UNIVERSIDADE FEDERAL DO CEARÁ
FACULDADE DE MEDICINA
DEPARTAMENTO DE MORFOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS MORFOFUNCIONAIS

RAQUEL FELIPE DE VASCONCELOS

AVALIAÇÃO DO EFEITO ANABÓLICO ÓSSEO DO KEFIR ASSOCIADO AO EXERCÍCIO RESISTIDO NO FÊMUR DE RATOS COM OSTEOPOROSE INDUZIDA POR GLICOCORTICOIDE

FORTALEZA
2021

RAQUEL FELIPE DE VASCONCELOS

AVALIAÇÃO DO EFEITO ANABÓLICO ÓSSEO DO KEFIR ASSOCIADO AO EXERCÍCIO RESISTIDO NO FÊMUR DE RATOS COM OSTEOPOROSE INDUZIDA POR GLICOCORTICOIDE

Tese de Doutorado apresentada como requisito parcial para obtenção do Título de Doutora em Ciências Morfofuncionais pelo Programa de Pós-Graduação em Ciências Morfofuncionais da Faculdade de Medicina da Universidade Federal do Ceará. Área de concentração: Morfofisiologia Óssea e Articular

Orientadora: Profa. Dra. Paula Goes Pinheiro Dutra
Coorientadora: Profa. Dra. Delane Viana Gondim

FORTALEZA

2021

Dados Internacionais de Catalogação na Publicação
Universidade Federal do Ceará
Biblioteca Universitária
Gerada automaticamente pelo módulo Catalog, mediante os dados fornecidos pelo(a) autor(a)

V451a Vasconcelos, Raquel Felipe de.
Avaliação do efeito anabólico ósseo do Kefir associado ao exercício resistido no fêmur de ratos com osteoporose induzida por glicocorticoide / Raquel Felipe de Vasconcelos. – 2021.
120 f. : il. color.

Tese (doutorado) – Universidade Federal do Ceará, Faculdade de Medicina, Programa de Pós-Graduação em Ciências Morfofuncionais, Fortaleza, 2021.
Orientação: Profa. Dra. Paula Goes Pinheiro Dutra.
Coorientação: Profa. Dra. Delane Viana Gondim .

1. Kefir. 2. exercício. 3. osteoporose. 4. glicocorticoides. 5. tecido ósseo. I. Título.

CDD 611

RAQUEL FELIPE DE VASCONCELOS

AVALIAÇÃO DO EFEITO ANABÓLICO ÓSSEO DO KEFIR ASSOCIADO AO EXERCÍCIO RESISTIDO NO FÊMUR DE RATOS COM OSTEOPOROSE INDUZIDA POR GLICOCORTICOIDE

Tese de Doutorado apresentada como requisito parcial para obtenção do Título de Doutora em Ciências Morfofuncionais pelo Programa de Pós-Graduação em Ciências Morfofuncionais da Faculdade de Medicina da Universidade Federal do Ceará. Área de concentração: Morfofisiologia Óssea e Articular

Aprovada em: 13/ 08/ 2021

BANCA EXAMINADORA

Profª. Dra. Paula Goes Pinheiro Dutra (Orientadora)
Universidade Federal do Ceará (UFC)

Profª. Dra. Renata Ferreira de Carvalho Leitão
Universidade Federal do Ceará (UFC)

Profª. Dra Hellíada Chaves Vasconcelos
Universidade Federal do Ceará (UFC)

Profª. Dra. Iracema Matos de Melo
Universidade Federal do Ceará (UFC)

Prof. Dr. Fabio Wildson Gurgel Costa
Universidade Federal do Ceará (UFC)

Aos meus pais, Eloneid e Dimas.

Ao meu marido, Daniel.

Aos filhos do coração, Caio e Luana.

Aos meus amados filhos, Isadora e Davi.

AGRADECIMENTOS

A essa força maior denominada **Deus** que me fez capaz e saudável para cumprir essa missão e chegar ao fim dessa jornada, vencendo todos os percalços e concretizando este sonho.

Aos meus pais**, Eloneid Nobre** e **Dimas Vasconcelos**, por terem sido os meus maiores exemplos de retidão e perseverança e por serem o meu porto seguro para onde eu sei que sempre posso voltar.

Aos meus irmãos, **Talita, Eduardo, Pedro Henrique e Gabriel**, por serem a certeza da presença amiga e do apoio mútuo que sempre posso contar.

A minha boadrasta, **Socorro Braun** e aos seus filhos, e também meus irmãos, **Jade, Átila** e **Kelly** por serem presença amorosa e exemplo de que família vai muito além de laços sanguíneos.

Aos meus sogros, **Luiz** e **Valdenira**, pelo auxílio constante e torcida frequente pela felicidade da nossa família.

Aos meus sobrinhos amados, **Thais, Alice, Matheus, Yasmin** e **Enzo**, por serem a alegria infantil que colore minha vida.

Às minhas cunhadas queridas, **Jana** e **Lorena**, e ao meu cunhado **Felipe,** pela presença amiga em todos os momentos.

Aos amigos do IFCE, de Canindé e da CAEF, em especial ao **Nilson, Thaidys, Samara, Rita, Ana Cláudia, Patrícia, Basílio e Andreyson** pelo apoio, parceria e torcida no fazer docente.

À minha orientadora, **Paula Goes,** por ser exemplo de professora e pesquisadora, por ser cobrança e compreensão na medida certa, por toda dedicação, paciência e ensinamentos ao me orientar durante todos esses anos de doutorado.

À minha coorientadora **Delane Gondim**, pelas críticas construtivas que me fizeram amadurecer como pesquisadora e por todo o seu apoio e colaboração na coorientação desde trabalho.

À professora **Karuza Alves,** pela análise histológica e por todo auxílio e contribuições para realização desta pesquisa.

Aos **professores** do Programa de Pós-Graduação em Ciências Morfofuncionais, por todos os ensinamentos transmitidos nesses anos.

Aos colegas de doutorado **Fátima Regina**, **Bárbara, Diego, Helson, Gisele, Lorena** e **Stephanie** pela ajuda na realização dos experimentos, e, em

especial, à amiga **Vanessa Costa** que foi presença, partilha, auxílio e ensinamentos nas mais diversas etapas dessa jornada.

Aos alunos de iniciação científica e voluntários da graduação que foram fundamentais para realização dos experimentos, em especial à **Thays Allane, Hugo** e **Romero Dias**.

À **Universidade Federal do Ceará,** por intermédio do seu Magnífico Reitor Professor Dr. Cândido Albuquerque; à **Faculdade de Medicina** (FAMED), por meio do seu diretor Prof. Dr. João Macedo; ao **Programa de Pós-Graduação em Ciências Morfofuncionais** na pessoa da sua coordenadora Profa. Dra. Antoniella Gomes Duarte, pela oportunidade de fazer parte desse programa e por todos os ensinamentos adquiridos.

Ao **Núcleo de Estudos em Microscopia e Processamento de Imagens – NEMPI da Universidade Federal do Ceará,** por meio da professora Doutora Gerly Anne de Castro Brito, pelo apoio técnico para a concretização deste trabalho.

À **Central Analítica-UFC**/CT-INFRA/MCTI-SISNANO/Pró-Equipamentos CAPES pelas excelentes imagens obtidas e pela aquisição das imagens no confocal e microscopia eletrônica de varredura.

Ao **Laboratório de Microscopia e Imagem Biológica – LABIM** da **Universidade Federal da Paraíba**, por meio do Professor Doutor Frederico Barbosa de Sousa e sua aluna Eugênia Dantas, pelas excelentes imagens e análises obtidas por meio da microtomografia computadorizada.

Ao professor Dr. **Alejandro Pedro Ayala** e seu aluno **Bruno Araújo**, por terem sido fundamentais na realização das coletas e análises da microscopia Raman.

Aos professores Dr. **Lúcio Kurita** e Dr. **Fabio Wildson** pela aquisição e análises das imagens radiográficas.

Ao **Núcleo de Estudos e Pesquisas em Dor, Inflamação e Osteoimunologia** – NEPDIO e todos os seus membros pelo compartilhamento engrandecedor do saber científico.

Aos queridos servidores técnicos do laboratório NEMPI, **Júnior e Flávia**, pelos serviços prestados com destreza e competência.

À técnica administrativa do Programa de Pós-Graduação em Ciências Morfofuncionais, **Laísa Albuquerque**, pela competência e disponibilidade em auxiliar.

Ao **Instituto Federal de Educação Ciências e Tecnologia do Ceará – IFCE,** por intermédio do seu Magnífico Reitor Professor Dr. José Wally Mendonça

Menezes; ao **Departamento de Educação Física e Esporte (DEFE)**, por meio do seu chefe Clautistony Pereira do Carmo; à **Coordenadoria da Área de Educação Física (CAEF),** por meio do meu coordenador Andreyson Calixto de Brito, pelo apoio a concretização deste trabalho e afastamento integral das minhas atividades como professora durante 1 ano e 5 meses para realização desta pesquisa.

Ao meu marido, **Daniel Gois**, por acreditar desde o princípio que eu seria capaz de chegar até aqui. Obrigada por ser o abraço forte na vitória, o ombro amigo na tristeza e, acima de tudo, por ser verdadeiramente meu parceiro e companheiro nessa e em todas as aventuras que eu ouso viver. Ter você em minha vida, sem dúvida, foi uma das minhas melhores escolhas. Te amo!

Aos filhos do Daniel e meus filhos do coração, meus enteados **Caio e Luana**, por serem a certeza de que o amor é a resposta, independente de qual seja a pergunta.

Aos meus filhos, **Isadora e Davi**, por serem a minha maior força e minha maior fraqueza, meus maiores sorrisos e minhas mais sofridas lágrimas, as minhas maiores certezas e as minhas profundas dúvidas. Obrigada por transformarem loucamente a minha vida de uma forma tão maravilhosamente única que eu não sei mais o que seria de mim sem vocês. Obrigada pela oportunidade de exercer com vocês a função mais importante da minha vida: ser sua mãe. Por vocês e para vocês que estou aqui!

A todos aqueles que, de alguma forma, contribuíram para minha caminhada no doutorado e conclusão deste trabalho, o meu muito obrigada!

Por tanto amor
Por tanta emoção
A vida me fez assim
Doce ou atroz
Manso ou feroz
Eu, caçador de mim
(…)
Nada a temer senão o correr da luta
Nada a fazer senão esquecer o medo
Abrir o peito a força, numa procura
Fugir às armadilhas da mata escura

Longe se vai
Sonhando demais
Mas onde se chega assim
Vou descobrir
O que me faz sentir
Eu, caçador de mim”

(Canção: Caçador de Mim/
Autores: Sérgio Magrão e Luiz Carlos Sá)

RESUMO

A osteoporose é uma doença esquelética multifatorial que altera microarquitetura e reduz massa óssea, causando perda de força mecânica e aumento do risco de fratura. Dentre as formas de osteoporose, a induzida por glicocorticoides (OIG) é o tipo mais frequente de osteoporose secundária. Sabendo que a prevenção e tratamento da OIG ainda permanecerem como um desafio, produtos naturais e exercícios físicos têm sido foco de estudo. Neste contexto, Kefir, um probiótico oral, destaca-se por apresentar benefícios ao osso e minimizar os efeitos colaterais farmacológicos das medicações. Assim, este estudo objetivou avaliar o efeito do exercício físico na osteoporose e o efeito do Kefir associado ao exercício resistido no tecido ósseo na OIG. Inicialmente, foram realizadas 2 revisões sistemáticas, com estudos pré-clínicos e clínicos, para analisar os efeitos do exercício no tecido ósseo durante a osteoporose. Foi observado que exercício de força aumentou densidade mineral óssea (DMO), volume ósseo e trabecular (BV/TV) e diminuiu separação trabecular (Tb.Sp), indicando efeitos benéficos do exercício para tecido ósseo em modelos animais. 60% dos estudos clínicos confirmaram associação positiva entre diminuição dos marcadores de reabsorção óssea e exercício, sugerindo redução da reabsorção em pacientes com osteoporose. Finalmente, para analisar o efeito da associação de exercício+Kefir no tecido ósseo submetido a OIG, 60 ratos Wistar machos foram divididos em 2 grupos: normal (N), e submetidos a OIG, que foi subdividido em 4 grupos de acordo com o tratamento: controle (C), Kefir (K), Exercício (Ex) e Exercício+Kefir (ExK). A OIG foi induzida por dexametasona (7mg/kg; i.m.). Kefir foi administrado por gavagem (0,7 ml/animal/dia) por 16 semanas. O exercício foi realizado 3 dias/semana, com intensidade de 20% a 80% da carga individual máxima, durante 16 semanas. Após eutanásia, o fêmur foi coletado para análises macro e microscópicas, exames de imagens e testes biomecânicos. À luz dos nossos conhecimentos, esta é a primeira vez que uma avaliação *in vivo* de exercício+Kefir em tecido ósseo de ratos com OIG foi realizada. OIG reduziu em 35% BV/TV, 33% espessura trabecular (Tb.Th), 26% conteúdo mineral e colágeno (56%). Houve ainda redução da resistência à flexão (81%), carga de fratura (80%) e número de osteócitos (84%) após OIG. Os glicocorticoides alteraram a remodelação óssea ($p < 0,05$), marcada pela redução no número de osteoblastos enquanto aumentaram número de osteoclastos, e rugosidade do tecido ósseo. Após a intervenção de 16 semanas, associação de ExK melhorou

significativamente microarquitetura e qualidade óssea, marcada pelo aumento na dimensão fractal (38%), volume cortical (34%), BV/TV (34%), Tb.Th (33%), conteúdo mineral e maturidade do colágeno, enquanto reduziu Tb.Sp (34%) e rugosidade óssea. Assim, ExK melhorou resistência óssea e biomecânica, além de estimular formação óssea e modular remodelação óssea. Conclui-se que o exercício apresentou efeitos antirreabsortivos no tecido ósseo e que Kefir potencializou os efeitos benéficos do exercício sobre o tecido ósseo, melhorando a microarquitetura, a qualidade do fêmur e as propriedades biomecânicas e estimulou a formação óssea. Assim, o tratamento com Kefir e exercícios físicos apresentam-se como possíveis ferramentas adjuvantes para melhorar o quadro de perda óssea provocados pela OIG.

Palavras-chave: Kefir; exercício; osteoporose; glicocorticoides; tecido ósseo.

ABSTRACT

Osteoporosis is a multifactorial skeletal disease that alters microarchitecture and reduces bone mass, causing loss of mechanical strength and increased risk of fracture. Among the forms of osteoporosis, glucocorticoid-induced (GIO) is the most frequent type of secondary osteoporosis. Knowing that the prevention and treatment of GIO will still remain a challenge, natural products and physical exercises have been the focus of study. In this context, Kefir, an oral probiotic, stands out for presenting benefits to the bone and minimizing the pharmacological effects of medications. Thus, this study aimed to evaluate the effect of physical exercise on osteoporosis and the effect of kefir associated with resistance exercise on bone tissue in the GIO. Initially, 2 systematic reviews were evaluated, with preclinical and clinical studies, to analyze the effects of exercise on bone tissue during osteoporosis. It was observed that exercise for enhanced bone mineral strength (BMD), bone and trabecular volume (BV/TV) and decreased trabecular separation (Tb.Sp), beneficial effects of exercise for bone tissue in animal models. 60% of clinical studies confirm a positive association between decreased bone resorption markers and exercise, suggesting reduced resorption in patients with osteoporosis. Finally, to analyze the effect of the association of exercise + kefir on bone tissue submitted to OIG, 60 male Wistar rats were divided into 2 groups: normal (N), and performing GIO, which was subdivided into 4 groups according to treatment: control (C), Kefir (K), Exercise (Ex) and Exercise+Kefir (ExK). GIO was induced by dexamethasone (7mg/kg; i.m.). Kefir was administered by gavage (0.7 ml/animal/day) for 16 weeks. The exercise was performed 3 days/week, with an intensity of 20% to 80% of the maximum individual load, for 16 weeks. After euthanasia, the femur was collected for macro and microscopic analyses, imaging examinations and biomechanical tests. In light of our knowledge, this is the first time that an in vivo evaluation of exercise + Kefir in bone tissue from rats with GIO has been performed. GIO reduced by 35% BV/TV, 33% trabecular thickness (Tb.Th), 26% mineral and collagen content (56%). There is also a reduction in flexion strength (81%), fracture load (80%) and number of osteocytes (84%) after GIO. Glucocorticoids altered bone remodeling ($p < 0.05$), marked by a reduction in the number of osteoblasts while an increased number of osteoclasts, and roughness of bone tissue. After the 16-week intervention, association of ExK improved microarchitecture evolution and bone quality, marked by an increase in fractal dimension (38%), cortical volume (34%),

BV/TV (34%), Tb.Th (33%), mineral content and collagen maturity, while reducing Tb.Sp (34%) and bone roughness. Thus, ExK improved bone strength and biomechanics, in addition to stimulating bone formation and modular bone remodeling. It is concluded that exercise had antiresorptive effects on bone tissue and that kefir potentiated the beneficial effects of exercise on bone tissue, improving microarchitecture, femur quality and biomechanical properties and stimulated bone formation. Thus, treatment with kefir and physical exercises are provided as adjuvant tools to improve the bone loss picture caused by GIO.

