]	0.720 [*]
Change	-33.17±95.18	-26.06±106.10	
p-value intra-group	0.691	0.160	
Sedentary time (hour)			
Pre-exercise	86.17±24.20 [d]	77.94±18.24 [b]	
Post-exercise	83.42±21.53 [e]	71.01±18.74 [g]	0.426 [‡]
Change	-3.87±11.10	-3.03±12.38	
p-value intra-group	0.690	0.161	
Sedentary time (counts/min)			
Pre-exercise	7.0±3.58 [d]	7.36±2.98 [b]	
Post-exercise	7.31±3.71 [e]	8.52±3.42 [g]	**0.041** [‡]
Change	-0.03±2.11	1.01±2.41	
p-value intra-group	0.735	0.242	
Light activity time (%/week)			
Pre-exercise	15.86±12.47 [d]	16.89±10.25 [b]	
Post-exercise	17.24±12.12 [e]	18.84±11.23 [g]	0.102 [‡]
Change	-0.11±4.25	1.48±6.87	
p-value intra-group	0.647	0.624	
Light activity time (min/day)			
Pre-exercise	139.09±110.09 [d]	140.53±96.08 [b]	
Post-exercise	149.65±112.44 [e]	142.42±81.78 [g]	0.185 [*]
Change	-4.45±35.92	-0.95±70.57	
p-value intra-group	0.417	0.564	
Light activity time (hour)			
Pre-exercise	16.24±12.85 [d]	16.40±11.21 [b]	
Post-exercise	17.47±13.12 [e]	16.62±9.54 [g]	0.148 [‡]
Change	-0.52±4.19	-0.11±8.23	
p-value intra-group	0.685	0.828	
Light activity time (counts/min)			
Pre-exercise	444.14±134.95 [d]	490.23±137.42 [b]	
Post-exercise	461.54±137.59 [e]	502.75±124.96 [g]	0.517 [‡]
Change	-1.23±56.95	2.02±87.60	
p-value intra-group	0.606	0.650	
Moderate activity time (%/week)			
Pre-exercise	0.57±0.95 [i]	1.09±1.63 [d]	
Post-exercise	0.54±0.78 [j]	1.29±2.26 [h]	0.269 [#]
Change	-0.07±0.52	0.23±1.01	
p-value intra-group	0.459	0.298	
Moderate activity time (min/day)			
Pre-exercise	4.94±8.23 [d]	8.78±13.62 [b]	
Post-exercise	4.49±6.25 [e]	9.77±17.57 [g]	0.261 [¶]
Change	-0.81±4.16	1.17±7.60	
p-value intra-group	0.681	0.993	
Moderate activity time (hour)			0.403 [π]

Pre-exercise	0.58±0.96 [d]	1.03±1.60 [b]	
Post-exercise	0.53±0.73 [e]	1.15±2.05 [g]	
Change	-0.10±0.48	0.14±0.89	
p-value intra-group	0.852	0.960	
Moderate activity time (counts/min)			
Pre-exercise	3,456±579.93 [i]	3,418±547.54 [d]	
Post-exercise	3,475±445.79 [j]	3,486±531.78 [h]	0.829 [#]
Change	-6.72±403.15	34.62±259.71	
p-value intra-group	0.820	0.587	
Vigorous activity time (%/week)			
Pre-exercise	0.02±0.06 [d]	0.03±0.11 [b]	
Post-exercise	0.01±0.01 [e]	0.08±0.28 [g]	0.075 [¤]
Change	-0.01±0.06	0.04±0.16	
p-value intra-group	0.060	0.177	
Vigorous activity time (min/day)			
Pre-exercise	0.14±0.56 [d]	0.23±0.82 [b]	
Post-exercise	0.05±0.13 [e]	0.53±1.58 [g]	0.117 [¤]
Change	-0.09±0.48	0.22±0.87	
p-value intra-group	**0.048**	0.285	
Vigorous activity time (hour)			
Pre-exercise	0.02±0.07 [d]	0.03±0.10 [b]	
Post-exercise	0.01±0.02 [e]	0.07±0.18 [g]	0.247 [§]
Change	-0.01±0.06	0.03±0.09	
p-value intra-group	0.545	0.644	
Vigorous activity time (counts/min)			
Pre-exercise	6,549±403.21 [m]	6,662±508.85 [k]	
Post-exercise	6,375±166.92 [l]	6,493±152.08 [l]	0.992 [¤]
Change	-33.08±411.54	-31.95±382.65	
p-value intra-group	0.475	0.411	

Note: Data is described as mean ± standard deviation. Sample size for for intergroup analysis: [†]n=36 iAET and n=29 iPRT; [‡]n=30 iAET and n=27 iPRT; [*]n=28 iAET and n=25 iPRT; [#]n=19 iAET and n=22 iPRT; [¶]=19 iAET and n=21 iPRT; [¤]n=18 iAET and n=19 iPRT; [¤]n=3 iAET and n=5 iPRT; [§]n=2 iAET and n=5 iPRT. Sample size for intra-group analysis: [a]n=38; [b]n=37; [c]n=36; [d]n=33; [e]n=32; [f]n=29; gn=27; [h]n=24; [i]n=22; [j]n=21; [k]n=7; [l]n=6; [m]n=3.

5.4.4. Comparison between control and exercise period

Bone Biomarkers. When compared control to exercise period, a significant increase in TRAP-5b levels (p= 0.006) was observed (table 5.9.).

Table 5.9. Comparison of changes in bone biomarkers between the 6-week run-in control period and 12-week grouped exercise period.

Variable	Control period	Exercise period	p-value
TRAP-5b (U/L)	0.22±1.01 [a]	0.93±1.16 [a]	**0.006** [a]
SOSt (pmol/L)	-1.13±21.12 [c]	5.05±27.59 [b]	0.103 [d]
RANKL (pg/mL)	-2.31±144.57 [f]	-21.37±83.50 [g]	0.314 [h]
OPG (pg/mL)	85.52±1,306 [e]	98.78±1,513 [d]	0.511 [e]
OC (pg/mL)	-4,840±16,026 [h]	-3,893±23,972 [g]	0.740 [i]
RANKL/OPG ratio	-0.00±0.04 [i]	-0.00±0.02 [j]	0.445 [k]

Note: TRAP, Tartrate-resistant acid phosphatase 5b; SOSt, Sclerostin; RANKL, receptor activator of nuclear factor kappa beta; OPG, Osteoprotegerin; OC, Osteocalcin.
Data described as mean ± standard deviation.
Sample size: [a]n=65; [b]n=62; [c]n=61; [d]n=60; [e]n=58; [f]n=49; [g]n=48; [h]n=47; [i]n=46; [j]n=45; [k]n=44.

Physical Function. The comparisons between the delta values of the control run-in period and the exercise period on physical function are fully described in Table. 5.10. A decreased in time walking on TUG (p=0.002) and in time spent performing STS-5 test (p<0.001) were observed after exercise period. Moreover, it was observed an increased in the number of repetitions performed on STS-60 (p=0.001) and in muscle strength on HGS (p=0.006) after exercise period.

Table 5. 10. Comparison of changes in physical function between the 6-week run-in control period and 12-week grouped exercise period.

Variable	Control period	Exercise period	p-value
ISWT (m)	-3.06±58.50 [a]	24.28±49.03 [g]	0.104 [h]
TUG (s)	0.38±1.17 [c]	-0.59±1.42 [g]	**0.002** [h]
STS-60 (reps)	-0.50±3.71 [d]	2.86±3.42 [g]	**0.001** [h]
Gait speed (m/s)	0.02±0.16 [b]	-0.11±0.7 [g]	0.604 [h]
HGS (Kg)	-0.55±3.94 [b]	1.42±3.46 [h]	**0.006** [j]
STS-5 (s)	-0.04±1.96 [e]	-1.48±1.85 [g]	**<0.001** [i]
Balance test (score)	-0.10±0.75 [f]	0.06±0.71 [g]	0.463 [h]

Note: ISWT, Incremental shuttle walk test; TUG, Time up and Go; STS-60, Sit-to-stand 60 seconds; HGS, Handgrip strength; STS-5, sit-to-stand 5 repetitions.
Data described as mean ± standard deviation.
Sample size: [a]n=78; [b]n=77; [c]n=76; [d]n=74; [e]=73; [f]=69; [g]n=64; [h]n=62; [i]n=61; [j]n=59.