Keywords: kefir; exercise; osteoporosis; glucocorticoids; bone tissue.

SUMÁRIO

Introdução Geral

1 INTRODUÇÃO GERAL

1.1 Tecido ósseo

O osso é um tecido dinâmico que apresenta múltiplas funções, dentre as quais o fornecimento de suporte estrutural para corpo; constituição do sistema de alavancas com os músculos, o que permite o movimento e a locomoção; proteção de órgãos e estruturas internas vitais; manutenção da homeostase mineral; e alojamento e proteção da medula óssea, fornecendo o ambiente para a hematopoiese (CLARKE, 2008; FENG; MCDONALD, 2011; LERNER, 2000).

Para suportar as forças, tais como os músculos em contração e a sustentação do peso corporal, os ossos necessitam ser fortes, porém não quebradiços. Essa característica é obtida por meio do componente inorgânico (ou mineral), composto por cristais de fosfato de cálcio na forma de hidroxiapatita, que oferecem ao osso a capacidade de suportar forças compressivas ou de esmagamento. Por outro lado, a capacidade de sustentar forças de tensão ou estiramento, tornando o osso menos vulnerável às fraturas, é obtida por meio do componente orgânico que consiste em fibras de colágeno embutidas em uma substância gelatinosa (ANDIA; CERRI; SPOLIDORIO, 2006; CLARKE, 2008). Os percentuais relativos de componentes inorgânicos e orgânicos variam de acordo com a idade. Em geral, apresenta-se por 60% a 70% de cristal inorgânico e 30% a 35% de material orgânico, sendo este último constituído em 90% por colágeno (ANDRADE *et al.*, 2007; CLARKE, 2008).

Macroscopicamente, é possível observar dois tipos ósseos diferentes: esponjoso e compacto. Embora os elementos que formam as duas substâncias sejam similares, elas apresentam algumas diferenças. A substância óssea esponjosa, também denominada trabecular ou reticular, apresenta lamínulas ósseas mais irregulares no que diz respeito a forma e tamanho e organizam-se deixando espaços entre si, formando lacunas que se comunicam e são preenchidas por medula óssea. Já a substância compacta, também chamada de cortical, possui lamínulas fortemente unidas, sem que haja espaço livre entre elas, proporcionando maior densidade e dureza à essa substância (CLARKE, 2008; DALMOLIN *et al.*, 2013; RALSTON, 2013). No osso longo, o predomínio de substância compacta ocorre na diáfise. Já nas

epífises, o osso esponjoso é mais evidente sendo revestido por fina camada de osso compacto (CLARKE, 2008; RALSTON, 2013).

A densidade mineral óssea (DMO), definida pela quantidade de massa mineral por área ou por volume ósseo, é determinada pelo equilíbrio entre os processos de remodelação óssea (LERNER, 2000; RALSTON, 2013; SOPHER; FENNOY; OBERFIELD, 2015). Essa característica faz do osso um tecido metabolicamente muito ativo que é constituído por uma população heterogênea de células (ANDRADE *et al.*, 2007). No esqueleto eutrófico, o osso está em processo contínuo de remodelação, que é um mecanismo de manutenção do metabolismo ósseo. Esse mecanismo que se dá pelo equilíbrio entre os processos de reabsorção e formação óssea, realizados por células ósseas especializadas conhecidas por osteoclastos e osteoblastos, respectivamente (ANDIA; CERRI; SPOLIDORIO, 2006; CLARKE, 2008; RALSTON, 2013). Quando ocorre o desequilíbrio entre a reabsorção óssea e formação óssea osteoblástica pode ocorrer uma condição patológica dentre as quais se destaca a osteoporose (FENG; MCDONALD, 2011).

1.2 Osteoporose

A osteoporose é definida como uma doença esquelética multifatorial que se caracteriza pela redução da massa óssea e ruptura da estrutura da microarquitetura do tecido ósseo, resultando em perda de força mecânica e aumento do risco de fratura (ARMAS; RECKER, 2012; PORFIRIO; FANARO, 2016).

Segundo a *International Osteoporosis Foundation* (COOPER; FERRARI, 2019), a osteoporose é uma condição muito comum. Uma em cada três mulheres e um a cada cinco homens com mais de 50 anos terão fratura por fragilidade decorrente dessa condição. Há uma grande variedade de estimativas para a prevalência de osteoporose (KÄRNSUND *et al.*, 2020), não tornando possível a identificação mais segura desses dados. Porém, estima-se que mais de 200 milhões de pessoas, ou seja aproximadamente 3% da população mundial, tem osteoporose (COOPER; FERRARI, 2019).

Entre os efeitos negativos da osteoporose sobre qualidade de vida dos pacientes, incluem-se deformidades, dor crônica, depressão, perda da independência e aumento da mortalidade e, ainda, as debilitantes fraturas (KANIS *et al.*, 2008). 1,4 milhões de indivíduos que sofreram fraturas osteoporóticas nas vértebras, por

exemplo, sofrem dores, perda de altura e muitos outros efeitos sobre a qualidade de suas vidas (COOPER; FERRARI, 2019). Surpreendentemente, em estudo de revisão sistemática analisando fraturas osteoporóticas em relação às taxas de incidência de fraturas de quadril em todo o mundo, verificou-se que taxas de incidência de fratura de quadril foram 2 a 10 vezes maiores nas categorias de idade mais jovens em comparação com as mais velhas e nas mulheres em comparação com os homens, com poucas exceções (CHAKHTOURA *et al.*, 2021).

A osteoporose, além de uma questão social, é um problema econômico, já que os cuidados com essa enfermidade geram altos custos (FONTES; ARAÚJO; SOARES, 2012). Na União Europeia, a osteoporose é um fator chave para o gasto elevado com cuidados médicos. Em 2010, a União Europeia gastou 40 bilhões de dólares e, em 2015, os Estados Unidos gastaram 20 bilhões de dólares com a osteoporose e suas consequências (COOPER; FERRARI, 2019). No Brasil, foram gastos mais 288 milhões de reais com tratamento de osteoporose no SUS entre os anos de 2008 e 2010 (MORAES *et al.*, 2014). Assim sendo, além de ser uma doença que reduz a qualidade de vida e que pode causar a morte, a osteoporose é uma doença que gera um alto custo para os orçamentos em saúde.

A osteoporose pode ser classificada em primária (tipo I ou tipo II) ou secundária. A osteoporose primária do tipo I é conhecida por tipo pós-menopausa e caracteriza-se por rápida perda de massa óssea. Já a osteoporose primária do tipo II é também denominada de senil, pois está associada ao envelhecimento, e surge pela deficiência crônica de cálcio, aumento da atividade do paratormônio e diminuição da formação óssea (RIGGS; MELTON, 1983). A osteoporose secundária, por sua vez, ocorre em virtude de processos inflamatórios, alterações endócrinas e desordens adrenais (LANE; NYDICK, 1999); mieloma múltiplo (MIRZA; CANALIS, 2015); por uso de drogas como heparina (RAJGOPAL *et al.*, 2008), álcool (CRUEL *et al.*, 2017), cigarro (YOON; MAALOUF; SAKHAEE, 2012), inatividade física (VOLPON *et al.*, 2008) e por glicocorticoides (GCs) (ADAMI; SAAG, 2019), sendo esta última, a causa mais frequente da osteoporose secundária (MESSINA *et al.*, 2021; WEINSTEIN, 2012).

A osteoporose secundária pode estar presente em mulheres pré- e pós-menopáusicas e também em homens. Até 30% das mulheres na pós-menopausa e 50 a 80% dos homens apresentam fatores que contribuem para o surgimento desse tipo de osteoporose (MIRZA; CANALIS, 2015). Apesar da osteoporose ser

comumente associada a um problema da saúde feminina, nos últimos 20 anos, essa doença tem sido considerada um problema de saúde pública entre os homens devido à ocorrência cada vez maior de fraturas por fragilidade nos mesmos (LOURES *et al.*, 2017).

A DMO pode ser afetada de forma negativa por várias doenças, como diabetes (SHAH et al., 2021) e artrite reumatoide (SILVA; PIPA; ZERBIN, 2007), e de forma frequente por drogas utilizadas nos tratamentos de doenças, tais como agonistas do hormônio liberador de gonadotrofinas (GnRHa), inibidores de aromatase, anticonvulsivantes, hormônios da tiroide em dose supressiva, uso prolongado de glitazonas, inibidores da bomba de prótons e por glicocorticoides (PINTO; ZERBINI, 2010). Os GCs são medicamentos de ampla utilização na prática clínica por apresentarem efeitos anti-inflamatórios e imunossupressores (MIRZA; CANALIS, 2015). A osteoporose tem sido observada em tratamentos crônicos com uso desses esteróides (KOMORI, 2016; WEI YAO *et al.*, 2008; CANALIS *et al.*, 2007) e sua utilização terapêutica está associada ao aumento no risco de fraturas (PINTO; ZERBINI, 2010; VAN STAA *et al.*, 2003; VAN STAA *et al.*, 2004). A osteoporose induzida por glicocorticoides (OIG), portanto, é uma doença sistêmica secundária que surge em indivíduos que fazem uso prolongado destes medicamentos, aumentando o risco de fraturas ósseas (MESSINA *et al.*, 2021; WEINSTEIN, 2012; SOUZA *et al.*, 2010).

1.3 Osteoporose induzida por glicocorticoide (OIG)

Os GCs são hormônios esteroides secretados pelo córtex suprarrenal que exercem múltiplos efeitos sistêmicos e locais (MILLER; AUCHUS, 2011). Em condições normais, no tecido ósseo, os GCs participam do metabolismo do cálcio (RUBIN; BILEZIKIAN, 2002) e participam da diferenciação e ativação dos osteoblastos (células que depositam matriz óssea), promovendo a formação da matriz óssea (BRIOT; ROUX, 2015). Todavia, o excesso de GCs altera a atividade metabólica e proliferativa das células do tecido ósseo (CANALIS *et al.*, 2007; HARTMANN *et al.*, 2016).

Os GCs em excesso interferem diretamente na proliferação, diferenciação e apoptose dos osteoblastos, inibindo a osteoblastogênese, reprimindo a função osteoblástica (CANALIS *et al.*, 2007; HENNEICKE *et al.*, 2011) e aumentando a

apoptose dos osteoblastos (KOMORI, 2016). Além disso, GCs geram autofagia, apoptose e/ou necrose dos osteócitos, dependendo da dose e tempo de exposição (YAO *et al.*, 2013). Os CGs em excesso também prolongam a vida útil dos osteoclastos (células que degradam a matriz óssea) (WEINSTEIN, 2012), aumentam a atividade osteoclástica (HENNEICKE *et al.*, 2011; ZHOU *et al.*, 2018), suprimem a inibição da osteoclastogênese (DOVIO *et al.*, 2006) e diminuem a apoptose de osteoclastos maduros (JIA *et al.*, 2006). Mais recentemente também foi verificado que os GCs reduzem a vascularização do osso (WANG; YU; HE, 2019) que, em conjunto com as demais alterações, pode ser a explicação para o fato de haver maior redução da resistência óssea do que da massa óssea, diferenciando a OIG dos demais tipos de osteoporose neste aspecto. Em conjunto, essas alterações promovem a degradação da matriz óssea (LANE, 2019).

Além disso, os GCs em excesso atuam no intestino, reduzindo a absorção de cálcio. Nos rins, o excesso desse esteroide aumenta a excreção de cálcio, o que eleva a secreção de hormônio da paratireoide (PTH) que, consequentemente, gera alteração dos níveis séricos de cálcio e de fosfatase alcalina óssea (FAO) (BORBA; LAZARETTI-CASTRO, 1999). Nas gônadas, o excesso de GCs reduz a secreção de hormônios sexuais (ZIEGLER; KASPERK, 1998); e nos músculos, promovem diminuição de massa e de força muscular (SZULC *et al.*, 2005), que é um fator que induz ao maior risco de queda e, consequentemente, de fraturas (CANALIS *et al.*, 2007; SZULC *et al.*, 2005). Assim, os GCs induzem um desequilíbrio no processo de remodelação óssea (SOUZA *et al.*, 2010; WEINSTEIN, 2012) que gera uma rápida perda óssea trabecular, deterioração da microarquitetura do tecido ósseo, diminui o *turnover* ósseo e aumenta o risco de fraturas (HENNEICKE *et al.*, 2011; LANE, 2019; WEINSTEIN, 2012).

Portanto, o uso prolongado de GCs tem sido considerado um fator de risco importante para osteoporose e consequente fratura osteoporótica (AMICHE *et al.*, 2018; BALASUBRAMANIAN *et al.*, 2016; SARINHO; PINHO; MELO, 2017). No entanto, apesar desse risco elevado, devido aos seus efeitos anti-inflamatórios e propriedades imunomoduladoras e antiproliferativas, os GCs têm sido utilizado como tratamento de doenças crônicas (HENNEICKE *et al.*, 2011) por praticamente todas as especialidades médicas (OVERMAN; YEH; DEAL, 2013; WALSH *et al.*, 2001). Aproximadamente 250.000 homens e mulheres usam GCs a longo prazo no Reino Unido e estão correndo risco significativamente aumentado de fraturas osteoporóticas,

independentes de fraturas prévias (KANIS *et al.*, 2011). A incidência de fraturas varia de 30 a 50% em pessoas que usam GCs por mais de três meses. Porém, além da duração prolongada do tratamento, o aumento da dosagem diária e dose cumulativa com vários cursos de alta dose oral ou terapia intravenosa com doses relativamente baixas também aumenta o risco para fraturas (WEINSTEIN, 2012). Além disso, é importante destacar que o risco de fratura em indivíduos com a mesma DMO é superior naqueles com OIG quando comparado aos pacientes com osteoporose pós-menopausa ou senil (KAJI *et al.*, 2006; LANE, 2019).

Com a instalação da atual pandemia de COVID-19 e a recomendação pela Organização Mundial da Saúde (OMS) do uso da dexametasona para os casos graves dessa doença (ROCHWERG *et al.*, 2020) e sabendo que esses esteróides tem sido utilizados contra a síndrome respiratória aguda grave do coronavírus (SARS-CoV-2), mesmo com uma conclusão ainda não confiável sobre o uso de GCs no tratamento de COVID-19 (CORDEIRO *et al.*, 2021), é provável que haja um aumento relevante de casos de OIG em um futuro próximo. Assim, com o aumento da sobrevida de pacientes com doenças crônicas, aumento da frequência de uso de GCs e consequente aumento da morbidade e mortalidade relacionadas ao seu uso (PEREIRA *et al.*, 2012), a OIG, e as fraturas decorrentes dela, deve ser melhor compreendidas, prevenidas e tratadas.

1.4 Prevenção e tratamento da OIG

Nos últimos anos, os programas de tratamento e prevenção da osteoporose tem focado em estratégias que visam minimizar a reabsorção e maximizar a formação óssea e, ainda, incluem estratégias que busquem diminuir os episódios de quedas e/ou outros incidentes que possam ocasionar fraturas (COHEN; ROE, 2000). Esse tratamento envolve ações farmacológicas e não farmacológicas (LANE, 2019).

A estratégia farmacológica prevê o uso de medicamentos tais como os bisfosfonatos, sendo esta a terapia mais amplamente usada na OIG (ADAMI; SAAG, 2019). Essa medicação visa a inibição da reabsorção óssea e atua primariamente nos osteoclastos (MARIE, 2006). Também são prescritos teriparatida (BRIOT; ROUX, 2015) e *denosumab* (BUCKLEY *et al.*, 2017), que visam promover a neoformação óssea, atuando primariamente nos osteoblastos (MARIE, 2006). Todos esses fármacos geram melhora no quadro de osteoporose reduzindo a ocorrência de

fraturas através de efeitos benéficos na massa, volume, tamanho, forma, *turnover*, microarquitetura e qualidade do osso (ALMEIDA *et al.*, 2017; CHO; KIM; JEON, 2012; NEWMAN *et al.*, 2016; ZHAO *et al.*, 2011). Porém, apesar dos relevantes benefícios, essas medicações estão associadas a efeitos adversos (ALMEIDA *et al.*, 2017), tais como intolerância gastrointestinal (SOUZA *et al.*, 2010) e osteonecrose mandibular (POZZI *et al.*, 2007; SOUSA FERREIRA *et al.*, 2021).

As intervenções não farmacológicas, por sua vez, incluem além da ingestão de vitamina D e cálcio, a prática de exercícios físicos (CORONADO-ZARCO; OLASCOAGA-G; MACÍAS-HERN, 2019; LANE, 2019), especialmente o treinamento resistido, com objetivo de prevenir a perda de massa e força muscular, e consequentemente evitar as quedas (LANE, 2019).

A inatividade física, e a consequente redução da carga mecânica sobre os ossos, é provavelmente um dos fatores que contribui para a osteoporose (VIANA *et al.*, 2013). Por outro lado, a atividade física regular pode prevenir a perda de massa óssea (YUAN *et al.*, 2016) e alguns exercícios físicos podem melhorar força e equilíbrio muscular, reduzindo o risco de quedas e fraturas (GIANOUDIS *et al.*, 2014). O exercício aumenta a DMO, massa e resistência óssea e melhora as propriedades mecânicas do osso, atuando nas células ósseas o que afeta os vários aspectos da remodelação (YUAN *et al.*, 2016). O exercício promove a formação óssea, estimulando diferenciação osteogênica e as atividades de osteoblastos e osteócitos, (HSU *et al.*, 2018; ZHANG *et al.*, 2017) e, paralelamente, inibe a osteoclastogênese e a reabsorção óssea (KANAZAWA *et al.*, 2020; ZHANG *et al.*, 2017).. Pichler e colaboradores (2013) identificaram que a estimulação mecânica por meio do exercício físico pode ser um possível tratamento terapêutico para OIG, aumentando a formação óssea e prevenindo fraturas. Porém, são necessários mais estudos para confirmar os benefícios da prática de exercícios físicos especialmente em pacientes com OIG (BUCKLEY *et al.*, 2017)

Sabendo que para pacientes que fazem uso de GCs a ingestão de cálcio é ainda mais necessária do que para mulheres com osteoporose pós-menopausa (RATERMAN; BULTINK; LEMS, 2019) e que o uso de produtos naturais, que apresentam menos efeitos adversos, tem ganhado destaque na prevenção e tratamento não apenas da osteoporose mas de todas as doenças (CHE *et al.*, 2016; CHO; KIM; JEON, 2012), o Kefir apresenta-se como uma relevante possibilidade de tratamento. Este probiótico se origina da ação da microbiota natural presente nos

grãos ou grumos de Kefir (MARCHIORI, 2007) e apresenta teores de cálcio semelhantes aos do leite após o processamento, o que o caracteriza como uma boa fonte desse mineral (LERNER, 2000).

Kefir é um leite fermentado de preparo fácil e economicamente acessível (AHMED *et al.*, 2013; MARCHIORI, 2007), que pode ser consumido fresco ou maturado. A maturação é uma fermentação secundária com duração de 24 horas, a 10°C, que tem o objetivo de promover o crescimento de leveduras e trazer sabor e aroma característicos à bebida (BESHKOVA et al., 2002). Como resultado desse processo, que pode ser repetido indefinidamente, ocorre a produção de um alimento rico em ácidos lático, acético e glicônico, gás carbônico, vitamina B12 e polissacarídeos que conferem ao Kefir características singulares e o caracterizam como um produto leve e de fácil digestão (RODRIGUES; CARVALHO; SCHNEEDORF, 2005).

Fina; Brun; Rigalli (2016) compararam leite caseiro enriquecido com casca de ovo e Kefir com leite não tratado e enriquecido com casca de ovo e observaram que houve menor conteúdo de lactose em ambos os leites com Kefir e maior concentração de cálcio no leite com Kefir e enriquecido devido ao menor pH que facilitou a dissolução da casca de ovo. Em adição, o efeito protetor de Kefir no tecido ósseo em ratas ovariectomizadas foi verificado através do aumento da captação de cálcio intracelular (CHEN *et al.*, 2015). Tu e colaboradores (2020), por sua vez, indicaram a função osteo-protetora do Kefir independente de suplementação de cálcio. Em um outro estudo realizado por esses autores, dessa vez em pacientes com osteoporose, observou-se que o consumo de leite fermentado com Kefir apresentou diminuição no marcador de reabsorção óssea nos pacientes que fizeram uso desse probiótico (TU *et al.*, 2015). Embora o Kefir venha conquistando adeptos e tenha apresentado resultados benéficos à saúde (GUZEL-SEYDIM; G¨OKIRMAKLI; GREENE, 2021; SILVA-CUTINI *et al.*, 2019; VASCONCELOS *et al.*, 2021), ainda são necessários mais estudos que investiguem o papel do Kefir no metabolismo ósseo na osteoporose, especialmente na OIG.

Proposições

2 PROPOSIÇÕES

Este trabalho teve como objetivos:

2.1 Objetivo geral

Avaliar o efeito do exercício físico em casos de osteoporose e o efeito do Kefir associado ao exercício resistido no tecido ósseo na osteoporose induzida por glicocorticóide.

2.2 Objetivos específicos

- Avaliar o efeito do exercício resistido no metabolismo ósseo, em modelos animais de osteoporose e estudos clínicos, por meio de revisão sistemática
- Avaliar o efeito anabólico ósseo de Kefir e do exercício resistido em ratos com osteoporose induzida por glicocorticoide na:
    - microarquitetura do fêmur;
    - força e qualidade do tecido ósseo do fêmur;
    - remodelação óssea no fêmur.

Capítulos

3. CAPÍTULOS

REGIMENTO INTERNO

Por se tratar de pesquisa envolvendo animais, o projeto de pesquisa referente a esta tese foi submetido à apreciação da Comissão de Ética no Uso de Animal (CEUA) da Universidade Federal do Ceará, tendo sido aprovado sob número de protocolo 137/17 (Anexo A). Esta Tese de Doutorado baseia-se no Artigo 37º do Regimento Interno do Programa de Pós-Graduação em Ciências Morfofuncionais da Universidade Federal do Ceará, que regulamenta o formato alternativo para dissertações de Mestrado e teses de Doutorado. Os resultados obtidos estão apresentados na forma de 3 artigos científicos, redigidos de acordo com as normas da revista científica escolhida para publicação.

Artigo 1: Effect of strength exercise on bone metabolism in osteoporosis: a systematic review of animal studies
Periódico: revista PLoS One* (ISSN: 1932-6203)
Qualis capes: A1
Fator de Impacto: 2.740

Artigo 2: Effect of physical exercise on bone tissue in patients with osteoporosis: a systematic review clinical study
Periódico: Bone Reports** (ISSN: 2352-1872)
Qualis capes: B2
Fator de Impacto: 3.710

Artigo 3: Kefir Treatment Enhanced Skeletal Response to Climb Exercise in Rats submitted to Glucocorticoid Induced-Osteoporosis
Periódico: Frontiers in Endocrinology*** (ISSN: 1664-2392)
Qualis capes: A1
Fator de Impacto: 4.850

Normas das revistas disponíveis em:

* https://journals.plos.org/plosone/

** https://www.elsevier.com/journals/bone-reports/2352-1872/guide-for-authors

*** https://www.frontiersin.org/journals/endocrinology

Capítulo 1

Artigo 1

Effect of strength exercise on bone metabolism in osteoporosis: a systematic review of animal studies

Raquel Vasconcelos[1¶*#a], Vanessa Ferreira[1¶], Delane Gondim[1&], Paula Goes[1&]

[1] Department of Morphology, Federal University of Ceará, Fortaleza, Ceará, Brazil

[#a]Current Address: Department of Morphology, Federal University of Ceará, Delmiro de Farias Street, Rodolfo Teófilo, Fortaleza, Ceará, Brazil

* Corresponding author
E-mail: paulagpinheiro@yahoo.com.br (PG)

[¶]These authors contributed equally to this work.
[&]These authors also contributed equally to this work.

Effect of strength exercise on bone metabolism in osteoporosis: a systematic review of animal studies

ABSTRACT

Osteoporosis is a crucial human, social and economic burden that has enormous personal and socioeconomic consequences for millions of people. This disease and its pathological changes have similarities between animal models and osteoporosis in humans. Therefore, animal models have been widely used as a clinically relevant model of bone loss. Thus, studies in animal models that address the treatment of osteoporosis are of great importance and interest to support future clinical studies that, in turn, may help to better choose treatments for patients with osteoporosis. In order to analyze the effects of strength exercise on bone tissue during osteoporosis, studies in animal models submitted to physical strength exercises have been searched at PubMed, Cochrane Library database, EMBASE, Ovid Medline, Scopus and Web of Science using a combination of terms: ((exercis*) OR (physical exercis*) OR (strength exercis*) OR (resistance exercis*)) AND ((bone tissue) AND (osteoporos*) AND (animal model)). Those which were pre-clinical trials, case-controlled studies, cross-sectional studies using animal models with osteoporosis and treated with therapeutic through physical strength exercises, published in Portuguese, English or Spanish language, in the last 10 years were included. The evaluation of the results considered the morphometric, histological, biomechanics, radiographic and/or laboratory parameters. It was seen that physical exercise can mitigate bone loss in animal models when compared to animals not submitted to exercise, either healthy or with osteoporosis. Exercises were related to an increase in bone mineral density (BMD) in 60% of the analyzed studies as well as increase in the maximum load, stiffness, toughness and fracture load. It was also seen that exercise increased bone and trabecular volume while decreased trabecular thickness, indicating beneficial effects of physical training on bone tissue. Together, these results support the choice of strength exercise as part of the treatment of patients with osteoporosis in future clinical studies that, in turn, can help in choosing the exercise as an adjunct tool for improving the bone loss process induced by osteoporosis.

Keywords: osteoporosis; exercise; bone tissue; animal models

1. Introduction

Osteoporosis is defined as a multifactorial skeletal disease that is characterized by the reduction of bone mass and disruption of the structure of bone tissue microarchitecture, resulting in loss of mechanical strength and increased risk of fracture (1). This disease is quite common since one in three women and one in five men over 50 has a fragility fracture as a result of osteoporosis. There is a prevision that prevalence increases significantly due to the aging process of the population. In addition, this disorder represents a crucial human, social and economic burden, as a result of fragility fractures, generating enormous personal and socioeconomic consequences for millions of people (2). Therefore, studies that address the treatment of osteoporosis are of high importance and interest.

Osteoporosis treatment and prevention programs have focused on strategies that aim to minimize resorption and maximize bone formation. They also include the reduction of falls and/or other incidents as an aim in order to lessen fractures (3). The mechanical load promoted by physical exercise increases bone mass (4-6), and it has been reported to be potentially safe and effective in preventing bone loss (6). So, physical exercise has been suggested as a valuable adjuvant tool for the treatment of osteoporosis.

Osteoporosis and its pathological changes have similarities between animal models and osteoporosis in humans. Therefore, animal models have been widely used as a model of bone loss clinically relevant (7–10). Thus, the analysis of the effects of strength exercise on the bone tissue of animals with osteoporosis is interesting to support the choice as part of the treatment of patients. So, in order to better understand physical exercises in the treatment of this disease, the aim of this study was to conduct a systematic review of the use of strength exercises in the treatment of osteoporosis with animal models and to investigate the effects of strength exercise on bone metabolism in osteoporosis.

2. Materials and methods

2. 1 Protocol and registration

This systematic review was carried out following the premises made by the Preferred Report Items for Systematic Reviews and Meta-Analysis Guidelines (PRISMA) (11). The protocol was elaborated and previously registered in the revision

database Reviews and Disclosure Center (PROSPERO) International property registration systematic reviews with several records CRD42020220192, updated November 30th, 2020.

2.2 Focused question

The focused-question “Does strength exercise alter bone metabolism in animal models of osteoporosis compared to animals not subjected to strength exercise?” was formatted. **Population (P)**: animal with osteoporosis; **Interventions (I)**: strength physical exercise; **Control intervention (C)**: animals not submitted to strength exercise; and **Outcome measured (O)**: the effect of physical strength exercise interventions on bone metabolism.

2.3 Search Strategy

This review study was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta Analyses Guidelines (11) and Cochrane Handbook for Systematic Reviews (12). The search strategy was constructed according to the Populations, Interventions, Comparison and Outcomes (PICO) principle. Individual search strategies were designed for the following electronic databases: PubMed, the Cochrane Library database, EMBASE, Ovid Medline, Scopus, Web of Science. Electronic databases were searched to identify relevant studies published up to and including November 2020. The searched publications were only considered in last ten years in Portuguese, English or Spanish language.

The search strategy contained a combination of controlled predefined Medical Subject Heading (MeSH) and/or Descriptors in Health Sciences (DeCS) terms and free terms using the Boolean operators (i.e., OR, AND), always adapted to the syntax rules of each bibliographic database. A combination of the following terms was used to identify relevant studies: ((exercis*) OR (physical exercis*) OR (strength exercis*) OR (resistance exercis*)) AND ((bone tissue) AND (osteoporos*) AND (animal model)). The Hooijmans (2010) and de Vries (2011) search filters for the identification of preclinical studies in PubMed and Embase, respectively, were applied to increase search efficiency (13,14). Additionally, it has also conducted a manual search of bibliographies and reference lists of the included studies to locate any potential unidentified study.

2.4 Selection criteria

It was included in vivo pre-clinical trials in animals such as rats, mice or rabbits, with osteoporosis, regardless of gender or age. The osteoporosis model should be induced by glucocorticoid, ovariectomy or oophorectomy, orquiectomia or orquidectomia, calcium restriction, hormone use or using senile rats. The studies must have used strength physical exercises independent of protocols, duration or intensity, combined or not with other non-surgical therapies or medications. Studies with a separate control group whose animals were received vehicle, were untreated, treated with simulation (for example, electrical stimulation), submitted to none treatment, without strength exercises in animals healthy or with osteoporosis were included. Studies should be published in Portuguese, English or Spanish, in the last ten years.

2.5 Exclusion criteria

In this study, all references related to (a) literature reviews, clinical trials, case reports or reviews, case studies, cross-over studies; (b) animals with comorbidities; pregnant; ex vivo, in vitro and in silico models; (c) exercise protocols that are not strength exercises or treatments that include strength exercises but are associated with any surgical treatment; (d) bone loss protocols that include animals in movement deprivation such as in the model of tail suspension and all other conditions of untreated control; (e) studies without a separated control group were excluded. Studies that present isolated non-relevant data, such as, for example, only animal weight variation, were not considered.

2.6 Types of outcome measures

For result evaluation, morphometric, histological, biomechanics, radiographic, and/or laboratory parameters were considered. The following parameters were adopted: bone structure parameters, as such histomorphometry, evaluation of bone resistance through biomechanical tests, bone mineral density (BMD); measurements of the area of bone loss, volumetric analysis, bone percentage, bone porosity and trabecular thickness (Tb. Th) and biochemical parameters (for example, serum levels of bone alkaline phosphatase [BALP], calcium and phosphorus; and bone biomarkers).

2.7 Screening methods, data extraction and risk-of-bias assessment

Titles and/or abstracts of the studies were selected using the search strategy with an electronic search algorithm considering the selection criteria. The algorithm

included the descriptors or keywords (DeCS and MeSH) already mentioned. The electronic search took place in the databases already reported and followed the selection criteria already specified. The full text of potentially eligible studies was retrieved and independently evaluated by two reviewers (VC, RF). The eligibility certification for the selected studies was carried out upon confirmation by a third reviewer. The Kappa (K) concordance test, also known as the Kappa coefficient, was used to measure the degree of agreement between the evaluators. Values greater than 0.80 had been considered for agreement between the evaluators.

A standardized pre-pilot form was used to extract data from the included studies in order to assess its quality and synthesize evidences. The information extracted included: study design; information about sample and experimental groups; osteoporosis induction protocol; details of exercise intervention and other interventions; information to assess the risk of bias, author, year of publication and research location. Missing data was requested from the authors. Information about controlled versus crossover, number of experimental groups, number of animals per group was also collected.

From the animal models, it was extracted variables such as age and weight, species, sex and the use of anesthetic agent. So, that we could evaluate the effect of strength exercise in different species and in both sexes, checking if there was a different performance with those variables. About the type of intervention, any exercise that was performed against resistance, in any medium (aerial or aquatic), using different implements (loads attached to the syrup, stairs, pools, among others), with any duration (daily, weekly, or monthly) was considered a strength exercise, in combination with non-surgical therapies and other drugs (combination of drugs).

The dichotomous data primary were extracted from different models of strength exercise, associated or not with other medications and/or non-surgical procedures, which caused an improvement in bone tissue with osteoporosis. The continuous data verified changes in the data of bone structure parameters, such as bone mineral density (BMD, g/cm²), bone volume (VB, mm³), percentage bone volume (BV/ TV, percentage), trabecular thickness (Tb.Th, mm), trabecular number (Tb.N), total porosity (Po (tot), percentage) and total volume of the porous space (Po.V (tot), mm³). The biomechanical outcomes had the following calculated values: stiffness (K; N mm^{-1}), maximum load (maximum load; N), work to fracture (Nmm) and post-flow

displacement (PYD; mm) and biochemical parameters such as serum levels of bone alkaline phosphatase, calcium, and phosphorus (mg/dL).

The secondary continuous data extracted were: type and duration of strength training per training session (in minutes); frequency of strength exercise (times per week); total duration of the strength exercise (in weeks); load used (in grams), number of sets and repetitions of the strength exercise; evolution of the animal weight (in grams); as well as bone structure parameters such as osteocyte, osteoblast and osteoclast counts (viable cells /mm²).