Physical Activity. The comparisons between the delta values of the control period versus the exercise period on PA are fully described in Table. 5.11. No significant changes were observed, except for wearable time (p=0.045).

Table 5. 11. Comparison of changes in physical activity level between the 6-week run-in control period and 12-week grouped exercise period.

Outcomes	Control period	Exercise period	p-value
Wearable time (h/day)	0.60±2.39 [a]	-0.44±2.58 [c]	**0.045 [d]**
Sedentary time (%/week)	1.65±4.95 [b]	-0.75±5.83 [e]	0.169 [f]
Sedentary time (min/day)	16.61±140.44 [b]	-29.80±99.66 [e]	0.051 [f]
Sedentary time (hour)	1.94±16.39 [b]	-3.47±11.63 [e]	0.050 [f]
Sedentary (counts/min)	-0.38±2.25 [b]	0.46±2.30 [e]	0.094 [f]
Light activity time (%/week)	-1.54±4.89 [b]	0.64±5.65 [e]	0.174 [f]
Light activity time (min/day)	-11.66±44.53 [b]	-2.79±54.62 [e]	0.564 [f]
Light activity time (hour)	-1.36±5.20 [b]	-0.33±6.37 [e]	0.573 [f]
Light activity (counts/min)	-8.94±59.97 [b]	0.31±72.42 [e]	0.844 [f]
Moderate activity time (%/week)	-0.09±0.63 [b]	0.07±0.80 [e]	0.128 [i]
Moderate activity time (min/day)	-0.76±5.70 [b]	1.12±6.06 [e]	0.222 [i]
Moderate activity time (hour)	-0.09±0.67 [b]	0.02±0.71 [e]	0.117 [j]
Moderate activity (counts/min)	-13.88±429.66 [g]	15.46±330.13 [h]	0.489 [i]
Vigorous activity time (%/week)	-0.00±0.10 [b]	0.01±0.12 [e]	0.555 [l]
Vigorous activity time (min/day)	-0.03±0.79 [b]	0.05±0.70 [e]	0.462 [l]
Vigorous activity time (hour)	0.00±0.09 [b]	0.00±0.08 [e]	0.978 [m]
Vigorous activity (counts/min)	40.16±440.92 [k]	-32.37±363.40 [k]	0.863 [l]

Note: Data is described as mean ± standard deviation.
Sample size: [a]n=73; [b]n=70; [c]n=65; [d]n=59; [e]n=57; [f]n=53; [g]n=52; [h]n=41; [i]n=40; [j]n=35; [k]n=8; [l]n=7 [m]n=4.

5.5. Discussion

Latest evidence revealed that HD patients should practice regular exercise to improve their bone quality and physical function [11]. However, according to the previous literature more studies are needed to better understand the effects of regular exercise and which type of exercise should be performed to induce positive effects on bone biomarkers in HD patients [11].

Six weeks of control period (non-exercise period) decreased OC levels, worse a submaximal effort, detected by the TUG, increased the percentage of time spent on sedentary behaviour and decreased the time spent on light and moderate PA. However, 12 weeks of iAET seems to increase a marker of bone resorption (TRAP-5b) as well as the number of repetitions performed on STS-60 test. In addition, patients who performed iAET decreased their time in vigorous activity. While iPRT seems to increase the distance walked in ISWT when compared to iAET. Furthermore, patients who performed iPRT had higher

counts/min in sedentary behaviour compared to those on iAET. However, when we compare the control period with the exercise period (despite exercise modality), bone resorption (TRAP-5b biomarker) increased after exercise intervention. Significant positive changes were observed in overall physical function, without changes in PA levels of patients.

In this sense, according to our findings, despite no changes on OC levels after 12 weeks of exercise intervention, there was a decreased in those levels after 6 weeks of control period, which may indicate that HD treatment may induce an inhibition of bone formation. In addition, the increased in TUG time as well as the decreased in light and moderate PA levels after control period may demonstrate poor physical function. These results are in line with previous literature [206,207].

According to our results, twelve weeks of iAET increased a marker of bone resorption, named TRAP-5b. It is known that iAET are more likely to induce effects on aerobic capacity, with poor evidence on its effects on bone health when compared to resistance exercise. The literature showed that resistance exercise promotes an osteogenic effect on a different population like in elderly [7] and osteoporotic [203] subjects. However, despite no clearly evidence about those effects on HD patients, one study demonstrated that 8 weeks of resistance exercise increased a marker of bone formation in HD patients [163]. Additionally, there is also evidence that 24 weeks of intradialytic resistance exercise improved OPG levels indicating an osteoclastogenesis inhibition [166].

Recently, a study demonstrated that resistance training performed with cluster set method (fractional sets with brief pauses interspersed every 3 or 4 repetitions) decreased SOSt levels in CKD patients [208], which may indicate an increase in osteoblast bone formation. This evidence allied with our results may suggest that iAET could not be the best type of exercise to potentiate osteogenic effect in those patients and may contribute to promote bone resorption. In parallel, we designed an exercise training programme to reach the osteogenic effect, however looking for these results we may infer that the intensity of exercises could also influence the results obtained. Hong et al. (2018) [116], suggest that higher intensities around 80-85% of 1-RM induce

greater skeletal benefits; however, intensities around 65-75% of 1-RM were applied in our study.

The achievement of higher exercise intensities during dialysis is difficult as the movement of those patients are limited to their position; however, our protocol applied higher intensities comparing to other protocols in the literature [11], although may not be sufficient. According to the literature, for better adaptative response of bone to resistance exercise training, protocols should include load progression, higher intensities and exercises must be dynamic rather than static [116]. Our resistance exercise training protocol included all these recommendations as well as is in accordance with American College of Sports Medicine guidelines [179]. However, it seems that this protocol was insufficient to induce osteogenic effects.

iAET seems to improve physical function detected by the number of repetitions performed on STS-60 test, while iPRT improved aerobic capacity throughout the ISWT when comparing to iAET. Despite those results are not in accordance with the literature, as aerobic exercise is used to improve aerobic capacity and resistance exercise to muscle strength, when we compared control period to exercise intervention, we observed that physical function improved after exercise intervention. Those results are in accordance with latest evidence reporting that regardless the exercise modality, exercise seems to improve overall physical function in those patients [15].

Looking for PA results, few significant changes were observed after both exercise programmes. However, according to the literature, it was expected an increase in PA levels of those patients [209]. Our results may indicate that these HD patients only exercised during dialysis session, which did not promote an increase in PA detected by the accelerometer. To explain the low engage in PA, there are several barriers to exercise that may contribute to this behaviour [105,107].

The authors recognized some limitations, bone biomarkers have circadian rhythm which is a potential source of variability special for bone resorption markers [57,210]. Despite the sample collections of each patient were performed at same conditions at each time point, some patients collected

blood during the morning others afternoon, which might introduce some bias in the analysis due to this circadian rhythm. In addition, the long control period potentially impacted withdrawal. Furthermore, 12 weeks of exercise intervention may not be sufficient to promote an osteogenic effect as well as the position of patients in some exercises may not be the best to develop muscle strength and to promote an activation in BMU in those patients.

5.6. Conclusion

Non-exercise seems to decrease bone formation and potentially induce poor physical function as well as decrease PA levels in HD patients. Moreover, exercise improved overall physical function and muscle strength. However, iAET seems to increase a marker of bone resorption.

Considering the expected osteogenic effect of resistance exercise, these results may suggest that 12 weeks of iPRT may not be enough to promote an osteogenic response in HD patients.

Chapter 6: Acute effects of resistance versus aerobic exercise on bone biomarkers in haemodialysis patients: a secondary analysis of a randomized clinical trial

6.1. Abstract

Introduction: HD patients frequently have low bone formation and resorption which may induce loss of bone**.** Exercise may induce positive effects on bone health in chronic kidney disease. However, the acute effects of exercise are poorly described. Thus, this study aims to compare the effects of an HD session with exercise (aerobic or resistance) versus an HD session without exercise on OPG, a regulator of bone turnover, and OC, a biomarker of bone formation.

Methods: This is a secondary analysis of a randomized clinical trial, which included fifty-two HD patients who performed a single bout of iAET or iPRT. Regulator of bone turnover (OPG) and a marker of bone formation (OC) were measured through DuoSet Enzyme-Linked Immunosorbent Assay kits in two moments (before and after the HD) in three different timepoints: HD session, HD session with acute exercise and HD session with chronic exercise (i.e., 12 weeks after iAET or iPRT exercise).

Results: The comparison of the blood biochemistry between different timepoints (HD-only, HD with acute and HD chronic exercise), showed that total calcium presented a statistical difference ($p=0.006$), with the values presented at the beginning of the study being lower than the values presented throughout the study. Regarding bone biomarkers, OPG (pg/ml) levels decrease both after the HD-only ($p<0.001$) and HD session with acute ($p<0.001$) and chronic ($p<0.001$) exercise. Also, OC (pg/ml) levels decrease after the HD-only, HD with acute and HD with chronic exercise ($p<0.001$ for all). However, the differences between the changes observed after the three timepoints did not represent significant changes. Furthermore, when the values of bone biomarkers were analysed separately before and after a HD session with aerobic or resistance exercise, the levels of OPG and OC showed the same behaviour as in the analysis of grouped exercise.

Conclusion: A single HD session, as well as a single bout of intradialytic aerobic or resistance exercise decreased a marker of bone formation (OC) and may promote osteoclastogenesis, due to OPG reduction, in HD patients. These results suggests that these exercise session, regardless modality, may not be adequate to promote an acute osteogenic effect in those patients.

Keywords: Acute exercises, bone biomarker, resistance training, aerobic training, haemodialysis.

6.2. Introduction

HD patients commonly have bone disorders characterized by low bone turnover where bone resorption exceeds bone formation [5]. Besides, those patients typically have low levels of PA which may exacerbate the deterioration of bone health [13]. Exercise has been pointing as a strategy to improve bone health-related outcomes in elderly [7,112], osteoporotic subjects [203] and recent evidence may also reveal some positive effects on bone in CKD patients [11]. However, it is well known that, to induce some osteogenic effects exercises must be dynamic, with load and intensity progression [116].

The few exercise interventions with HD patients have low intensities with most of them being performed through rate of perceived exertion [111,211], which may not achieve sufficient loads to promote an osteogenic effect. Despite of this, the little evidence revealed that there is a positive effect of resistance exercise on bone health in CKD patients [11]. However, so far, little is known about the effects of an acute bout of exercise in CKD patients. According to the literature, acute exercise may induce an increasing in bone resorption markers in healthy individuals [10]. So, the same is expected to occur in HD patients.

An acute bout of exercise may activate BMU such as osteoclasts, osteoblasts and osteocytes leading to an increase in osteoclast activity and consequently an increase in markers of bone resorption [10]. Normal bone is constantly undergoing remodelling in which bone resorption is balance by bone formation [112]. As bone resorption is the initial phase of bone remodelling [10], exercise may help to keep this balance between bone formation and resorption. Of note that, bone formation markers can also be responsiveness to exercise, however are less sensitive to acute bouts [10].

In parallel with chronic effects of exercise it may be expected that acute bout of exercise may elicit positive effects on bone health of HD patients. Thus, our study aims to compare the effects of an HD session with exercise (aerobic and resistance) in untrained (acute effects) and trained (chronic effects) individuals versus an HD session without exercise on OPG, a regulator of bone turnover, and OC, a biomarker of bone formation.

6.3. Methods

6.3.1. Study design and participants

This is a secondary analysis of a randomized clinical trial that included patients who had OPG or OC collected in two different moments, i.e., before and after de HD treatment in three difference timepoints (HD-only, HD with acute exercise and HD with chronic exercise, i.e., 12 weeks after iAET or iPRT).

The recruitment was in accordance with the exclusion and inclusion criteria previously listed in section 2: general methods, page 44 and 45. Study flow chart is depicted in Figure 6.1.

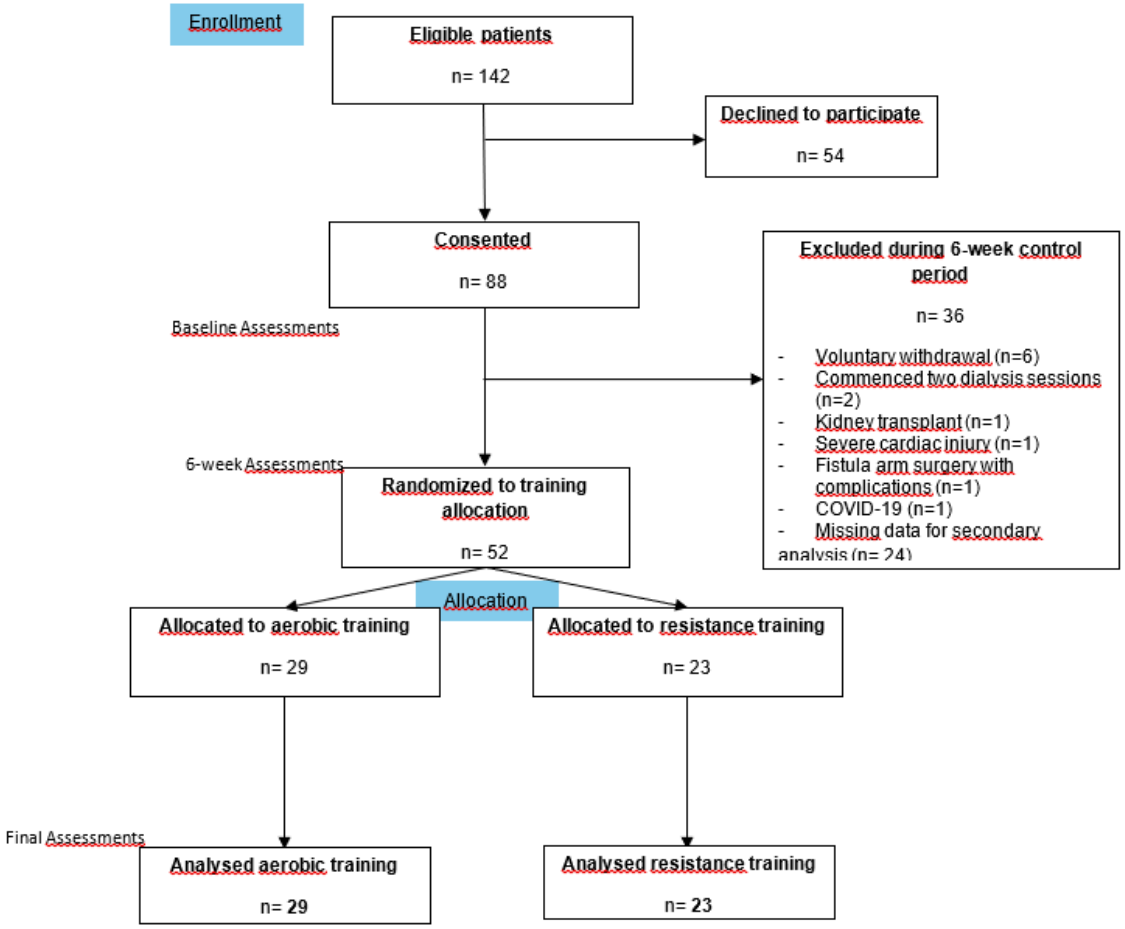

Figure 6.1. Study flow chart.