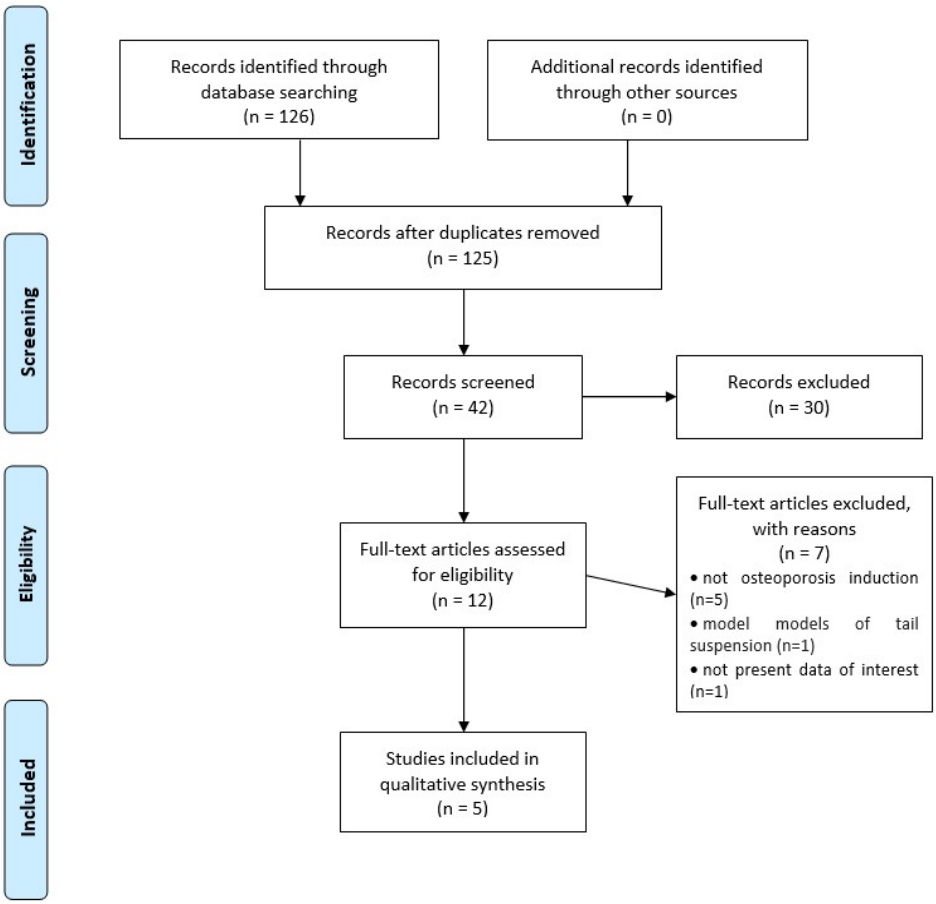
We used the RoB tool for animal intervention studies (SYRCLE RoB tool), without adaptations, to assess the risk of bias. The CAMARADES (Collaborative Approach to Meta-Analysis and Review of Animal Data in Experiment Studies) (15) checklist was used to analyze for study quality.

3. Results

3.1. Search Results

A total of 126 studies were identified (Figure 1). Of these, a total of 84 studies were excluded, one was duplicated and 83 it did not evaluate bone tissue in animal models. After reading the 42 selected articles, it was found that 30 of these did not meet the eligibility criteria, and seven articles were excluded because they did not induced osteoporosis (n=5), or included animals in motion deprivation, such in model of tail suspension (n=1) or did not present data of interest for the study (n=1); thus, five complete articles were included in this systematic review. The Kappa agreement test (K) showed a value of 0.88, which means a perfect agreement level (threshold > 0.8).

Figure 1. PRISMA flowchart. Studies included for qualitative assessment based upon the Preferred Reporting Items for Systematic Reviews and Meta-analysis guidelines.



3.2. Characteristics of the Included Studies

Four studies aimed to investigate the effects of exercise training, combined or not with other intervention (16–19). One study aimed to investigate whether swimming can prevent bone loss caused by osteoporosis (20).

General information and technical features of the included studies are summarized in Table 1. All studies were published in English. Two studies were randomized controlled (18,20) and three others studies were randomized placebo-controlled study (16,17,19).

The studies used between 24 to 66 adult rats, among them, 3 studies used Wistar rats (17,18,20), 1 study used Sprague-Dawley (19) and 1 study used Holstman rats (16). Four studies (16,17,19,20) used females while 1 study used male animals

(18). The initial weight ranged between 100 to 300 g. The studies had 4 to 6 experimental groups with a range of 6 to 11 animals per group. Four studies used the ovariectomy-induced osteoporosis model (16,17,19,20) while 1 study used glucocorticoid-induced osteoporosis model (18).

About the exercise intervention, three studies used treadmill exercise (17–19), one study used climb with an 80º inclination (16) and 1 study used swimming (20). Four studies used another intervention associated with exercise: vibration stimulation (18), creatine-supplemented (17), hormone replacement (16) and Icariin (19). Only 1 study did not perform any other type of intervention in addition to the physical exercise (20).

Table 1. General characteristics of the included studies.

Author, year	Research location	Study design	Sample (age, weight, gender, species)	Experimental groups and animals per group	Osteoporosis induction protocol	Exercise Intervention	Others Intervention
Pichler et al. 2013	Austria	RC	N=40 (12-week-old 240±20g male Wistar rats)	5 groups (n=8 per group) • control; • osteoporosis + prednisolone • prednisolone + treadmill training • prednisolone +vibration stimulation • prednisolone + treadmill + vibration stimulation	glucocorticoid (prednisolone)	treadmill exercise	vibration stimulation (13x28x15 cm compartments attached to Pneu-Vibe vibration plataform; 30 min of whole-body vibration/d, 5 d/wk, for 12 wk)
Murai et al. 2015	Brazil	RPC	N=55, (15-week-old 250–300 g female Wistar rats)	5 groups (n=11 per group) • sham-operated • OVX non-trained placebo-supplemented • OVX non-trained creatine-supplemented • OVX exercise-trained placebo-supplemented • OVX exercise-trained creatine-supplemented	ovariectomy	treadmill exercise	creatine-supplemented (300 mg/kg body weight)
Souza et al. 2017	Brazil	RPC	N=66 (adult, 220 ± 12 g female Holstman rats)	6 groups (n=11 per group) • sham-operated sedentary • OVX sedentary • sham-operated resistance training • OVX resistance training • OVX sedentary hormone replacement • OVX resistance training hormone replacement	ovariectomy	climbing	hormone replacement (sc. implanted silastic capsules with 5% solution of 17β-estradiol (50 mg 17β-estradiol/ml of sunflower oil), volume/ capsule (10 µL)
Souza et al. 2016	Brazil	RC	N=24 (adult, ~ 100g, female, Wistar rats)	4 groups (n=6 per group) • no surgery and sedentary • no surgery and trained • OVX and sedentary • OVX and trained	ovariectomy	swimming	No
Zhao; Bu; Chen 2019	China	RPC	N=32, (3-month old, 210-235g, female, SD rats)	4 groups (n=8 per group) • sham-operated • OVX group • OVX + exercise • OVX + exercise + Icariin	ovariectomy	treadmill exercise	Icariin (50 mg/kg/day by gavage)

Source: survey data. RC: randomized controlled; RPC: randomized placebo-controlled; SD: Sprague Dawley; OVX: ovariectomized.

3.3 Main outcome variables of studies

The morphological and histological parameters were tibial weights in one study (16); visibility and organization of chondrocytes and structural organization of tissues in a study (20); bone volume in one study (18); trabecular bone volume (BV/TV), trabecular number (Tb.N) and trabecular thickness (Tb.Th) in two studies (17, 19). This last study (17), in addition to the parameters reported above, also analyzed the relationship between the volume of the osteoid and the total volume of the bone (OV/BV) and the thickness of the osteoid (O.Th), the areas of erosion surface (ES/BS) and osteoid surface (OS/BS), the osteoclast surface (Oc.S/BS), the osteoblast surface (Ob.S/BS), osteoid thickness (O.Th), mineral apposition rate (MAR), mineralizing surface area (MS/BS) and bone formation rate (BFR / BS).

Regarding bone parameters, two studies analyzed only bone mineral density (BMD) (18,19), one study (17) analyzed BMD and bone mineral content (BMC), one study (16) analyzed bone density (BD) and BMC; and another study analyzed only BD and bone mass (20). The biomechanical parameters of maximum load, stiffness, and tenacity (17) and stiffness, fracture load, and maximum load (16) were verified. The biochemical parameters analyzed were the plasma level of kappa B nuclear activator receptor (RANKL) and osteoprotegerin (OPG) ligand in 1 study (18); calcium and phosphorus levels from bones in 1 study (16); dosage serum of calcium and alkaline phosphatase in another study (20) and serum concentrations of estradiol, alkaline phosphatase and tartrate-resistant acid phosphatase (TRAP) in one study (19).

3.4 Primary outcomes: effects of exercise treatment in the bone loss

The characteristics of exercise in the included studies are summarized in Table 2. The treatment with exercise, alone or associated with another intervention, was able to decreased Tb.Sp (17) and increase tibia weight (14), bone volume (16), BV/TV (17) as well as increase the number of chondrocytes and improve observation of the structural organization of the tissue, suggesting an increased in bone formation, despite fewer gaps in the proximal cancellous bone diaphysis microarray, indicating that bone was strengthened (20). Only one study did not show relevant changes due to exercise in histological parameters (17). Exercise, associated or not with another treatment, increased BMD (17-19), BMC (16, 17), BD (16, 20) and bone mass (20).

The biochemical results of the selected articles indicate that exercise, associated or not with another intervention, was able to decrease the serum levels of RANKL (18), serum levels of alkaline phosphatase and TRAP (19) and increased serum OPG (18), E2 concentrations (19), both protein and mRNA expression of ERα, p-Akt (Akt) or Akt, ß-catenin, and Runx2 (19), and levels of calcium and phosphorus in the bone (16) when compared with animals with osteoporosis. One study did not observe changes in serum levels of serum calcium and phosphorus induced by exercise (20).

Biomechanical results were identified in two studies. Exercise, associated or not with another intervention, was able to increase maximal load, stiffness, toughness (17), and fracture load (16) indicating beneficial effects of exercise training on bone tissue.

All studies claim that exercise was effective in preventing deleterious changes in bone tissue and that exercise can be promising tool in preventing bone loss and promoting bone formation.

Table 2. Characteristics of exercise intervention the studies included (primary outcomes).

Author, year	Morfological and Histological outcomes	Bone parameters outcomes	Biomechanical and biochemical outcomes	Conclusions
Pichler et al. 2013	• EX and EI increased the bone volume by 12.9 and 42.8% compared with osteoporosis group. • Increase of EI was significant compared with osteoporosis and control group.	• EX produced variation in BMD compared to control rats after 12 weeks. • EI showed BMD no significant difference compared to control after 12 weeks.	• EX increased RANKL compared to the control group. • EI decreased RANKL compared to osteoporosis group, equated to the controls. • EI increased OPG compared to osteoporosis, and equated controls.	Mechanical stimulation (exercise and/or vibration) could be a possible therapeutic treatment, through lowering the RANKL level, thereby increasing bone formation and preventing fractures in osteoporotic bone.
Murai et al. 2015	• No main or interaction effects were observed for any of the histomorphometric parameters evaluated.	• increased BMC and BMD, femoral and lumbar spine, suggesting that the trained animals had higher values, irrespective of other intervention.	• Maximal load ($p<0.001$), stiffness ($p<0.001$), and toughness ($p=0.046$) indicated beneficial effects of exercise training on bone tissue.	Exercise training does improve overall bone health in this experimental model.
Souza et al. 2017	• Greater tibia weight in the EI (0.61 ± 0.0 g) compared to healthy sedentary (0.57 ± 0.0 g)	• BD and BMC were significantly higher in trained groups compared to groups without training • The association between exercise and hormone replacement induced higher values of BD and BMC	• EX and EI increase the calcium and phosphorus concentrations in bones. • EX and EI induced significantly higher stiffness, fracture load and maximum load.	Exercise was effective in preventing the deleterious changes on the bone tissue of OVX rats. Exercise associated with hormone were even more favorable to the health of the bone tissue.
Souza et al. 2016	• EX showed better organization of the tissue and the gaps when compared to sedentary group. • The cancellous bone of EX has fewer gaps than in any other groups, especially compared to the OVX group. • EX was able to prevent the disintegration of the general arrangement of the bone.	• EX had higher BD in the diaphysis and epiphysis when compared to any other group. • EX bone mass was higher when compared to sedentary	• Groups did not present a statistically significant difference on alkaline phosphatase (U/L at 37°C). • No group presented abnormal values of serum calcium and alkaline phosphatase compared to the reference values.	Through the parameters used, swimming promotes greater bone formation and proved to be effective to prevent the loss of bone mass, the disorganization of bone structure and the proliferation of osteoclasts and adipocytes.
Zhao; Bu; Chen 2019	• OVX significantly decreased BV/TV, Tb.N and Tb.Th, and increased Tb. Sp. Both EX and EI improved these adverse changes. • EI generated greater BV/TV gains and decreased more Tb.Sp compared with EX.	• OVX induced a significant bone loss, but exercise alone (0.162 ± 0.01 g/cm²) and associated (0.171 ± 0.01 g/cm²) markedly alleviated bone wasting, with more BMD increment.	• EX and EI decreased serum alkaline phosphatase and tartrate-resistant acid phosphatase and elevated estradiol. • These changes were greater in EI compared with EX.	The combined benefits of exercise and Icariin improved OVX-induced bone loss by up-regulating osteoblastic formation

Source: survey data. Note: OVX: ovariectomy; EX: exercise intervention alone; EI: exercise with other intervention; BMD: bone mineral density; BMC: bone mineral content; BD: bone density; BV/TV: trabecular bone volume; Tb.N: trabecular number; Tb.Th: trabecular thickness; RANKL: nuclear factor kappa B activator ligand; OPG: osteoprotegerin

3.5 Secondary outcomes

Secondary outcomes of the included studies are summarized in Table 3. Body weight was recorded in all studies, except by one (18). Three studies (16, 19, 20) showed greater weight gain in the group of animals with osteoporosis and without treatment. In one study (17), all groups gained weight regardless of the intervention. One study (18) did not present data about animal weight.

Three studies used running exercise as a treatment (17-19), one of them being downhill running (17); one study used climbing exercise with a load attached to the tail (16), and one study used swimming as a treatment for osteoporosis (20).

The total duration of treatment with exercise was 12 weeks in three studies (16-18) and eight weeks in two studies (19, 20). Training session duration was 30 min in two studies (17, 18): a single session in one study (18) and two 15 min sessions (one in the morning and one in the afternoon) in the other study (17). Two other studies had a total training session time of 60 min in a single session (19, 20). One study did not show the duration of the training session (16). The weekly frequency was 5 days a week in four studies (17-20) and 2 days a week in a study (16).

Table 3. Secondary outcomes.

Author, year	Body weight	Type of exercise	Total training duration (weeks)	Training session duration (minutes)	Weekly frequency (days per week)	Exercise intensity
Pichler et al. 2013	---	Treadmill exercise	12	30	5	10-speed m/min with incline at 2° set
Murai et al. 2015	Irrespective of the interventions, all of the animals increased their body weight across time	Treadmill exercise	12	30 (2 sessions of 15)	5	20-speed m/min downhill running The trained groups demonstrate a significantly progressive increases in the workload
Souza et al. 2017	Greater weight gain has been observed in the OVX group, exception of exercise with the hormone group	Climbing	12	--	2	1,1m vertical ladder, 80º incline, 50%, 75%, 90%, and 100% of the rat's MCL, 27 sessions de 4–9 climbs
Souza et al. 2016	Greater weight gain has been observed in the OVX group	Swimming	8	60	5	---
Zhao; Bu; Chen; 2019	Greater weight gains have been found in all groups, exception control group, with the largest increment in OVX	Treadmill exercise	8	60	5	18-speed m/ min and grade of 5%

Source: survey data. Note: OVX: ovariectomy; MCL: maximal carrying load

The intensity of the exercise on the treadmill was 10, 18 and 20 m/min of speed, respectively, with inclination (17-19). One study used downhill running (17). The intensity of the climbing exercise with an inclination of 80º gradually increased by 50%, 75%, 90% and 100% of the maximal carrying load (MCL) of the rat, measured in the previous training. There were 27 training sessions with 4 to 9 climbs each session (16). The study that used swimming exercises did not present specific information on training intensity.

Only one study presented information about bone cells, claiming to have observed a greater number of osteoclasts in the osteoporotic and sedentary group (20) A single study (18) showed less RANKL immunoexpression when compared exercise with another intervention (vibration stimulation) to the osteoporosis sedentary group, and it also showed high OPG immunoexpression when compared to osteoporosis sedentary group. The exercise alone did not show the same results.

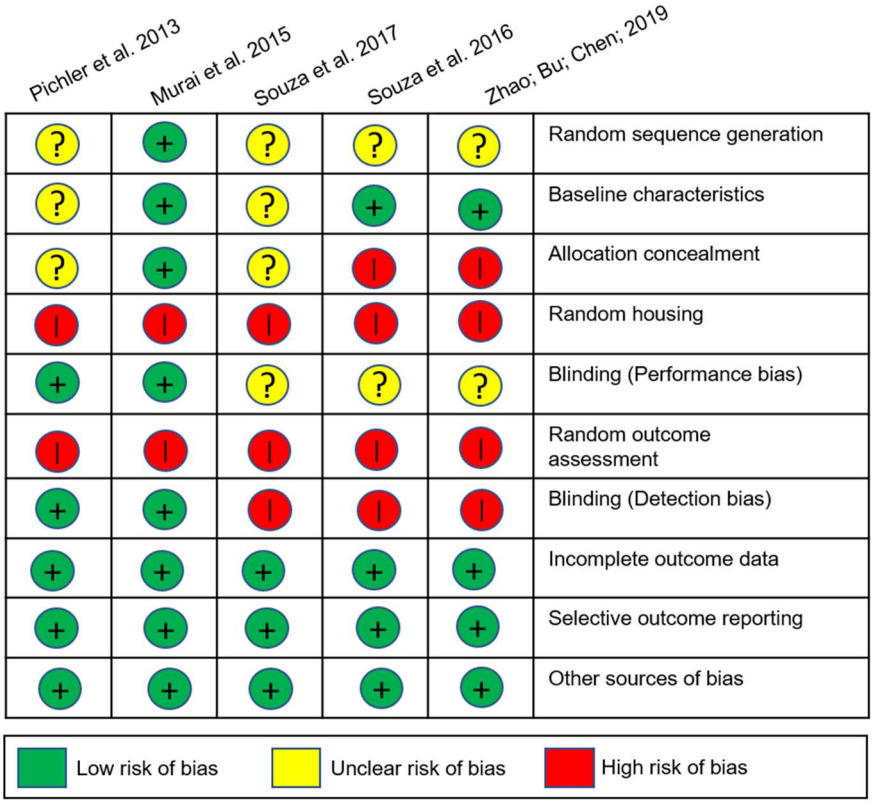
3.6 Risk-of-bias assessment

The risk of bias assessment of the included studies was assessed, using the RoB tool for animal intervention studies (SYRCLE RoB tool) (Figure 2). About selection bias, one study had a low risk of bias for random sequence generation (17) and four studies were classified as unclear for this item, as they claimed to be randomized, but did not describe the methods used to generate the allocation sequence in sufficient detail (16, 18-20). About allocation concealment, one study presented low risk of bias (17), two studies were classified as unclear because did not describe the method used to conceal the allocation sequence in sufficient (16,18) and two studies showed high risk of bias because did not present any description about allocation concealment (19,20). Three studies have a low risk of bias in the item baseline characteristics (17,19,20) and two studies did not be clear about if control and intervention groups were similar at the beginning of the experiment (16,18).

About Performance and Detection bias, all studies presented a high risk of bias in the items Random housing and Random evaluation of results because they do not demonstrate if the animals were randomly housed and if the animals were randomly selected to evaluate the results. Two studies (17,18) low risk of bias for blinding (performance bias), but three studies (16,19,20) were unclear about blinding the care givers or researchers. Two studies (17,18) showed low risk of blinding bias (detection

bias), but three studies (16,19,20) presented a high risk of bias because they did not present describe all measures used for the blind result evaluators to know which intervention each animal. Regarding the Incomplete outcome data, selective outcome reporting aspects and other sources of bias, all studies showed low risk of bias.

Figure 2. Risk of bias assessment. The risk-of-bias of the included studies was assessed using the SYRCLE RoB tool.



Source: elaborated by the authors.

3.7 Quality Assessment

A summary of the quality assessment using the CAMARADES Checklist (15) is shown in Table 4. Total scores showed that the quality of the 5 studies with a minimum of 5 points and a maximum of 9 points, out of 10 points. One study had 5 points (16), 3 studies had 6 points (18–20) and 1 study had 9 points (17).

Table 4. Camarades Checklist.

Article	Pichler et al. 2013	Murai et al. 2015	Souza et al. 2017	Souza et al. 2016	Zhao; Bu; Chen 2019
Publication in peer-reviewed journal	Y	Y	Y	Y	Y
Statement of control of temperature	Y	Y	Y	Y	Y
Randomization of treatment or control	Y	Y	Y	Y	Y
Allocation concealment	N	Y	N	N	N
Blinded assessment of outcome	N	Y	N	N	N
Avoidance of anesthetics with marked intrinsic properties	Y	Y	Y	Y	Y
Use of animals with hypertension or diabetes	N	N	N	N	N
Sample size calculation	N	Y	N	N	N
Statement of compliance with regulatory requirements	Y	Y	Y	Y	Y
Statement regarding possible conflict of interest	Y	Y	N	Y	Y
Total (on 10)	**6**	**9**	**5**	**6**	**6**

Source: survey data. Note: Y: yes; N: no.

4. Discussion

The studies analyzed in this systematic review showed that physical strength exercise can reduce the level of bone loss progression in animal, when compared to animals without exercise, either healthy or with osteoporosis.

After induction of osteoporosis through ovariectomy (16,17,19,20) or glucocorticoids (18), treatment with exercise alone was able to increase bone mineral density (BMD) (17-19). When associated with other interventions, such as vibration stimulation (18) and creatine-supplemented (17), these effects were

increased, helping to support the choice of strength exercise as part of the treatment of patients with osteoporosis in future clinical studies.

One of the main benefits of evaluating BMD is that the amount of bone mass is an excellent predictor of the risk of osteoporosis fracture (21). The vibration stimulation or creatine-supplementation favored the exercise of strength which, in turn, played a protective bone effect in these animal models. BMD measures the quantity of bone density; however other factors need to be considered to assess bone quality.

In order to assess bone quality, its material and structural properties must be taken into account. Material properties include mineral and collagen composition and micro-damages and respective repairs. The structural properties include bone geometry and microarchitecture (22). A better tissue organization was observed in the femoral head in both groups of healthy animals and in animals with osteoporosis submitted to exercise (20), suggesting that exercise was also able to promote changes in quality at the tissue level. Thus, exercise was able to prevent the disintegration of the general bone arrangement caused by osteoporosis, improving the structural properties of bone tissue.

Reinforcing the findings of bone quality, bone mechanical properties were verified through biomechanical tests. Treatment with exercise increased maximal load, stiffness, toughness (16,17), as well as fracture load (16) indicating beneficial effects of exercise training on bone tissue. When associated with hormone replacement, this effect was enhanced (16). Treatment with exercise also increased bone volume (18) and trabecular volume and decreased trabecular thickness (19) reinforcing the positive impacts of physical exercise on bone quality.

However, the study with high quality based on the measurement of quality scores by the CAMARADES Checklist and the lowest risk of bias analyzed via the RoB tool for animal intervention studies (SYRCLE RoB tool), did not show any relevant changes in the histological parameters caused by exercise (17). These results may have occurred due to the methodological difference in the preparation of the histological material. In two studies (16,17), the hematoxylin and eosin (H&E) histological evaluation routine was used and, in another study (15), there was the application of a bone marker (oxytetracycline) and, later, decalcified distal femurs were dissecated for histological analysis. However, this last study (15) pointed out changes

in other parameters caused by physical training, such as increased bone mineral content which was also observed in the study by Souza et al. (2017) (14), increased bone density (also observed in the study by Souza et al. [18]), and an increase in BMD already mentioned.

A decrease in serum RANKL levels and an increase in OPG (18) was observed only when exercise was associated with vibration training. The immunoexpression of RANKL was also low only in the group that associated exercise with vibration training. Neither exercise alone nor vibration training alone was able to cause these changes. Vibration training is a mechanical stimulus that has been used in the training and rehabilitation of the physical capacities of individuals of different levels of physical conditioning and age group (23). Therefore, it appears that the vibration training potentiated the effects of exercise, promoting a decrease in bone resorption since the positive regulation of RANKL is an essential factor in the increase in bone resorption (24). The high levels of OPG, a regulator of the RANK-RANKL-OPG pathway, reinforce these findings favoring the synthesis of the bone matrix (22). It should be noted, however, that these findings refer to only one study.

However, the study by Zhao; Bu; Chen (2019) (19) showed that exercise, alone or in combination with another treatment, increased serum estradiol levels. The exercise associated with Icariin potentiated this finding. RANKL expression is positively regulated two to three times by estrogen deficiency and directly correlated with increases in bone resorption markers, indicating that the increase in estradiol (estrogen) levels, promoted by exercise, can result in a decrease in bone absorption mediated by the RANK-RANKL-OPG route (25).

The RANK-RANKL-OPG pathway is one of the routes that regulate bone remodeling (26). The positive regulation of RANKL and the negative regulation of OPG can be one of the means that generate bone loss caused by osteoporosis (27). Exercises and physical activities promote bone health, increasing OPG levels, and decreasing RANKL levels (28), corroborating what was identified in this review study.

Zhao; Bu; Chen (2019) (19) reinforces their own findings as they identified the decrease in serum levels of TRAP and alkaline phosphatase promoted by treatment with exercise, alone or in combination. Similar to the other findings already mentioned, the exercise associated with Icariin also potentiated this finding. TRAP and an alkaline

phosphatase were measured as markers of bone remodeling. Thus, the decrease in the two markers indicates the anti-resorptive effect.

When analyzing calcium and phosphorus levels in blood and bone (16,20), only in bone tissue did physical exercise increase the concentrations of calcium and phosphorus on bone, which may contribute to greater resistance and tissue remodeling (16). Ovariectomy contributed to increasing the body weight in three studies (16,19,20) corroborating the literature in the castrated rat is also a model for inducing obesity (7,10,29).

In this systematic review, treadmill running (17-19), climbing (16), and swimming (20) were used as treatment for osteoporosis. In a systematic review that analyzed different types of exercise as treatment for postmenopausal women (a situation similar to most osteoporosis-inducing animal models in this review), the positive impact of exercise on bone health was undeniable (30). In this review, three studies used the weight resistance of the animal's own body associated with the impact of running (17-19), one study used weight resistance of the body of animals associated with external loads coupled to the tail (16), and a study used water resistance in a reduced impact environment (20). Thus, regardless of the type of resistance used, the exercises, associated or not with other types of treatment, were effective in preventing deleterious changes caused by animal models of osteoporosis induction, corroborating with the existing literature.

The intensity, volume, and density of training are important variables of physical exercise. The intensity includes load, speed, and power; the volume includes duration, distance, repetitions and the density are the training frequency. Generally, volume and intensity are inversely related, and the higher the density, the shorter the recovery time (31). Therefore, the exercises applied as treatment in the studies analyzed are of high intensity, short duration and high density. It is noteworthy that there was an indirect relationship between intensity and training volume since the treatments that were performed five days a week lasting 30 min per session in a total duration of 12 weeks (17,18). The treatments that were carried out five days a week with a duration of 60 min per session had a total duration of less than eight weeks (19,20). And the treatment that was performed twice a week for 12 weeks had a high load intensity, reaching 100% of the maximum load (16).

American College of Sports Medicine, when it comes to physical exercise for bone health, recommends moderate to high intensity, weekly frequency of 2 to 5 days a week, and duration of each session ranging from 30 to 60 minutes (32). Therefore, the treatment with exercise in animal models used in the analyzed studies is adequate to the recommended.

The strengths of this review include the use of a well-established system of methodology, the processes for checking eligibility, sorting, and abstraction of information that were carried out in duplicates to minimize all possible deficiencies. In addition, the fact that the experimental models of included studies used animals of the same species facilitates the extrapolation of the results in humans.

The total duration of treatment with exercise in the studies analyzed ranged from 8 to 12 weeks. Despite the short duration, the benefits for bone tissue could be revealed. However, it is necessary to investigate treatment models with longer duration to analyze the maintenance of these results over time, since the aging process is harmful to bone tissue.

All studies in this review used young adult animals in models that simulated postmenopausal osteoporosis (16,17,19,20) or induced osteoporosis by glucocorticoids (18). Thus, the non-inclusion of studies with aging animal models was a gap in this review, and it may not be possible to analyze the effects of treatment with physical exercise on senile osteoporosis.

Other limitations of this review are due to the different outcome variables in the different studies since only one study presented information about bone cells (20); a single study showed immunohistochemical results (18) and only two studies presented biomechanical data (16,17). The date limitation in the study selection process may have generated this limitation. Research with these more comprehensive inclusion criteria in future studies is suggested.

However, despite these different outcome variables, their findings complemented each other, demonstrating the anti-resorptive effects on bone tissue. Thus, the repercussions of strength exercise on the bone tissue of animals with osteoporosis analyzed in this systematic review showed the beneficial effects of exercise treatment in animal models, helping to substantiate the choice of resistance exercise as part of patients with osteoporosis treatment.

Conflicts of interest: None.

References

1. Porfirio E, Fanaro GB. Suplementação com colágeno como terapia complementar na prevenção e tratamento de osteoporose e osteoartrite : uma revisão sistemática. Rev Bras Geriatr e Gerontol. 2016;19(1):153–64.
2. Cooper C, S Ferrari, Reginster J, Hughes BD, Rizzoli R, Kanis J, et al. IOF Compendium of Osteoporosis About IOF. Honor Pres. 2017;
3. Cohen AJ, Roe FJC. Review of risk factors for osteoporosis with particular reference to a possible aetiological role of dietary salt. Food Chem Toxicol. 2000;38(2–3):237–53.
4. Akkawi I, Zmerly H. Osteoporosis : Current Concepts. 2018;122–7.
5. Bielemann RM, da Silva BGC, Coll C de VN, Xavier MO, da Silva SG. Burden of physical inactivity and hospitalization costs due to chronic diseases. Rev Saude Publica. 2015;49.
6. Howe TE, Shea B, Dawson LJ, Downie F, Murray A, Ross C, et al. Exercise for preventing and treating osteoporosis in postmenopausal women. Cochrane Database Syst Rev. 2011;2011(7):1–167.
7. Cho D, Kim K, Jeon Y. A synergistic bone sparing effect of curcumin and alendronate in ovariectomized rat. Acta Neurochir. 2012;154:2215–23.
8. Lucinda LMF, Aarestrup BJV, Peters VM, De Paula Reis JE, De Oliveira RSMF, De Oliveira Guerra M. The effect of the ginkgo biloba extract in the expression of Bax, Bcl-2 and bone mineral content of wistar rats with glucocorticoid-induced osteoporosis. Phyther Res. 2013;27(4):515–20.
9. Bragine D, Junior F, Ramos V, Toledo T, Oliveira D, Cho D, et al. Perfil biométrico , histomorfométrico e bioquímico no tratamento com atorvastatina cálcica de ratas com osteoporose induzida com dexametasona ☪. Rev Bras Ortop [Internet]. 2017;53(5):2215–23. Available from: https://doi.org/10.1016/j.rbo.2017.06.018
10. Beattie JR, Sophocleous A, Caraher MC, Driscoll OO, Cummins NM, Bell SEJ,

et al. Raman spectroscopy as a predictive tool for monitoring osteoporosis therapy in a rat model of postmenopausal osteoporosis. J Mater Sci Mater Med [Internet]. 2019; Available from: http://dx.doi.org/10.1007/s10856-019-6226-x

11. Moher D, Liberati A, Tetzlaff J AD. Itens de relatório preferidos para revisões sistemáticas e meta-análises: a declaração PRISMA. Ann Intern Med. 2009;151:264–9.

12. Noyes J, Booth A, Cargo M, Flemming K, Harden A, Harris J, et al. Cochrane Handbook for Systematic Reviews of Interventions. 2019;(October).

13. de Vries RBM, Hooijmans CR, Tillema A, Leenaars M, Ritskes-Hoitinga M. A search filter for increasing the retrieval of animal studies in Embase. Lab Anim. 2011;45(4):268–70.

14. Hooijmans CR, Tillema A, Leenaars M, Ritskes-Hoitinga M. Enhancing search efficiency by means of a search filter for finding all studies on animal experimentation in PubMed. Lab Anim. 2010;44(3):170–5.

15. Macleod MR, Fisher M, Collins VO, Sena ES, Dirnagl U, Bath PMW, et al. Preventing Introduction of Bias at the Bench. 2009;50–2.

16. Souza MVC, Lino ADS, Ruffoni LGD, Domingos MM, Barbosa MR, Rodrigues MFC, et al. Resistance training and hormone replacement increase MMP-2 activity , quality and quantity of bone in ovariectomized rats. 2017;1–8.

17. Murai I, Roschel L, Pabis L, Takayama L, de Oliveira R, dos Santos Pereira R, et al. Exercise training, creatine supplementation, and bone health in ovariectomized rats. 2013;26:1185–96.

18. Pichler K, Loreto C, Leonardi R, Reuber T, Weinberg AM, Musumeci G. RANKL is downregulated in bone cells by physical activity (treadmill and vibration stimulation training) in rat with glucocorticoid-induced osteoporosis. Histol Histopathol. 2013;28:1185–96.

19. Zhao R, Bu W, Chen Y. Icariin Treatment Enhanced the Skeletal Response to Exercise in Estrogen-Deficient Rats. 2019;16(3779):1–11.

20. Souza HR, Girol AP, Schiaveto APS, Geromel MR, Iyomasa MM, Arruda MF. Biochemical analysis and bone remodeling in response to oophorectomy and

aquatic training. 2016;24(5):235–9.

21. Fontes TMP, Araújo LFB, Soares PRG. Osteoporose no climatério I : epidemiologia , definição , rastreio e diagnóstico. Rev Fem. 2012;40(2):109–16.

22. Martin RM, Correa PHS. Bone quality and osteoporosis therapy. Arq Bras Endocrinol Metabol. 2010;54(2):186–99.

23. Hallal CZ, Marques NR, Gonçalves M. O uso da vibração como método auxiliar no treinamento de capacidades físicas: uma revisão da literatura. Motriz Rev Educ Física UNESP. 2010;16(2):527–33.