6.3.2. Exercise intervention

iPRT session consisted of 10 exercises (2 sets of 12 repetitions) for upper and lower limbs, performed at 5-eRM, using a weight machine and free-

weight dumbbells. iAET was performed on a cycle ergometer at 50-70 rpm for 30 minutes at an RPE between 12-14. Exercise training protocol is fully detailed on Section 2: General Methods, pages 46-50.

6.3.3. Outcome measures

Blood samples were collected before and after the second weekly HD session at three timepoints (baseline, post 6 weeks run-in control period and post 12 weeks of exercise).

All analytic procedures are fully described on Section 2: General Methods, pages 54-57. Albumin, iPTH, total calcium, phosphorus, phosphorus-calcium product, and magnesium were measured through the routine clinical laboratory. Plasma levels of OPG (R&D Systems, USA) and OC (R&D Systems, USA) were assessed in duplicate through ELISA DuoSet kits, as previously mentioned.

6.3.4. Statistical analysis

Normality was tested through the Shapiro Wilk test. Bone biomarkers data were transformed using logarithmic (LOG10) transformation for parametrical statistical analysis, but the original data were presented. Data are expressed as mean ± SD. To compare the blood biochemistry between three different timepoints (HD-only, HD with acute exercise and HD with chronic exercise) the analysis of variance (ANOVA) one-way Repeated Measures was used for parametrical data while the Friedman test was used for non-parametrical data.

ANOVA Two-Way Repeated Measures followed by Bonferroni post-hoc were performed to compare bone biomarkers at three timepoints: HD-only, HD with acute exercise (aerobic or resistance), and HD with chronic exercise (aerobic or resistance), across two measurement moments (pre and post HD). Additionally, ANOVA one-way repeated measure followed by Bonferroni post-hoc were performed to compare delta values of bone biomarkers also in the three timepoints.

Finally, to compare the influence of aerobic and resistance training before and after the HD with acute (untrained) and chronic (trained) exercise,

the ANOVA two-way followed Bonferroni post-hoc test was also used. Statistical significance was set at p≤0.050. All analyses were carried out using IBM SPSS (IBM SPSS Statistics, version 29.0).

6.4. Results

Patients' characteristics are presented in table 6.1 and blood biochemistry on the HD-only session compared with the HD session with exercise is presented in table 6.2. The comparisons between blood biochemistry at three moments showed a statistical difference in total calcium (p=0.006), especially between HD-only *versus* HD with acute exercise (p=0.024) and HD with chronic exercise (p=0.016).

Table 6. 1. Baseline patients' characteristics**.**

Variable	N=52
Age (years)	66.40±14.28
Dialysis Vintage (months)	27.96±32.48
Male, n (%)	35 (67,6)
BMI (kg/m^2)	26.78±4.62
Charlson Comorbidity Index Score	3.54±1.80
Age-adjusted Charlson Comorbidity Index Score	5.90±2.62
Dry Weight (Kg)	71.67±14.36
Dialysis Time (min)	244.17±5.64
Kt/V	1.78±0.32

Note: Data is presented in mean±SD. BMI, Body mass index; Kt/V, Dialysis efficiency.

Table 6.2. Blood biochemistry comparisons.

Variable	HD-only	HD with acute exercise	HD with chronic exercise	p-value
Albumin (g/dL) ¥	3.97±0.23	5.07±7.99	3.98±0.33	0.536
iPTH (ng/dL) ¥	315.29±211.02	331.89±217.30	308.17±204.20	0.127
Total Calcium (mg/dL)	8.92±0.47 †,‡	9.10±0.46 †	9.10±0.51 ‡	**0.006***
Phosphorus (mg/dL)	4.47±0.94	4.59±1.15	4.50±1.21	0.665
Phosphorus-Calcium Product (mg/dL^2)	39.83±8.45	41.80±10.65	40.89±11.59	0.317
Magnesium (mg/dL) ¥	2.44±0.42	2.43±0.43	2.44±0.39	0.832
Haematocrit (%) ¥	34.38±4.75	32.52±4.66 [a]	34.16±5.76 [a]	0.159
Haemoglobin (g/dL) ¥	10.95±1.31	10.49±1.59 [a]	11.20±1.55 [a]	0.244

Note: iPTH, Parathyroid hormone.
Sample size: [a]n=44.
¥ parametrical data.
† significant difference between HD-only and HD with acute exercise.
‡ significant difference between HD-only and HD with chronic exercise.
* significant difference between HD with acute and HD with chronic exercise.

Table 6.3. demonstrates the comparison between a single HD session with and without an exercise session (acute and chronic). A significant decrease in OPG (pg/ml) levels after a single session of HD ($p<0.001$), of acute ($p<0.001$) and chronic ($p<0.001$) exercise session. However, there was no significant difference observed in this biomarker when compared changes in the three timepoints ($p=0.073$). Moreover, the post-hoc analyses carried out indicated that there was a significant difference between the HD-only and the HD with chronic exercise ($p=0.021$), suggesting a specific variation between these two moments that was not captured by the general analysis.

Additionally, there was also a significant decrease in OC (pg/ml) levels after a single HD session ($p<0.001$), of acute ($p<0.001$) and chronic ($p<0.001$) exercise session. However, there was no significant difference observed in the comparison between the three timepoints ($p=0.456$).

Table 6.3. Comparison between differences in bone biomarkers in haemodialysis sessions with and without exercise.

Variables	HD-only	HD with acute exercise	HD with chronic exercise	Delta p-value
OPG, pg/ml [a]				
Pre-HD	6,022±2,531	6,192±2,687	6,362±2,666	
Post-HD	4,652±1,844	4,532±1,947	4,495±1,822	0.073
Change	-1,370±1,440 †	-1,660±1,408	-1,867±1,702 †	
Intra-analysis p-value	**<0.001**	**<0.001**	**<0.001**	
OC, pg/ml [b]				
Pre-HD	69,717±28,048	63,121±28,531	59,422±29,569	
Post-HD	22,421±17,137	20,869±13,911	20,621±16,765	0.456
Change	-50,133±21,030	-42,252±21,024	-38,801±26,229	
Intra-analysis p-value	**<0.001**	**<0.001**	**<0.001**	

Note: Data as mean ± standard deviation.
OPG, Osteoprotegerin; OC, Osteocalcin.
Sample size: [a]n=52, [b]n=40.
† significant difference between the delta value of HD-only versus HD with chronic exercise.

Figure 6.2. and 6.3. demonstrate OPG and OC levels, respectively, at the three timepoints (after a single HD session, HD session with acute exercise session and HD session after chronic exercise).

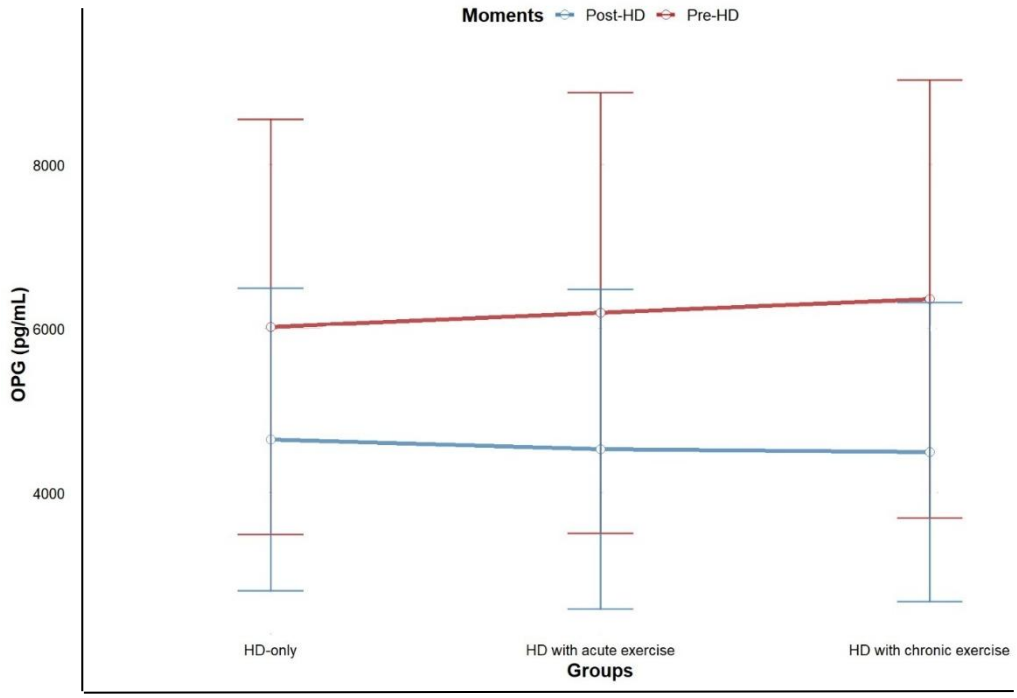

Figure 6.2. OPG levels at the three timepoints.

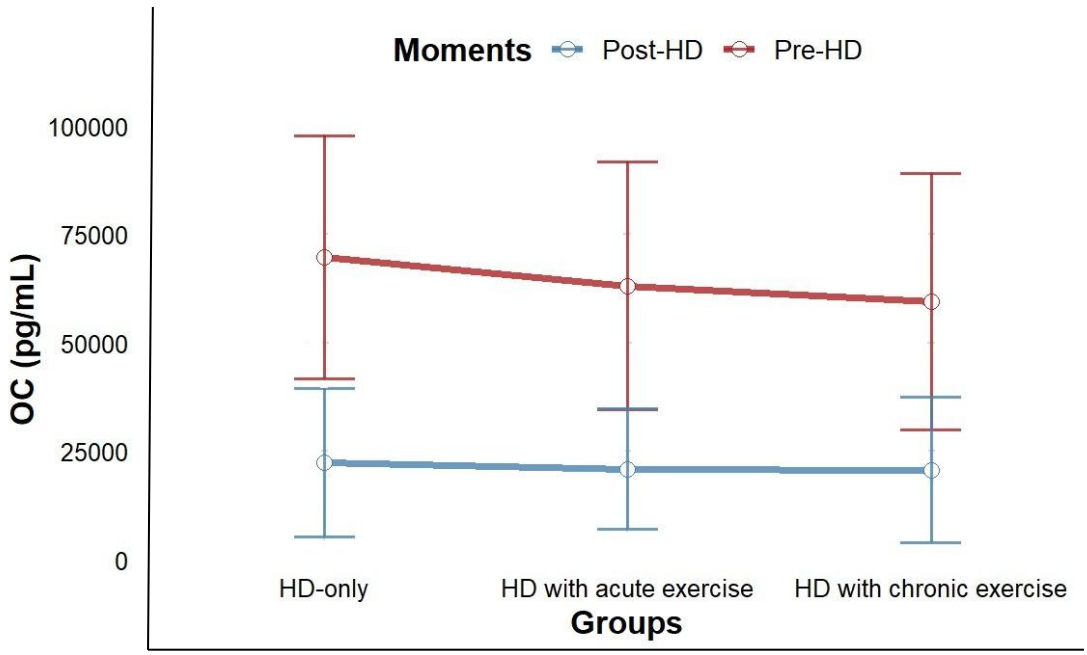

Figure 6 3. OC levels at the three timepoints.

Table 6.4. present the comparison between the effects of acute exercise (untrained patients) and chronic exercise (trained patients) in a separate analysis by modality (aerobic and resistance) before and after a single HD session on OPG and OC levels. There were no differences between the comparison of delta values for acute versus chronic exercise in any bone biomarker for both exercise modalities. However, an intra-analysis showed that even after a single session of acute and chronic exercise, whether aerobic or resistance, OPG and OC levels appear to decrease ($p<0.001$ for all) in HD patients.

Table 6. 4. Effects of acute versus chronic aerobic and resistance exercise before and after a HD session on bone biomarkers.

Variables	Aerobic exercise			Resistance exercise			p-value *	p-value #
	Acute	Chronic	p-value †	Acute	Chronic	p-value †		
OPG, pg/ml								
Pre-HD	6,312±2,937 [a]	6,639±2,944 [a]		6,039±2,391 [c]	6,012±2,283 [c]			
Post-HD	4,662±2,015 [a]	4,728±2,015 [a]	0.498	4,368±1,890 [c]	4,201±1,540 [c]	0.743	0.522	0.776
Change	-1,651±1,505	-1,911±1,801		-1,672±1,310	-1,811±1,606			
Intra-analysis p-value	**<0.001**	**<0.001**		**<0.001**	**<0.001**			
OC, pg/ml								
Pre-HD	58,173±28,263 [b]	56,401±31,501 [b]		73,398±27,300 [d]	65,694±25,068 [d]			
Post-HD	18,214±12,426 [b]	18,259±16,821 [b]	0.562	26,383±15,670 [d]	25,525±16,179 [d]	0.862	0.223	0.874
Change	-39,959±20,373	-38,142±30,824		-47,015±22,377	-40,169±13,198			
Intra-analysis p-value	**<0.001**	**<0.001**		**<0.001**	**<0.001**			

Note: OPG, Osteoprotegerin; OC, Osteocalcin.

Data as mean ± standard deviation.

Samples size: [a]n=29, [b]n=27, [c]n=23, [d]n=13.

† p-value for comparing delta values of untrained *versus* trained patients.

* p-value for comparing delta values of aerobic *versus* resistance in untrained patients.

p-value for comparing delta values of aerobic *versus* resistance in trained patients.

6.5. Discussion

The present study reveals that a single HD session decreased a marker of bone formation (OC) and may promote osteoclastogenesis due to OPG reduction in HD patients. In addition, acute exercise session, whether in trained or untrained patients and regardless exercise modality, were unable to stimulate bone formation and promote osteoclasts differentiation in those patients. According to the literature bone resorption biomarkers are expected to increase after a single session of exercise [10]. Despite we do not analysed markers of bone resorption in our study, we may have the same results, as bone resorption could be increased throughout OPG and OC reduction. Thus, we believe that a single bout of exercise, regardless modality, may not be enough to promote an acute osteogenic effect in HD patients.

HD patients commonly have high levels of OPG [212]. However, according to the evidence, OPG seems to decrease around 15% after a HD treatment [88], which may be expected as the blood is filtrated during the HD treatment. This evidence may explain the reduction in OPG levels after a single session of HD, observed in our study. Despite, OPG may be elevated in HD patients with an increased age and as well as due to an imbalance between bone formation and resorption [88]. Vik et al. (2017) [213], revelled that OPG is inversely associated to eGFR in older patients with reduced renal function. In addition, there is also a significant positive correlation between this biomarker and dialysis vintage in HD patients [214]. According to the evidence, these levels tend to be much higher in patients with low and normal bone turnover (characterized by low PTH levels) when compared to patients with high bone turnover (characterized by high PTH levels) [215]. High levels of PTH may downregulate OPG levels, promoting bone resorption [216]. Thus, we can infer that a decrease in OPG levels after a single bout of exercise (regardless modality) may indicate an increase in bone resorption in HD patients. According to a secondary analysis from data of our RCT study (RCT in chapter 5), exercise promote an increase in bone resorption biomarkers in patients with normal and low PTH levels, while any those effects were observed in patients with high levels of PTH [217]. Despite, this analysis was conducted with a long-term intervention (12 weeks) and it was considered other bone biomarkers, we

can infer that reduced OPG levels might be clinically relevant in HD patients, as may revelled the protective effect of exercise contributing to prevent bone loss through osteoclastogenesis inhibition which may indicate a compensatory mechanism of bone remodelling process [88].

OC is higher in those patients [72], mainly due to poor renal clearance and an increase of bone metabolism [218]. OC is a marker of osteoblast activity and bone formation [219]; thus, high levels may indicate an increase in bone turnover and consequently induce bone health imbalance [218,220]. OC is also released during bone resorption which may suggest that this biomarker could also indicate overall bone remodelling [56]. The latest evidence revealed that high levels of both biomarkers (OPG and OC) may decrease bone remodelling and may inhibit bone resorption [88]. Furthermore, despite non-significant changes, OC levels tend to decrease after a HD session [77], which is in line with our results. Serum OC concentrations seem to be positively associated with intact PTH in CKD patients [221], thus low values may indicate low bone turnover representing a decreased in bone formation.