24. Graves DT, Oates T, Garlet GP. Review of osteoimmunology and the host response in endodontic and periodontal lesions. J Oral Microbiol. 2011;3(2011):1–15.

25. Eghbali-Fatourechi G, Khosla S, Sanyal A, Boyle WJ, Lacey DL, Riggs BL. Role of RANK ligand in mediating increased bone resorption in early postmenopausal women. J Clin Invest. 2003;111(8):1221–30.

26. Trip TR, Sobacchi C, Menale C, Villa A. The RANKL-RANK Axis : A Bone to. 2019;10(March):1–10.

27. Kostenuik PJ. Osteoprotegerin and RANKL regulate bone resorption, density, geometry and strength. Curr Opin Pharmacol. 2005;5(6 SPEC. ISS.):618–25.

28. Yuan Y, Chen X, Zhang L, Wu J, Guo J, Zou D, et al. The roles of exercise in bone remodeling and in prevention and treatment of osteoporosis. Prog Biophys Mol Biol [Internet]. 2016;122(2):122–30. Available from: http://dx.doi.org/10.1016/j.pbiomolbio.2015.11.005

29. Almeida JE, Cúneo F, Amaya-Farfan J, de Assunção J, Quintaes K. A food supplement of hydrolyzed collagen improves compositional and biodynamic characteristics of vertebrae in ovariectomized rats. J Med Food. 2010;13(6):1385–90.

30. Tobeiha M, Moghadasian MH, Amin N, Jafarnejad S. RANKL/RANK/OPG Pathway: A Mechanism Involved in Exercise-Induced Bone Remodeling. Biomed Res Int. 2020;2020.

31. Bompa T, Haff GG. Periodização: Teoria e Metodologia do Treinamento. 5a ed. Phorte, editor. São Paulo: 2013;

32. Kohrt WM, Bloomfield SA, Little KD, Nelson ME, Yingling VR. Physical activity and bone health. Med Sci Sports Exerc. 2004;36(11):1985–96.

Supporting information captions

Prisma Checklist

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).	8
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	not applicable
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.	8-9
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.	9-10
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).	11-12
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.	12-16
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	not applicable
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	18-19
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	16-19
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).	19-24
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).	24-25
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	25
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.	not applicable

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	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.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	2-3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	3-4
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.	4
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.	5
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.	4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	4
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).	6-8
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.	6-8
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
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.	6, 8
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	not applicable
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.	not applicable

Capítulo 2

Artigo 2

Effect of physical exercise on bone tissue in patients with osteoporosis: a systematic review clinical study

Raquel Vasconcelos[1¶*#a], Vanessa Ferreira[1¶], Delane Gondim[1&], Paula Goes[1&]

[1] Department of Morphology, Federal University of Ceará, Fortaleza, Ceará, Brazil

[#a]Current Address: Department of Morphology, Federal University of Ceará, Delmiro de Farias Street, Rodolfo Teófilo, Fortaleza, Ceará, Brazil

* Corresponding author
E-mail: paulagpinheiro@yahoo.com.br (PG)

[¶]These authors contributed equally to this work.
[&]These authors also contributed equally to this work

Effect of physical exercise on bone tissue in patients with osteoporosis: a systematic review clinical study

ABSTRACT

Osteoporosis is a skeletal muscle disorder that is characterized by low bone mass and structural deterioration of bone tissue, causing an increased risk of fractures due to fragility. Although exercise is indicated as part of the treatment for patients with osteoporosis, it is necessary to better understand how these benefits occur in bone metabolism in order to indicate the best type, frequency and duration of exercise to treat osteoporosis. This study aims investigate the effects of physical exercise on bone tissue of patients with osteoporosis. A systematic review was carried out using PubMed, the Cochrane Library database, EMBASE, Ovid Medline, Scopus, Web of Science databases, using a combination of the following terms: exercise OR physical exercise AND bone tissue AND osteoporosis AND clinical trials. The methodological quality of the included studies was assessed with the checklist CONSORT statement and the Cochrane Collaboration for clinical trials. After application of predetermined inclusion and exclusion criteria compatible with the PICOS process, a total of five suitable articles were identified. Three studies confirmed a positive association between the decreased levels of markers of bone resorption and physical exercise. Only one study showed increased BMD in patients of exercise group. However, the number the studies that investigated the physical exercise in bone metabolism in osteoporosis was small. So, the evidence is enough to just hypothesize that physical exercise can reduce bone resorption, decreasing the level of bone loss progression, in patients with osteoporosis compared to sedentary people, helping to substantiate the choice of resistance exercise as part of the treatment of patients with osteoporosis.

Key words: osteoporosis; exercise; bone tissue; clinical study.

1. Introduction

Osteoporosis is a very common condition (1) that is characterized by the patient having low bone and structural deterioration of bone tissue, causing an increased risk of fractures due to frailty (2). High impacts on the economy and loss of quality of life for patients and their families are important burdens generated by these fragility fractures (1). Thus, the effort in the search for effective treatments for osteoporosis is extremely important. Thereby, osteoporosis treatment and prevention programs should focus on strategies that aim to minimize resorption and maximize bone formation, and also include strategies that seek to reduce episodes of falls that may cause fractures (1,3).

Although there are anti-resorptive and anabolic medications available to treat osteoporosis, this disease can´t be completely cured (4). Therefore, physical exercise presents itself as an economical, effective and safe way to prevent the development of osteoporosis (5), presenting itself as a valuable resource in the prevention and treatment of osteoporosis (6).

Physical activity can prevent bone loss (4), improve quality of life and muscle strength (7) and balance, and reduce the risk of falls (8). Although exercise is indicated as part of the treatment for patients with osteoporosis (1), it is necessary to understand how these benefits occur in bone metabolism to better indicate the type, frequency and duration of treatment through physical exercise.

Therefore, long-term studies examining the effect of treatment of osteoporosis with physical exercise is invaluable importance. Thus, in order to better include physical exercises in the treatment of osteoporosis, this study aims to investigate the effects of physical exercise on bone tissue in patients with osteoporosis.

2. Materials and methods

2.1 Protocol and registration

This systematic review was carried out following the premises made by the Preferred Report Items for systematic reviews and meta-analysis guidelines (PRISMA) (9) and a Cochrane handbook for systematic reviews (10). The protocol was elaborated and previously registered in the revision database Reviews and Disclosure Center (PROSPERO) International property registration systematic reviews with a number of records CRD42021224368, updated January 16, 2021.

2.2 Focused question

The focused-question "Does physical exercise reduce the level of bone loss progression in patients with osteoporosis compared to sedentary people?" was formatted. Population (P): patients with osteoporosis; Interventions (I): physical exercise; Control intervention (C): sedentary patients and Measured result (O): reduction in the level of bone loss.

2.3 Search Strategy

According to the Populations, Interventions, Comparison and Outcomes (PICO) principle, the search strategy was constructed. Individual search strategies were designed for the following electronic databases: PubMed, the Cochrane Library database EMBASE, Ovid Medline, Scopus, Web of Science. The electronic databases were searched to identify relevant studies published up to and including January 2021. The searched publications were only considered in last ten years with no restrictions on idiom.

The search strategy contained a combination of controlled predefined Medical Subject Heading (MeSH) terms and free terms using the Boolean operators (i.e., OR, AND), always adapted to the syntax rules of each bibliographic database. A combination of the following terms was used to identify relevant studies: ((exercis*) OR (physical exercis*) AND ((bone tissue) OR (bone)) AND (osteoporos*) AND ((clinical trial*). Additionally, it was also conducted a manual search of bibliographies and reference lists of the included studies to locate any potential unidentified study.

2.4 Selection criteria

Inclusion criteria were used: (a) randomized clinical trials; (b) adults, aged 18 years or older, both genders, without distinction of ethnicity, with osteoporosis; c) therapeutic intervention through physical exercises was included, regardless of the exercise frequency, duration and intensity, combined or not with other therapies or non-surgical medications; (d) articles published in any idiom; and (e) articles published in the last ten years.

2.5 Exclusion criteria

In this study, all references related to (a) case studies, case reports, cross-over studies, studies without a separate control group and preclinical trials; (b) people with

disabilities and pregnancy; (c) treatments that include physical exercises, but which are associated with some surgical treatment; (d) studies without a separate control group; (e) any other study that does not address the use of physical exercise in patients with osteoporosis were excluded. Studies that presented isolated non-relevant data, such as, for example, only weight variation, were not considered.

2.6 Types of outcome measures

In order to evaluate the results, morphometric, biomechanics, radiographic and/or laboratory parameters were considered. The following parameters were adopted: bone mineral density (BMD) (spine, hip or forearm), bone microarchitecture (assessed by high-resolution peripheral quantitative CT (HRpQCT) or bone biopsy), bone turnover markers (carboxy-terminal collagen cross-link (CTX) (bone resorption) or amino-terminal propeptide of type I collagen (P1NP) (bone formation) or calcaneal quantitative ultrasound and biochemical parameters like serum levels of alkaline phosphatase, bone alkaline phosphatase, calcium, and phosphorus.

2.7 Screening methods, data extraction and risk-of-bias assessment

Initially, studies were included based on data from titles and abstract screening. The final selection phase involved the full-text reading by two reviewers (VC, RF) using a predetermined data extraction form to confirm eligibility of each study based on the inclusion and exclusion mentioned criterion. Studies that present isolated non-relevant data were not considered. Titles and/or abstracts of studies were selected through the search strategy that included the construction of the algorithm, the electronic search and the selection criteria. The algorithm included the descriptors or keywords already mentioned. The electronic search took place in the databases already reported and will follow the selection criteria already specified. The full text of potentially eligible studies was retrieved and independently evaluated by two independent reviewers. The Jadad scale was used to assess the methodological quality of the selected studies. Any disagreement between them about the eligibility for specific studies was resolved through discussion with a third reviewer. The Kappa (K) concordance test, also known as the Kappa coefficient, was used to measure the degree of agreement between the evaluators. Values greater than 0.80 were considered for agreement between the evaluators.

A standardized pre-pilot form was used to extract data from the included studies to assess the study's quality and synthesize evidence. The information extracted was included: details of the intervention and control conditions; suggested intervention action mechanisms; information to assess the risk of bias, author and year of publication. Missing data were requested from the study authors.

To evaluate the results of clinical trials, considering the sample number and the follow-up period of the volunteers, in addition to clinical parameters for improving bone loss, radiographic and/or biochemical data, and aspects of gender, age, underlying diseases, and adverse effects and morphometric, histological, radiographic and/or laboratory analyzes were consider. For data about intervention of interest for performance measurements, any exercise, in any medium (aerial or aquatic), using different implements (loads attached to the syrup, stairs, pools, among others), with any duration (daily, weekly or monthly) were considered, in combination with non-surgical therapies and other drugs (combination of drugs).

The criteria used to evaluate the quality were adopted from the checklist CONSORT statement, providing guidelines for the following parameters: (a) sequence generation; (b) allocation concealment method; (c) masking of the examiner; (d) address of incomplete outcome data; and (e) free of selective outcome reporting. The degree of bias was categorized as low risk if all the criteria were met, moderate risk when only one criterion was missing, and high risk if two or more criteria were missing. Potential impact of risk of bias for sample size calculation, patient selection, and reporting was considered for each selected study. The potential risk of bias was considered low if a study provided detailed data on all the parameters. A study was considered to have a moderate risk if it does not provide information about just one of the parameters; however, when a study lacked information about more than two parameters, was designed to have a high risk of bias. Two independent reviewers assessed he eligibility and extraction of the results of the articles. Any disagreements were resolved with third reviewer.

3. Results

3.1 Search Results

A total of 88 studies were identified. Of these, a total of 42 studies were excluded because they did not evaluate physical exercise treatment on bone tissue in

patients with osteoporosis. After reading the 46 selected articles, it was found that 39 of these did not meet the inclusion and/or exclusion criteria because patients did not diagnose with osteoporosis. Seven studies were assessed for eligibility, but two articles were excluded because did not present data of interest for the study (n=2). Thus, five complete articles were included in this systematic review (Figure 1). The Kappa agreement test (K) showed a value of 0.91, which means a perfect agreement level (threshold > 0.8).

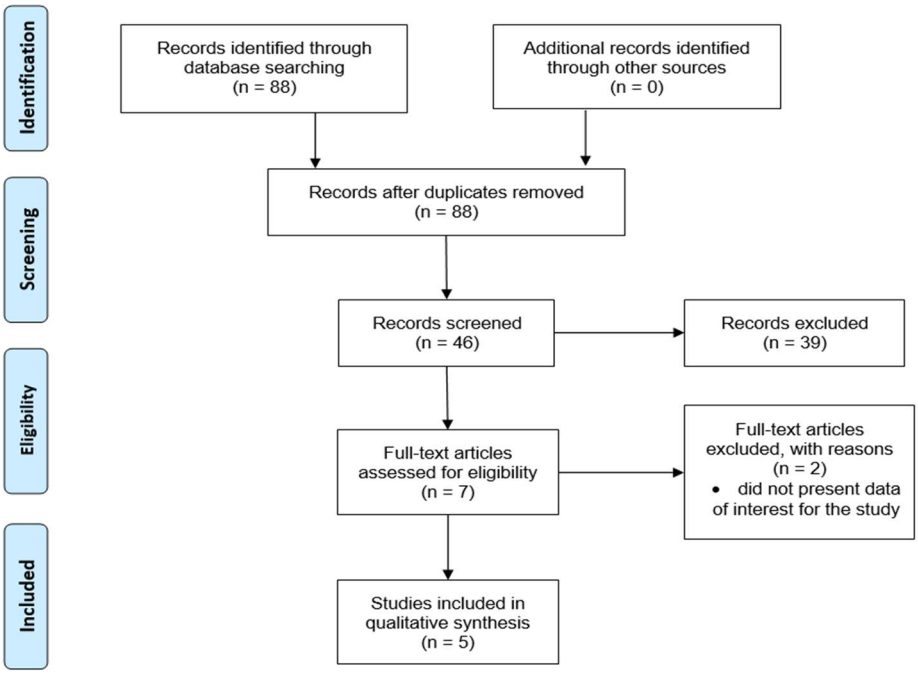


Figure 1. PRISMA flowchart. Studies included for qualitative assessment based upon the Preferred Reporting Items for Systematic Reviews and Meta-analysis guidelines**.**

3.2. Characteristics of the Included Studies

General information and technical features of the included studies are summarized in Table 1. All studies were published in English and were randomized controlled study. These studies were conducted in the Turkey (11), Hungary (12), Serbia (13), India (14), and Iran (15).

One study aimed to investigate the effects of single session of exercise (12). Two studies investigated the effects of exercise training, combined or not with other intervention (11,14). And two others studies inquired different kinds of exercise on osteoporosis treatment (12,13,15).

All studies used group control, and all had comparable demographic characteristics (age, BMI and sex). In all studies, patients were overweight with a BMI between 25.79 and 31.23 kg/m². The studies included women between 40 to 75 years old with postmenopausal osteoporosis (11–15). Two studies included women with osteoporosis and osteopenia, without difference between groups (12,14). Postmenopausal osteoporosis was diagnosed by bone mineral density (BMD), according to densitometry T-score value (≤ − 2, 5) in four studies (11–13,15). One study used BMD measured using quantitative ultrasound densitometry (SOS) with values between 3864 m/s and 3740 m/s (14).

Four studies used three experimental groups with many patients between 19 and 50 per group in three of these (11,12,14), and one study used small number of patients per group: between 8 and 10 (15). One study used two experimental groups with a number of patients between 31 and 37 per group (13).

About the exercise intervention, three studies (12,13,15) used two different kinds of exercise in osteoporosis treatment: resistance and aerobic exercise in one study (12), resistance, aerobic and balance exercise aerobic exercise in one study (13), and aerobic exercise with additional load attached to the body in another study (15). One study used resistance exercise (14), and one study used high-impact exercise (with jumps) (11). One study lasted 24 weeks (11), two studies lasted 12 weeks (13,14), one study lasted six weeks (15), and only one study carried out single session of physical exercises (12).

Table 1. General characteristics of the included studies.