It has been reported that exercise may improve bone health-related biomarkers, with a balance between bone formation and resorption, even in healthy subjects as well as in CKD patients [10,11]. Chronic exercise may elicit changes in bone formation, while a single bout of exercise is likely to affect bone resorption markers [10]. Despite no evidence about the acute effects of exercise on bone biomarkers in HD patients, it was expected that markers of bone resorption decreased after an exercise session. It seems that markers of bone resorption are more responsiveness to acute exercise than biomarkers of bone formation, as it has been reported with healthy subjects [10]. Bone resorption is the first phase of bone remodelling due to the activation of the basic multicellular units by an acute bout of exercise [10]. Therefore, it is expected that its response is not altered by chronic exercise, but rather by acute. A systematic review and meta-analysis reported that a single bout of cycling may increase bone resorption marker specifically Type I Collagen C-Telopeptide [117]. While inconsistent and small changes were observed in bone formation biomarkers [117]. The authors also pointing the importance of type of exercise as well as dose and the amount of load. Higher volume and intensity

seem to influence bone biomarker response to exercise [117]. In our study, a single bout of aerobic and resistance exercise decreases bone formation marker (OC), and a regulator of bone turnover, OPG. Despite no evidence about the effect of acute exercise on these bone biomarkers in HD patients [11], our results were not expected as the literature report that OPG levels increased significantly after 24 weeks of intradialytic resistance exercise [166] while OC was unchanged following any type of exercise [11].

Despite, our study has some limitations, this is a secondary analysis with no statistical power calculation which may impact the results obtained. Additionally, there were many patients excluded during 6 weeks of control period which reduced the sample size analysed. Moreover, we only analysed two biomarkers and there is a range of bone biomarkers in CKD setting that must be analysed.

6.6. Conclusion

A single HD treatment decreased bone formation and may promote osteoclastogenesis in HD patients. Additionally, an acute bout of aerobic or resistance exercise, even in trained and untrained patients, were unable to stimulate bone formation and promote osteoclasts differentiation in those patients.

However, more studies, with other bone biomarkers of bone resorption and formation, are needed to better understand the effects of a single bout of exercise in these populations.

Chapter 7: General discussion

The key findings of studies are: (i) RANKL levels are inversely associated with OPG, while there is also an inverse association between SOSt and OC levels; (ii) increase aerobic capacity and muscle strength is associated with a reduction in bone resorption; (iii) increases in light PA may induce bone formation; (iv) better physical health is associated with osteoclastogenesis; (v) non-exercise seems to promote a decrease in bone formation and physical activity and function; (vi) twelve weeks of intradialytic aerobic or resistance exercise training improve physical function and muscle strength; (vii) 12 weeks of intradialytic aerobic exercise increased a marker of bone resorption (TRAP-5b) and maintained stable OC levels, and improved lower muscle strength; while intradialytic resistance exercise increased aerobic capacity when compared to aerobic training; (viii) a single HD session decrease marker of bone formation (OC) and promote osteoclastogenesis through the decrease of OPG; (ix) a single bout of intradialytic aerobic or resistance exercise may have the same impact on bone.

Based on the literature, HD patients have low levels of PA that reducing with HD-vintage [13,101]. Low levels of PA have been associated with low physical function [222]. Furthermore, low physical function may decrease quality of life and bone health in CKD patients [196,223]. Thus, the results in or study are in line with the current literature. As we demonstrate that non-exercise induces poor physical function, PA and it was associated with a decrease bone formation and an increase in bone resorption in HD patients. Looking for the interaction between bone biomarkers and physical function in CKD patients, TRAP-5b seems to be inversely associated with aerobic capacity, as measured by 6-min walk test [161]. Therefore, our findings corroborate with the literature, since we demonstrated an inverse relationship between aerobic capacity, measured by ISWT, and TRAP-5b levels. This result may indicate that poor aerobic capacity may induce changes in bone resorption in HD patients. Additionally, our results also shown that an increase in a marker of bone formation (OC) may decrease a marker of bone formation inhibition (SOSt). It is well known that HD patients have high levels of OC maybe due to high bone metabolism and a decreased in renal clearance [218,224]. A study conducted with frail obese adults revelled levels of OC in those patients around 4.7 ng/ml

[225]; while HD patients had 10.1ng/ml of this biomarker [166]. Even so, high levels may reflect an increase in bone turnover [218,220]. A study with HD treatment indicates a 19% decreased in OC concentration after 6h of treatment, despite non-significant [77]. Recent evidence demonstrates that TRAP-5b and OC are positively associated in HD patients [81], however, we do not observe this result on our study. Additionally, high levels of TRAP-5b and OC revealed high rate of cortical bone loss [81]. As TRAP-5b is a bone resorption biomarker secreted by osteoclasts [226], it may indicate that this biomarker specifies osteoclast activity. Low TRAP-5b concentrations, when not accompanied by other biomarkers of bone formation, is associated with low bone turnover characterized through a decreased bone resorption and formation [227,228]. However, these levels are commonly higher in CKD patients when compared to healthy subjects [81], and according to the literature, a single HD session did not affect TRAP-5b concentrations [81]. RANKL has been reported with inconsistent results in CKD setting [14]. Similarly to TRAP-5b, RANKL also correlates with OC [76]. We also observed that high levels of OPG is associated with changes in osteoclastogenesis. OPG binds with RANKL to block osteoclastogenesis [229], high levels of OPG may reduce RANKL levels inhibiting osteoclastogenesis and consequently protect bone from excessive loss [230]. Thus, RANKL/OPG ratio may indicate bone resorption [5]. OPG levels are also typically elevated in HD patients increasing with HD-vintage [212]. Considering bone health in HD patients, it is surprising our results. HD patients tend to have high levels of OC, TRAP-5b, RANKL and OPG biomarkers, thus the decreased observed after 6-week of control period in OC as well as after a single HD session in OPG and OC, may indicate a compensatory mechanism to restore balance between bone formation and resorption in those patients. Looking for the effects of exercise, a single bout of intradialytic aerobic or resistance exercise also seems to reduce OC and OPG levels. Bone resorption biomarkers seem to be more responsiveness to acute exercise bouts [10], despite we did not analyse resorption biomarkers in our study, we observed a reduced bone formation. The only two studies investigating the exercise effects on OC did not reveal any significant change after intervention as well as in control group [161,166]. However, OC alone may

not provide sufficient evidence about histologic changes of skeletal diseases [72].

One the other hand, 12 weeks of intradialytic aerobic exercise seems to increase TRAP-5b levels. We also considered that such result occurs because aerobic exercise does not promote an osteogenic effect to induce changes in bone in those patients. Latest studies did not find any significant difference in TRAP-5b and OC after 24 weeks of aerobic and resistance exercise training [161,166]. However, Marinho et al. [166] report a significant increase in OPG levels after 24 weeks of intradialytic resistance exercise, suggesting an inhibition in osteoclastogenesis. This may raise the question that 12 weeks of exercise intervention may not be sufficient to promote osteogenic effects on bone in HD patients. Thus, further investigation will be needed to better understand the exercise time-effect on those patients.

Some limitations in our studies were recognized. Firstly, it was only measured bone biomarkers, and these data were not compared with histologic and imaging resources. In addition, more bone biomarkers should be measured to better understand exercise effects in a wide range of bone biomarkers. Moreover, we may recognize that 12 weeks of intradialytic aerobic and resistance exercise were not sufficient to promote osteogenic effects in these patients. Furthermore, pre-analytical sample handling might have introduced some bias due to some factors that can affect results such as sample type, sampling time, sample handling, patient's preparation, and the nutritional status of the patient. Knowing the circadian rhythm of bone biomarkers (64), all samples should be collected during morning, especially when assessing bone resorption biomarkers, however this was not considered due to HD shifts. In addition, the loss of some blood sample collection reduced the number of patients analysed, which may have impacted the results. Lastly, 6 weeks of control period it may be a long-time without any type of exercise which might potentiate some dropouts.

Future researchers should deeply investigate the effects of acute and chronic exercise in a wide range of bone biomarkers of HD patients. Intradialytic exercise programmes longer than 12 weeks might be considered. In addition,

the effects of intradialytic and extradialytic exercise should be compared on bone biomarkers of those patients. Furthermore, a cohort study should be done to investigate the long-term effects of HD treatment in biomarkers of bone formation, resorption, and regulators of bone turnover.

In conclusion strategies to improve an overall physical function, PA and consequently bone turnover should be considered in HD setting. Farther, structure exercise programmes should also be included in daily life of those patients.

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Attachments

Attachment 1: Written informed consent

CONSENTIMENTO INFORMADO, LIVRE E ESCLARECIDO PARA PARTICIPAÇÃO EM INVESTIGAÇÃO
de acordo com a Declaração de Helsínquia[1] e a Convenção de Oviedo[2]

Se concordar, pedimos que:
- Preencha o consentimento informado.
- Permita a colheita de sangue e saliva para avaliação das variáveis propostas. A colheita não comporta qualquer risco para si e será efetuada por um profissional de saúde habilitado para esta competência.
- Efetue os testes de aptidão física propostos pelos investigadores.

Saiba que:
- É livre de solicitar qualquer informação/esclarecimento ao investigador, em qualquer momento antes, durante e/ou após a sua participação.
- Toda a informação recolhida será confidencial de acordo com a legislação aplicável. As amostras de sangue e saliva, serão codificadas e tornadas anónimas no ato de colheita, passando a sua identificação pessoal a estar codificada e não acessível aos utilizadores das amostras/dados. Apenas o Investigador Principal abaixo-assinado, sujeito a sigilo profissional, terá acesso à chave de codificação, armazenada em local diferente do material recolhido. Este estudo foi autorizado pela Comissão Nacional de Proteção de Dados, que é a autoridade nacional nesta matéria.
- Este estudo foi revisto pela Comissão de Ética para a Saúde da Nephrocare Portugal, S.A., que reviu os objetivos e a conduta proposta para este estudo e deu a sua opinião favorável. Para sua segurança, a Comissão de Ética para a Saúde da Nephrocare Portugal, S.A. poderá monitorizar a implementação do estudo e, neste âmbito, poderá aceder à sua informação de saúde que está armazenada nos respetivos ficheiros. Ao assinar esta declaração de consentimento está a autorizar o acesso a esta informação confidencial.
- Os resultados serão também consultados pelos responsáveis científicos do estudo e ser objeto de publicação científica, mas de uma forma anónima e agregada, isto é, em termos de percentagens ou de dados numéricos, nunca individualmente e não poderão ser relacionados consigo, ou seja, com a sua identificação.
- O consentimento informado é um direito dos doentes e um dever dos profissionais de saúde, assegurando o respeito à integridade moral e física do participante.

Muito obrigado pela sua colaboração

O Investigador Principal

Nome (1º e último): _____

Assinatura: _____

-o-o-o-o-o-o-o-o-o-o-o-o-o-o-o-o-o-o-o-o-o-o-o

Declaro ter lido e compreendido este documento, bem como as informações verbais que me foram fornecidas pela pessoa que acima assina. Compreendo que a minha participação é voluntária e que sou

[1] http://www.uc.pt/fcdef/Comissao_de_etica/Documentos/Nova_Helsinquia

[2] http://www.gddc.pt/siii/docs/oviedo.pdf

livre de desistir a qualquer momento, sem apresentar qualquer justificação, e que tal não afetará os meus cuidados médicos. Compreendo que o meu processo clínico será revisto pelo médico do estudo, e que a Comissão de Ética para a Saúde da Nephrocare Portugal, S.A. também poderá aceder a esta informação. Autorizo o investigador do estudo a aceder à minha informação de saúde. Desta forma, aceito participar neste estudo e permito a utilização dos dados que de forma voluntária forneço, confiando em que apenas serão utilizados para esta investigação e nas garantias de confidencialidade e anonimato que me são dadas pelo investigador principal.

Nome (1º e último): ________________________________

Assinatura: _______________________________________ Data: …… /…… /………..

SE NÃO FOR O PRÓPRIO A ASSINAR POR INCAPACIDADE
NOME: …
BI/CD Nº: .... DATA OU VALIDADE: ….. /.…. /….....
GRAU DE PARENTESCO OU TIPO DE REPRESENTAÇÃO: ....
ASSINATURA: …

ESTE DOCUMENTO É COMPOSTO DE 2 PÁGINA/S E FEITO EM DUPLICADO:
UMA VIA PARA O/A INVESTIGADOR/A, OUTRA PARA A PESSOA QUE CONSENTE

Attachment 2: PRISMA 2009 checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Page 1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	Page 2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	Page 3 and 4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	Page 4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	-
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	Page 5 and 6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Page 6 and 7
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Additional file 2
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Page 6 and 7
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	Page 7

Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Page 7
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Page 7 and 8
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	-
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	-
Section/topic	**#**	**Checklist item**	**Reported on page #**
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	-
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	-
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Page 8 and figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Page 8-11
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Page 11
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	-
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Page 12 and 13
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	-
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	-
DISCUSSION			

Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	Page 13 and 14
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	Page 14-17
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	Page 17-19
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	Page 21

Attachment 3: Search strategy: EBSCO

01: Exercise AND bone AND CKD

02: Exercise AND bone AND “renal function”

03: Exercise AND bone AND hemodialysis

04: Exercise AND bone AND dialysis

05: Exercise AND bone AND “glomerular filtration rate”

06: Exercise AND bone AND renal

07: “physical activity” AND bone AND CKD

08: “physical activity” AND bone AND “renal function”

09: “physical activity” AND bone AND hemodialysis

10: “physical activity” AND bone AND dialysis

11: “physical activity” AND bone AND “glomerular filtration rate”

12: “physical activity” AND bone AND renal

13: “physical performance AND bone AND CKD

14: “physical performance” AND bone AND “renal function”

15: “physical performance” AND bone AND hemodialysis

16: “physical performance” AND bone AND dialysis

17: “physical performance” AND bone AND “glomerular filtration rate”

18: “physical performance” AND bone AND renal

19: “physical function” AND bone AND CKD

20: “physical function” AND bone AND “renal function”

21: “physical function” AND bone AND hemodialysis

22: “physical function” AND bone AND dialysis

23: “physical function” AND bone AND “glomerular filtration rate”

24: “physical function” AND bone AND renal

25: “fitness” AND bone AND CKD

26: “fitness” AND bone AND “renal function”

27: “fitness” AND bone AND hemodialysis

28: “fitness” AND bone AND dialysis

29: “fitness” AND bone AND “glomerular filtration rate”

30: “fitness” AND bone AND renal

31: “functional ability” AND bone AND CKD

32: “functional ability” AND bone AND “renal function”

33: “functional ability” AND bone AND hemodialysis

34: “functional ability” AND bone AND dialysis

35: “functional ability” AND bone AND “glomerular filtration rate”

36: “functional ability” AND bone AND renal

Attachment 4: Tool for assessing risk of bias- Newcastle-Ottawa Scale modified

1- Selection Bias – Is the source population (cases, controls, cohorts) appropriate and representative of the population of interested? (Methods for selection study participants).