Author, year	Location	Study design	Sample (age and sex)	Experimental groups and patients per group	Osteoporosis diagnosis	Type of osteoporosis	Exercise Intervention	Other Interventions
Sen; Esmaeilzade; Eskiyurt, 2020	Turkey	RCT	N=58 40–65 years women	3 groups •CTRL group (*n* = 20) •WBV training group (*n* = 19) •HIT group (*n* = 19)	BMD T-scores	PM	• HIT: 20–60 min, 3 d/wk, for 24 wk	WBV exercises 20–60 min, 3 days/ week, for 24 weeks
Gombos et al. 2016	Hungary	RCT	N=150 58.5±7.5 years women	3 groups •CTRL group (CG; n = 50) •RE group (RG; n = 50) •W group (WG; n = 50)	BMD T-scores	PM	• RE: 30 min, muscle-strengthening and core stabilization elements, single session • W: moderate intensity (~ 3–6 metabolic equivalents), 100 steps/min for 46 min in outdoors on even ground, single session	---
Filipovi´c et al. 2019	Serbia	RCT	N=68 64.27±5.61 years women	2 groups • CTRL group (CG, n=31) • Ex group (EG; n=37)	BMD T-scores	PM	• RE: muscles of the upper and lower extremities, 3 d/wk, each of 70-min duration, for 12 wk. • W: rapid walking, 3–5 km/h,70% MHR, 5 d/wk, 50 min/d, for 12 wk.	---
Shenoy, Bedi, Sandhu 2013	India	RCT	N=60 45-65 years women	3 groups • CTRL (n = 20) • Soy isolate protein alone (PG; n = 20) • soy isolate protein+RE (PEG; n = 20)	Quantitative ultrasound densitometry (SOS)	PM	• RE: 10 dynamic exercises for both lower and upper limbs, 8–15 repetitions at 40–80% of 1RM, 40-50 min, 4 d/wk, for 12 wk.	40g/day of soy protein oral incorporated into 200–250 ml of milk twice a day.
Roghani et al. 2013	Iran	RCT	N=27 45-65 years women	3 groups • CTRL group (n=10) • AE group (n=8) • WV group (n=9)	BMD T-scores	PM	• AE: 18 sessions of submaximal treadmill walking, 30 min, 3 days/ week, for 6 weeks. • WV: identical to that of the aerobic with weighted vest (4–8 % of body weight), for 6 weeks.	---

RCS: randomized controlled trial; WBV: Whole body vibration; BMD: bone mineral density; MHR: maximum heart rate, RM: repetition maximum; HIT: high-impact training; PM: Postmenopausal; RE: resistance exercise; W: walking; CTRL: control; Ex: exercise; AE: aerobic exercise; WV: weighted-vest

Two studies used another intervention associated with exercise: whole body vibration with the same session duration, weekly frequency and total time to exercise sessions (11), and soy protein supplemented orally, 40g per day incorporated into 200 to 250 ml of milk twice a day (14).

3.3 Quality Assessment

A summary of the quality assessment was adopted from the checklist of the Cochrane Center and the CONSORT (Consolidated Standards of Reporting Trials) statement is shown in Table 2.

Table 2: Parameters evaluated the quality of randomized controlled trials (RCT) according to the Cochrane Center and CONSORT guidelines (RoB 2.0) 38.

Publication	Random Sequence Generation	Allocation Concealment	Blinding of Participants	Blinding of outcomes Assessment	Incomplete Outcome Data Addresses	Selective Reporting	Other Biases	Overall Risk
Sen; Esmaeilzade; Eskiyurt, 2020	(+)	(+)	(+)	(-)	(x)	(+)	(+)	Moderate
Gombos et al. 2016	(-)	(+)	(+)	(-)	(+)	(+)	(+)	Moderate
Filipovi´c et al. 2019	(+)	(+)	(+)	(-)	(+)	(+)	(+)	Low
Shenoy, Bedi, Sandhu, 2013	(-)	(x)	(+)	(-)	(+)	(+)	(+)	High
Roghani et al. 2013	(-)	(-)	(+)	(-)	(x)	(+)	(+)	High

(+): Low bias risk; (-): unclear bias risk; (x): high bias risk

100% of studies with low risk of bias for blinding participants, selective reporting and other biases. The included studies included 40% of the random sequence generation (11,13), 60% demonstrated confidential allocation (11–13), in 100% there is not enough information about the blinding of outcomes assessment (11–15), 60% describe the number of losses of the participants (12–14). Thus, 40% of the studies

had a moderate risk of bias (11,12), 40% a high risk of bias (14,15) and 20% a low risk of bias (15).

3.4 Main outcome variables of studies

The parameters bone mineral density (BMD) were adopted for three studies (11,14). The dual-energy x-ray absorptiometry (DXA) based BMD value of the femoral neck was determined for two studies (11,13). One of these (13) was used lumbar spine T-score too. BMD of left distal radius and midshaft tibia was used in one another study (14).

The biochemical parameters were verified for four studies (12,13,15). The concentrations of markers of bone metabolism carboxy-terminal cross-linked telopeptide of type I collagen (CTX) was collected in three studies (11,12,15) and serum bone-specific alkaline phosphatase (BALP) was measured for two studies (12,15). Indicators for altered bone homeostasis in the serum level of matrix metalloproteinases (MMP-9) and their tissue inhibitors (TIMP-1) were detected in one study (13). The measurement of serum calcium (Ca), phosphorus (P) and total alkaline phosphatase (TALP) was realized for one study (15) and serum osteocalcin (OC) for other study (11).

Three studies evaluated others parameters: functional mobility, fall index, health-related quality of life and depressive symptoms by Quality of Life Questionnaire of the European Foundation for Osteoporosis (QUALEFFO) and Beck Depression Inventory (BDI) (11); isokinetic muscle performance measurements (14); and balance measurement by near tandem stand (NTS) and star-excursion (SE) test (15).

3.5 Primary outcomes: effects of exercise treatment in the bone loss

The characteristics of exercise intervention the studies included are summarized in Table 3. The treatment with exercise associated with another intervention, was able to increase increased significantly the BMD T-score in only one study (11), suggesting an increase in bone formation. In one other study, vibration exercise was able to increased BMD T-score, but not in high impact exercise group (14). Two studies measured the BMD T-score before intervention, but did not after.

The biochemical results of the selected articles indicate that exercise was able to decreased the serum levels of NTX and CTX in two studies, respectively (12,15), indicating that a single session of resistance exercise (12) and aerobic exercise, with

or without weighted vest (15), reduces bone resorption. However, other study did not find changes in serum CTX due to treatment with exercise (11). There was a significant decrease in the enzyme activity of serum MMP-9 and increased in the TIMP-1 inhibitor in the exercised group, which did not occur in the control group (13), reinforcing the results that indicate that exercise reduces bone resorption.

Specific serum bone alkaline phosphatase (BALP) increased due to aerobic exercise and a decrease in the control group in one study (15), and there was no change in these levels in another study (12). Serum calcium (Ca) levels increased with aerobic exercise with weighted vest (15). There was no significant change as a result of treatment with physical exercise in the levels of osteocalcin (OC) (11), sclerostin (SOST) (12), total alkaline phosphatase (TALP) and phosphorus (P) (15).

3.6 Secondary outcomes

High impact exercise was able decreased QUALEFFO-41 scores, and functional mobility test called Timed Up and Go (TUG) scores compared with control group, meaning improvement on categories pain, physical function, social function, general health perception, mental function and functional mobility. There was no significant change in fall index scores (11).

Resistance exercise improved muscle strength parameters for knee and elbow before and post-exercise, but it was not different from the control group (14). Aerobic exercise, with or without weighted-vest, decreased fat mass and increased fat-free mass. Aerobic exercise with weighted-vest increased NTS and SE score and decreased in CG, showing significant improvements in all balance criteria (NTS test and all directions of SE) (15).

Table 3: Characteristics of exercise intervention the studies included (primary outcomes)

Author, year	Bone parameters	Biochemicals parameters	Others	Conclusions
Sen; Esmaeilzade; Eskiyurt, 2020	• VG: BMD increased in L2–L4 (+1.3%, p = 0.005) and femoral neck (+5.0%, p = 0.003) • HG: no significant change in BMD	• VG: OC -31.3% • No significant change in serum CTX levels	• VG and HG: decreased QUALEFFO-41 scores BDI and TUG score. • no significant change in fall index scores.	WBV exercises prevents bone loss, functional mobility, and the HRQoL
Gombos et al. 2016	• BMD T-scores varied between −4.7 and −1.0 • Post-intervention BMD has not been performed	• RG, WG and CG: BALP post-intervention not differ • RG and CG: non-significant in sclerostin • WG: significant increase in sclerostin • RG: decrease in CTX post-intervention c • WG or CG: no change CTX	----	Physical activity can influence bone turnover, causing change in serum concentrations of biochemical markers of bone metabolism and serum SOST levels.
Filipovi´c et al. 2019	• Post-intervention BMD has not been performed	• EG: decrease in the enzyme activity of serum MMP-9 and increased TIMP-1 • CG: no differences	----	Exercise has significant effects on the enzyme activity of serum MMP-9 and TIMP-1 in humans, in vivo.
Shenoy, Bedi, Sandhu 2013	• PG and PEG: BMD increased significantly • CG: there was not change	---	• PG and PEG: improved muscle strength before and after intervention • CG: it was not different	Soy protein supplemented increased in muscle performance and bone health. The addition of resistance exercises enhanced these effects.
Roghani et al. 2013	---	• AG and WVG: BALP increased • CG: BALP decreased and CTX increased • AG and WVG: NTX decreased • WVG: Ca was increased compared to CG • No differences in TALP and P in the three groups	• AG and WVG: Fat mass decreased and NTS score increased • WVG: Fat-free mass increased, and SE values increased • AG: SE values was not significant • CG: decrease in all SE values	Exercise decrease NTX and increase BALP levels. Besides being an effective nonpharmacologic intervention to protect bone integrity exercise improves balance and physical fitness in postmenopausal women with OP.

VG: vibration exercise group; HG: High impact exercise group; WG: Walking group; RG: Resistance group; EG: Exercise group; PG: Protein group; PEG: Protein+exercise group; AG: Aerobic group; WVG: Weighted-vest group; CG: Control group; OC: osteocalcin; CTX: carboxy-terminal cross-linked telopeptide of type I collagen; NTX: N-terminal telopeptide of type 1 collagen; WBV: Whole body vibration; BMD: bone mineral density; SOST: sclerostin, BALP: bone alkaline phosphatase; QUALEFFO: Quality of Life Questionnaire of the European Foundation for Osteoporosis; HRQoL: health-related quality of life; Ca: calcium; TALP: total alkaline phosphatase; P: serum phosphorus; NTS: near tandem stand; SE: star-excursion; OP: Osteoporosis.

4. Discussion

The studies analyzed in this systematic review have shown that physical exercise can reduces bone resorption, decreasing the level of bone loss progression in patients with osteoporosis compared to sedentary people.

The reduction in bone absorption was seen in three studies by decreasing the biomarkers of bone resorption: NTX (15), CTX (12) and MMP-9 (13). To accurately examine bone structure and resistance in living tissue, biochemical markers of bone metabolism (16–18) have been used. These markers are some substances produced during bone remodeling and are divided into two categories: markers of bone resorption, which reflect the activity of osteoclasts, and markers of bone formation, reflecting the activity of osteoblasts (19,20) .

The markers of bone resorption are mostly the products of type 1 collagen degradation and NTX and CTX are included in this group of biomarkers. Thus, the reduction in NTX and CTX levels observed in groups of women with osteoporosis who practiced physical exercise (12,15) indicates a reduction in bone resorption, which means an improvement in osteoporosis since in this disease there is a decrease in formation and an increase in bone resorption with consequent loss of bone mass (21).

The results about reduction in bone absorption are reinforced for a decrease of MMP-9 and increase of its TIMP-1 (13). The association between MMP-1, MMP-2, MMP-3, MMP-9, and MMP-13 with the pathogenesis of osteoporosis has been demonstrated in different studies (22–24). These proteolytic enzymes are responsible for early bone resorption because, before demineralization begins, they degrade the collagen layer of the bone surface (23,24). Increased MMP-9 concentration was correlated with increased bone reabsorption (23) and was negatively correlated with BMD (25). In addition, MMP-9 is inhibited by TIMP-1 (24). Thus, the decrease in MMP-9 and the increase in the TIMP-1 inhibitor indicate that exercise can prevent an accelerated loss of BMD caused by osteoporosis (26).

The markers of bone formation, in its turn, that are the byproducts of collagen synthesis (matrix proteins or osteoblastic enzymes), which showed change in one study: increased values BALP (15). These results corroborate with previous results. But, in other three studies of this systematic review, there was no change in other

markers of bone formation: osteocalcin (11), SOST (12) and total alkaline phosphatase TALP (15).

The response of biochemical markers of bone metabolism to exercise depends on the type and intensity of the exercise performed (27). This may justify the different results on bone metabolism biomarkers because different types, intensity, and duration of exercises were used as a treatment for osteoporosis in the studies included in this systematic review.

The effect of exercise on serum calcium and phosphorus levels was performed in only one study and calcium levels increased only in aerobic exercise with load (15). Thus, there is an inability to assess the effects of exercise on these biochemical parameters in osteoporosis patients who exercised. The same was observed about BMD T-score that increased in only one study (14) because the exercise intervention and in another study only in vibration exercise group (11). Therefore, it is not possible to make any conclusion reliable favorable bone health outcomes in these aspects.

The patients that practiced exercise to treat the multiple aspects of osteoporosis were able to improve on categories pain, physical function, social function, general health perception, mental function and functional mobility (11); muscle strength parameters (14); decreased fat mass and increased fat-free mass and improvements in balance criteria (15). These secondary outcomes were identified in three studies (11,14,15), demonstrating that physical exercise contributes to the improvement of positive aspects about the quality of life of patients.

The strengths of this review are the all groups had comparable demographic characteristics and most of the studies have a high sample size and it did not have large sample losses. In addition, trials are based on many centers' experiences, different countries, thus reducing regional and temporal bias regarding data collection and interpretation.

All the studies evaluated showed favorable results for bone health as a result of physical exercise. However, the parameters assessed differed from one another, hindering more concrete evidence on these aspects. Only three of the reviewed studies examined the effects of bone biomarker levels as a result of physical exercise, of which three showed positive effects on bone health. Studies show the positive relationship between physical exercise and bone mass gain in healthy patients (without

osteoporosis) (28,29) or with low bone mass (7,30). However, few have carried out this investigation in groups of patients diagnosed with osteoporosis (as shown in this review). Studies included in this systematic review presented some bias, which is also a limitation of this systematic review. More research with patients with osteoporosis that aim to investigate effects of physical exercise on bone metabolism is needed.

The final aim of the review was to investigate of effects of physical exercise on bone metabolism of patients with osteoporosis. Unfortunately, the number the studies that investigated the physical exercise in osteoporosis was small, and only five of these were included in this review. Of these, three confirmed a positive association between the decreased levels of markers of bone resorption and physical exercise. In addition, the outcome variables of studies were different. It is not possible to reach any reliable conclusion. Thus, this review can only raise the hypothesis that physical exercise can reduce bone resorption, decreasing the level of bone loss progression, in patients with osteoporosis when compared to sedentary people, helping to support the choice of exercise as part of treatment of patients with osteoporosis.

References

1. Cooper C, Ferrari S. IOF Compendium of Osteoporosis. Int Osteoporos Found [Internet]. 2019;2nd Editio:1–76. Available from: https://www.iofbonehealth.org/compendium-of-osteoporosis

2. Organization WH. Who Scientific Group on the Assessment of Osteoporosis At Primary Health. World Heal Meet Rep Brussels, Belgium, 2004 [Internet]. 2007;May(May 2004):1–13. Available from: http://www.who.int/chp/topics/Osteoporosis.pdf

3. Cohen AJ, Roe FJC. Review of risk factors for osteoporosis with particular reference to a possible aetiological role of dietary salt. Food Chem Toxicol. 2000;38(2–3):237–53.

4. Yuan Y, Chen X, Zhang L, Wu J, Guo J, Zou D, et al. The roles of exercise in bone remodeling and in prevention and treatment of osteoporosis. Prog Biophys Mol Biol [Internet]. 2016;122(2):122–30. Available from: http://dx.doi.org/10.1016/j.pbiomolbio.2015.11.005

5. Santos L, Elliott-Sale KJ, Sale C. Exercise and bone health across the lifespan. Biogerontology. 2017;18(6):931–46.

6. Porto EF, Filoni E. Efeito da atividade física sobre densidade mineral óssea de mulheres pós menopausa Effect of physical activity on bone mineral density in postmenopausal women. 2020;

7. Pasqualini L, Ministrini S, Lombardini R, Bagaglia F, Paltriccia R, Pippi R, et al. Effects of a 3-month weight-bearing and resistance exercise training on circulating osteogenic cells and bone formation markers in postmenopausal women with low bone mass. Osteoporos Int. 2019;30(4):797–806.

8. Madureira MM, Bonfá E, Takayama L, Pereira RMR. A 12-month randomized controlled trial of balance training in elderly women with osteoporosis: Improvement of quality of life. Maturitas. 2010;66(2):206–11.

9. Moher D, Liberati A, Tetzlaff J AD. Itens de relatório preferidos para revisões sistemáticas e meta-análises: a declaração PRISMA. Ann Intern Med. 2009;151:264–9.

10. Noyes J, Booth A, Cargo M, Flemming K, Harden A, Harris J, et al. Cochrane Handbook for Systematic Reviews of Interventions. 2019;(October).

11. Sen EI, Esmaeilzadeh S, Eskiyurt N. Effects of whole-body vibration and high impact exercises on the bone metabolism and functional mobility in postmenopausal women. J Bone Miner Metab [Internet]. 2020;38(3):392–404. Available from: https://doi.org/10.1007/s00774-019-01072-2

12. Gombos GC, Bajsz V, Pék E, Schmidt B, Sió E, Molics B, et al. Direct effects of physical training on markers of bone metabolism and serum sclerostin concentrations in older adults with low bone mass. BMC Musculoskelet Disord. 2016;17(1):1–8.

13. Filipović T, Gopčević K, Dimitrijević S, Hrković M, Backović A, Lazović M. Effects of 12-Week Exercise Program on Enzyme Activity of Serum Matrix Metalloproteinase-9 and Tissue Inhibitor of Metalloproteinase-1 in Female Patients with Postmenopausal Osteoporosis: A Randomized Control Study.