1 (low risk of bias) ☐

The investigators describe a:

- random selection from a population that is representative of the condition under study ☐
- A consecutive sample from a population that is representative of the condition under study ☐
- Eligible, exposed individuals from a defined population ☐

☐ Quote: " "

2 (moderate risk of bias) ☐

· A consecutive sample from a population that is not highly representative of the outcome of interest ☐

· A random selection from a population that is not highly representative of the outcome of interest☐

☐ Quote: " "

3 (high risk of bias) ☐

Non-random approach, for example:

· The source population cannot be defined or enumerated ☐

· Volunteering or self-recruitment ☐

· No description of the derivation of the cohort ☐

☐ Quote: " "

? (unclear risk of bias) ☐

· Insufficient information to permit judgment of '1', '2' or '3' ☐

2- Performance Bias (Methods to control for confounding)

2.1. Is the sample size sufficient and is there sufficient power to detect a meaningful difference in the outcome of interest?

1 (low risk of bias) ☐

· Sample size was adequate and there was sufficient power to detect a difference in the outcome ☐

☐ Quote: " "

2 (moderate risk of bias) ☐

· Sample size may not be adequate and study may have been slightly underpowered ☐

☐ Quote: " "

3 (high risk of bias) ☐

· Sample size was small and there was not enough power to test the outcome of interest ☐

☐ Quote: " "

? (unclear risk of bias) ☐

· Insufficient information to permit judgment of '1', '2' or '3' ☐

2.2. Did the study adjust for any variables or confounders that may influence the outcome?

1 (low risk of bias) ☐

· The study identified and adjusted for all possible confounders that may influence the estimates of association between exposure and outcome ☐

☐ Quote: " "

2 (moderate risk of bias) ☐

· The study identified and reported possible variables that may influence the outcome but did not statistically explore their influence☐

☐ Quote: “ ”

3 (high risk of bias) ☐

· The study either did not report any variables of influence or acknowledge any variables of influence when it was clear they were present.☐

☐ Quote: “ ”

? (unclear risk of bias) ☐

· Insufficient information to permit judgment of ‘1’, ‘2’ or ‘3’ ☐

3- Detection bias (Statistical methods)

3.1. Did the study use appropriate statistical analysis methods relative to the outcome of interest?

1 (low risk of bias) ☐

Any one of the following:

· The study reported use of appropriate statistical analysis as required ☐

☐ Quote: “ ”

2 (moderate risk of bias) ☐

· The study used either correct statistical methods but did not report them well, or used the incorrect methods but reported them in detail ☐

☐ Quote: “ ”

3 (high risk of bias) ☐

· The study did not use appropriate statistical analysis as required ☐

☐ Quote: “ ”

? (unclear risk of bias) ☐

· Insufficient information to permit judgment of ‘1’, ‘2’ or ‘3’ ☐

3.2. Is there little missing data and did the study handle it accordingly?

1 (low risk of bias) ☐

· The study acknowledged missing data to be less than 10% and specified the method of handling it ☐

· No missing data ☐

☐ Quote: “ ”

2 (moderate risk of bias) ☐

· The study either had greater than 15% of missing data but they specified the method used to handle it ☐

· Missing data is not excessive, and they specified the method used to handle it ☐

☐ Quote: “ ”

3 (high risk of bias) ☐

· The study had greater than 15% of missing data and did not handle it at all ☐

☐ Quote: “ ”

? (unclear risk of bias) ☐

· Insufficient information to permit judgment of ‘1’, ‘2’ or ‘3’ ☐

4- Information bias (Methods of measuring outcome variables)

4.1. Is the methodology of the outcome measurement explicitly stated and is it appropriate?

1 (low risk of bias) ☐

· The study provides a detailed description of the outcome measure(s) which are appropriate for the outcome of interest ☐

☐ Quote: " "

2 (moderate risk of bias) ☐

· The study provides a somewhat complete description of outcome measurements that are justified ☐

☐ Quote: " "

3 (high risk of bias) ☐

· The study provides limited information on the methods of measuring the outcome and the measure is not appropriate considering the outcome ☐

☐ Quote: " "

? (unclear risk of bias) ☐

· Insufficient information to permit judgment of '1', '2' or '3' ☐

4.2. Is there an objective assessment of the outcome of interest?

1 (low risk of bias) ☐

· The study used objective methods to discern the outcome status of participants (i.e. laboratory measurements, medical records) ☐

☐ Quote: " "

2 (moderate risk of bias) ☐

· The study relied on subjective data as the primary method to discern the outcome status of participants (i.e. self-report) ☐

☐ Quote: “ ”

3 (high risk of bias) ☐

· The study had limited reporting about assessment of outcomes ☐

· No description ☐

☐ Quote: “ ”

? (unclear risk of bias) ☐

· Insufficient information to permit judgment of ‘1’, ‘2’ or ‘3’ ☐

5- Attrition bias (Subject follow-up)

5.1. **Was the follow-up sufficiently long enough for the outcome to occur?**

1 (low risk of bias) ☐

· Follow-up was sufficiently long enough for the outcome to occur ☐

☐ Quote: “ ”

3 (high risk of bias) ☐

· Follow-up was not sufficiently long enough for the outcome to occur ☐

☐ Quote: “ ”

? (unclear risk of bias) ☐

· Insufficient information to permit judgment of ‘1’ or ‘3’ ☐

5.2. Was there minimal loss to follow-up and are subjects lost to follow-up unlikely to

introduce bias?

1 (low risk of bias) ☐

· Follow-up was completed for all, or nearly all subjects, and reasons for losses to follow-up were well documented.☐

☐ Quote: " "

2 (moderate risk of bias) ☐

· Losses to follow-up are not excessive, and reasons for losses to follow-up are well documented and mostly unrelated to the outcome ☐

☐ Quote: " "

3 (high risk of bias) ☐

· Significant loss to follow-up, reasons for losses to follow-up not reported, suspect that reasons for dropouts are related to the outcome ☐

☐ Quote: " "

? (unclear risk of bias) ☐

· Insufficient information to permit judgment of '1', '2' or '3' ☐

Attachment 6: Checklist of items that should be included in reports of cross-sectional studies

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	79
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	80/81
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	82
Objectives	3	State specific objectives, including any prespecified hypotheses	82
Methods			
Study design	4	Present key elements of study design early in the paper	83

Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	45/46
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	45
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	83
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	83
Bias	9	Describe any efforts to address potential sources of bias	-
Study size	10	Explain how the study size was arrived at	101
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	-
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	83/84
		(*b*) Describe any methods used to examine subgroups and interactions	83/84
		(*c*) Explain how missing data were addressed	-
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	-
		(*e*) Describe any sensitivity analyses	-
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	84
		(b) Give reasons for non-participation at each stage	-
		(c) Consider use of a flow diagram	84
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	85
		(b) Indicate number of participants with missing data for each variable of interest	84
Outcome data	15*	Report numbers of outcome events or summary measures	85-92
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for	-

		and why they were included	
		(*b*) Report category boundaries when continuous variables were categorized	-
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	-
Discussion			
Key results	18	Summarise key results with reference to study objectives	93-95
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	95
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	95
Generalisability	21	Discuss the generalisability (external validity) of the study results	95
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	ii

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Attachment 7: CONSORT 2010 checklist of information to include when reporting a randomised trial.

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	96
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	97/98
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	99
	2b	Specific objectives or hypotheses	100
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	100
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	45/46
Participants	4a	Eligibility criteria for participants	45/46
	4b	Settings and locations where the data were collected	45/46
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	47-51
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	51-58
	6b	Any changes to trial outcomes after the trial commenced, with reasons	-

Sample size	7a	How sample size was determined	101
	7b	When applicable, explanation of any interim analyses and stopping guidelines	-
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	46
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	46
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	46
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	46
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	-
	11b	If relevant, description of the similarity of interventions	-
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	101
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	101
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	102
	13b	For each group, losses and exclusions after randomisation, together with reasons	102
Recruitment	14a	Dates defining the periods of recruitment and follow-up	46
	14b	Why the trial ended or was stopped	-

Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	103
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	103-112
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	103-112
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	-
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	103-112
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	-
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	113-115
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	113-115
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	113-115
Other information			
Registration	23	Registration number and name of trial registry	
Protocol	24	Where the full trial protocol can be accessed, if available	
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	Ii

Citation: Schulz KF, Altman DG, Moher D, for the CONSORT Group. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. BMC Medicine. 2010;8:18.