Biomed Res Int. 2020;2020.

14. Shenoy S, Bedi R, Sandhu JS. Effect of Soy Isolate Protein and Resistance Exercises on Muscle Performance and Bone Health of Osteopenic/Osteoporotic Post-Menopausal Women. J Women Aging. 2013;25(2):183–98.

15. Roghani T, Torkaman G, Movasseghe S, Hedayati M, Goosheh B, Bayat N. Effects of short-term aerobic exercise with and without external loading on bone metabolism and balance in postmenopausal women with osteoporosis. Rheumatol Int. 2013;33(2):291–8.

16. Cabral HWS, Andolphi BFG, Ferreira BVC, Alves DCF, Morelato RL, Chambo Filho A, et al. The use of biomarkers in clinical osteoporosis. Rev Assoc Med Bras. 2016;62(4):368–76.

17. Zhang P, Pan L, Luo Z, Zhao H, Cai S. Interrelationship of Circulating Matrix Metalloproteinase-9, TNF-α, and OPG/RANK/RANKL Systems in COPD Patients with Osteoporosis. COPD J Chronic Obstr Pulm Dis [Internet]. 2013 Dec 11;10(6):650–6. Available from: https://www.tandfonline.com/doi/full/10.3109/15412555.2013.813928

18. Lv M, Cui C, Chen P, Li Z. Identification of osteoporosis markers through bioinformatic functional analysis of serum proteome. Medicine (Baltimore). 2020;99(39):e22172.

19. Milat F, Ng KW. Is Wnt signalling the final common pathway leading to bone formation? Mol Cell Endocrinol. 2009;310(1–2):52–62.

20. Bolton C, Stone M, Edwards P, Duckers J, Evans W, Shale D. Circulating matrix metalloproteinase-9 and osteoporosis in patients with chronic obstructive pulmonary disease. Chron Respir Dis. 2009;6(2):81–7.

21. El-Gazzar A, Högler W. Mechanisms of bone fragility: From osteogenesis imperfecta to secondary osteoporosis. Int J Mol Sci. 2021;22(2):1–18.

22. Sun B, Sun J, Han X, Liu H, Li J, Du J, et al. Immunolocalization ofMMP2, 9

and 13 in prednisolone induced osteoporosis in mice. Histol Histopathol. 2016;31:647–56.

23. Zheng X, Zhang Y, Guo S, Zhang W, Wang J, Lin Y. Dynamic expression of matrix metalloproteinases 2, 9 and 13 in ovariectomy-induced osteoporosis rats. Exp erapeutic Med. 2018;16(3):1807–13.

24. Grigoryan A. Serum concentrations of matrix metalloproteinase-9, -13 and TIMP-1 in an ovariectomized wistar rat model of osteoporosis. Arch Balk Med Union. 2017;52(4):391–6.

25. Andrea KK, Kurt H. Extracellular matrix networks in bone remodeling. Int J Biochem Cell Biol. 2015;65:20–31.

26. MB D, J B, C S. Osteoporosis: the increasing role of the orthopaedist. Iowa Orthop J. 1999;19:43–52.

27. Banfi G, LOMBARDI G, Colombini A, Lippi G. Bone Metabolism Markers in Sports This material is the copyright of the original publisher . Unauthorised copying and distribution is prohibited . This material is the copyright of the original publisher . Unauthorised copying and distribution. 2010;40(8):697–714.

28. Multanen J, Rantalainen T, Kautiainen H, Ahola R, Jämsä T, Nieminen MT, et al. Multanen et al., 2017. Osteoporos Int. 2017;28(1323–1333).

29. Tønnesen R, Schwarz P, Hovind PH, Jensen LT. Physical exercise associated with improved BMD independently of sex and vitamin D levels in young adults. Eur J Appl Physiol. 2016;116(7):1297–304.

30. Watson SL, Weeks BK, Weis LJ, Harding AT, Horan SA, Beck BR. High-intensity exercise did not cause vertebral fractures and improves thoracic kyphosis in postmenopausal women with low to very low bone mass: the LIFTMOR trial. Osteoporos Int. 2019;30(5):957–64.

Capítulo 3

Artigo 3

Kefir Treatment Enhanced Skeletal Response to Climb Exercise in Rats submitted to Glucocorticoid Induced-Osteoporosis

Raquel Felipe Vasconcelos[1,2], Vanessa Costa[1,2], Bruno Araujo[3], Thays Allane Cordeiro Maia[2], Romero Dias[2], Lorena Vasconcelos[1,2], Helson Silveira[1,2], Barbara Carneiro[1,2], Diego Thiers[1,2], Fabio Wildson Gurgel Costa[4], Lúcio Mitsuo Kurita[4], Alejandro Pedro Ayala[3], Renata Leitão[1,5], Delane Gondim[1,2,5], Karuza Alves[1,2,5], Paula Goes[1,2,6*]

[1]Post-graduation Program in Morphofunctional Science, Department of Morphology, School of Medicine, Federal University of Ceará, Fortaleza, Brazil

[2] Nucleus of Study and Research in Pain, Inflammation and Osteoimmunology (NEPDIO), Medical School, Federal University of Ceará, Fortaleza, Brazil

[3]Post-graduation Program in Physics, Department of Physics, Federal University of Ceará, Fortaleza, Brazil

[4]Department of Dental Clinic, Faculty of Pharmacy, Dentistry and Nursing, Federal University of Ceará, Fortaleza, Brazil

[5]Department of Morphology, Medical School, Federal University of Ceará, Fortaleza, Brazil

[6]Department of Pathology and Legal Medicine, Medical School, Federal University of Ceará, Fortaleza, Brazil

*** Correspondence:**
Corresponding Author
paulagoes@ufc.br

Abstract

Introduction: Glucocorticoid-induced osteoporosis (GIO) has emerged as a challenge after long-term administration of glucocorticoids (GC). Although some candidates for the treatment of GIO have been studied, there is still a lack of studies that support these guidelines. Exercise has been pointed out as an important non-pharmacological option, while medications have been used with the objective of modulating bone remodeling despite their important adverse effects. Therefore, the use of a natural product, such as Kefir milk, can be an alternative to minimize these side effects. In this context, our study aimed to investigate the combined effect of physical exercise and Kefir milk on bone loss in rats submitted to the GIO model.

Methods: For this study, it was used male Wistar rats divided into 5 groups: normal (N), and subjected to GIO which was subdivided into 4 groups according to the treatment: control (C), Kefir (K), Exercise (Ex), and Exercise+Kefir (ExK). GIO was induced by dexamethasone (7 mg/kg – i.m.) administered 1x/wk, during 5 wk. Kefir milk was administered orally 1x/d, 7d/wk, at 0.7 ml/animal/d for 16 wk. The climb exercise with load was performed 1x/day, 3d/wk, with progressive loads from 20% to 80% of the maximum individual load for 16 wk. After euthanasia, femur was collected for morphological, radiographic, biomechanical, histopathological, histometric, confocal and scanning electron microscopy analyses. Microarchitecture of femurs was evaluated by micro-CT, and micro-Raman spectroscopy was used to analyze its bone composition.

Results: GIO markedly affected bone, reducing trabecular bone volume density (BV/TV) (-35%), trabecular thickness (Tb.Th) (-33%), mineral content of femur (-26%) as well as the content of bone collagen (-56%) while increased bone tissue roughness. Bone strength and its biomechanical properties given by flexural strength (-81%), fracture load (-80%) and the number of osteocytes (-84%) were lowered after GIO. GCs directly reduced osteoblast number and function while increased osteoclast number, altering bone remodeling ($p<0.05$). After a 16-week intervention, when compared to GIO, the association of ExK significantly improved bone microarchitecture and quality, marked by an increase in fractal dimension (+38%), cortical volume (34%), BV/TV (34%), Tb.Th (33%), mineral content and collagen maturity, while reduced trabecular separation (34%), and bone roughness. The association of exercise+Kefir ameliorates bone strength and biomechanics ($p<0.05$). Besides that, exercise+Kefir stimulated bone formation and modulated bone remodeling ($p<0.05$).

Conclusion: Our results showed that Kefir treatment enhanced skeletal response to climb exercise in rats with glucocorticoid induced-osteoporosis. Therefore, these therapies may be an interesting tool to improve bone microarchitecture and quality, bone strength and biomechanical properties, and stimulated bone formation.

Keywords: Kefir; exercise; osteoporosis; glucocorticoid; bone loss

1 Introduction

Glucocorticoids (GCs) have been used as a treatment for chronic diseases, due to their anti-inflammatory and immunomodulating effects, and antiproliferative properties (1). However, the prolonged use of GCs has been considered a crucial risk factor for osteoporosis and subconsequent osteoporotic fracture, which is an important health problem (2). The incidence of fractures varies from 30% to 50% in individuals who use GCs for more than three months (3).

The pathophysiology of glucocorticoid induced osteoporosis is complex. This occurs due to their effects on both hematopoietic and mesenchymal-derived bone cells. GCs cause initial increases in osteoclast activity followed by a delayed and sustained reduction in osteoblast action, reducing osteogenesis leading to decreased osteoblast maturation, life expectancy and function (3,4). GCs also promote apoptosis of osteoblasts and osteocytes (5). These changes can eventually cause bone loss and an increased risk of fractures (3). However, at GIO, the risk of increased fracture is not partly related to loss of bone density (6).It is important to notice that subjects treated with GCs often present fracture in bones with high density when compared to postmenopausal or senile osteoporosis (3,7). Thus, the increased risk of fracture is not fully evaluated by bone mineral density measurements, as it is also related to changes in bone quality (8) that included bone morphology, microarchitecture, and material properties (9).

In 2017, the American College of Rheumatology revised their guidelines and indicated pharmacological and non-pharmacological options for prevention and treatment of GIO. Non-pharmacologic treatments include adequate intake of calcium and vitamin D and exercise. Regular weight-bearing exercise with an emphasis on strength training may prevent loss of muscle mass and strength, preventing falls, an important triggering factor that leds to bone fracture in individuals under GCs therapy. Despite the benefits of exercise, studies that can confirm the utility of these non-pharmacological recommendations are lacking (10). Among the pharmacological treatments, bisphosphonates, hPTH and denosumab have been pointed out. The use of anti-resorptive agents in GCs-treated subjects has been shown to prevent additional bone loss and improve bone strength, without reversing changes in the bone structure induced by glucocorticoid treatment. In addition, considering that these drugs have

also been related to important adverse effects (3,10), the use of natural products has gained prominence in terms of prevention and treatment of diseases (11,12).

Kefir is a probiotic derivate from the natural microbiota present in Kefir grains or groats (13). It presents calcium levels similar to that of milk after processing, is therefore characterized as a good source of this mineral (14). This probiotic is also considered an easily accessible and affordable fermented milk (8,13). Kefir has already shown promising effects in animal models of osteoporosis induced by ovariectomy (12,15) as well as in clinical studies over postmenopausal osteoporosis. These findings suggest that administration of Kefir has an anabolic activity, increasing osteocalcin levels, a bone formation marker, which is strongly associated with changes in total hip and femoral neck bone mineral density (BMD) (16). Nevertheless, to the best of our knowledge, there is no study evaluating the effect of Kefir milk on glucocorticoid-induced osteoporosis. Taking together, the study aimed to evaluate the effect of the combination between Kefir milk and physical exercises on bone loss caused by glucocorticoid-induced osteoporosis in rats. We hypothesize that the treatments with exercise and Kefir would be able to reduce the deleterious effects of GCs in the bone tissue and that the associated treatment with exercise and Kefir would be as more efficient as the isolated treatments.

2 Materials and Methods

2.1 Animals and study design

This study started only after approval by the Institutional Ethics Committee for Animal Research from the Federal University of Ceará (UFC) (protocol number 137/2017). Sixty male Wistar rats (Rattus norvegicus) (12 weeks old, ±200g) from our own facility, were used in this study. Throughout the whole experiment, the animals were kept in cages with temperature-controlled rooms, food, free water, and with a 12-h light-dark cycle.

To determine the sample size, it was performed a power calculation. The sample size was determined to provide 80% power to recognize a significant difference of 20% among groups and the standard deviation of 15% with a 95% confidence interval ($p = 0.05$), considering the changes in bone microarchitecture parameters,

identify by micro-computed microtomography (micro-CT) analysis, as the primary outcome variable. Therefore, a sample size of 6 rats per group was required.

After two weeks of acclimation to the laboratory environment, 60 animals were randomly divided, in a blind manner, into two experimental main groups: Normal (N), and glucocorticoid–induced osteoporosis (GIO). Animals with GIO were then subdivided into Control (C), Kefir (K), Exercise (Ex), and Exercise+Kefir (ExK) (n=6 animals per group). Two experiments were performed, both lasting 22 weeks. Initially, GIO was induced by the administration of dexamethasone (Decadron® - 4 mg/ml – i.m.) at 7 mg/kg of body weight, 1x/wk, during 5 wk. Animals from Normal group received 0.9% saline solution in the same regimen (17,18).

At week 6, resistance climbing exercises were introduced according to the groups (19). Initially, the animals were subjected to an adaptation, in which they were encouraged to climb the ladder without any kind of painful stimulus. On the first day of week 7, a maximum load test was performed (19). Then, exercise was performed 3x/wk, with one rest day between the exercise days, during 120 days. The exercise started with 20% of the maximum load and the load was increased by 10% per week to reach the maximum of 80% at week 13. From weeks 14 to 21, the load of 80% was maintained. Maximum load tests took place monthly (at weeks 7, 11, 15 and 19) to readjust the load. Each exercise session consisted of 4 sets of 5 climbs and a 2-minute rest period between each set. The ascent time of climbing exercise of each animal was measured. The timer was started when the animal was positioned at the base of the stairs and stopped when the hind legs of the animal reached the base of the house at the top of the stairs (Figure 1).

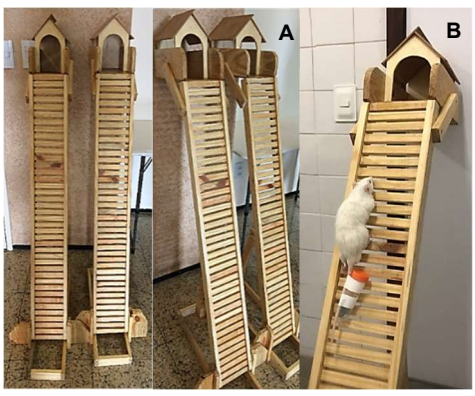


FIGURE 1 | Ladder used in the resistance exercise treatment (A) and resisted climbing exercise being performed by the animal (B). Source: author's personal file

At week 6, the oral treatment with Kefir was initiated according to the groups. Kefir particles (Kefir grains), donated by the UFC Dairy Laboratory (Fortaleza, Ceará, Brazil), were prepared according to Urdaneta et al. (2007) to obtain Kefir milk. The preparation process was repeated daily throughout 16 weeks of the experimental period. Kefir was administered at 0.7ml/animal/day by gavage daily (20). Animals from groups N, C and Ex received the same volume of saline solution.

Exercise and Kefir administration were performed until week 21. All animals were euthanized, at week 22, 72 hours after the last exercise session, using overdose anesthetics (ketamine and xylazine administered intraperitoneally). All animals were weighed weekly. Both femurs were excised and dissecated for posterior analysis.

2.2 Morphometry

After euthanasia, bone morphometry of the animals was performed to identify any macroscopic differences. All right femurs free of muscular, capsular and tendon structures in all their extension were measured and weighed. The length measurement was performed with a precision pachymeter, taking as parameters the large trochanter and the lateral condyle of the femurs. The weight of each femur was acquired using a precision analytical balance. The ratio of the femur weight/body weight of each animal was calculated.

2.3 Radiographic images

The same right femurs were submitted to radiography in order to see the global structures of cortical and trabecular bones at anatomical sites of proximal-metaphysis, diaphysis, distal-metaphysis, and femoral neck. A single investigator obtained all images. The images were acquired and processed using the Express system (Instrumentarium Dental Inc. Milwaukee, WI - U.S.A) to estimate fractal dimension through the use of TACT1 workbench software (Verity Software Systems, Winston-Salem, NC, USA).The region of interest (ROI) was selected, analyzed and fractal dimensions was calculated according to Pornprasertsuk et al (21). The data were expressed as mean±S.E.M.

2.4 Micro-computed tomography analysis

The left femurs were scanned using a high-resolution Micro-CT system (Skyscan 1172®; Bruker, Belgium). The scanning parameters were adjusted to 50kVp, 800μA, 180º rotation with 0.8º rotation angle, 1021x1300 pixels and 14μm3 voxel. A 0.5mm aluminum filter was used to minimize the beam hardening effects and reduce noise in the images. The Data Viewer1 software (version 1.5.0, Bruker, Kontich, Belgium) was used to reconstruct the images with an isotropic voxel size of 14μm^3. The images were manually registered using the MeVisLab 2.8.1 platform (MeVis Medical Solutions Ag, Germany) and then automatically registered using the software (MTM Scaffold, KU Leuven, Belgium). The CTanalyzer® software (Bruker, Belgium) was used for image segmentation and the calculation of three-dimensional morphometric parameters. The volume of interest (VOI) of the femur was delimited including the trabecular bone of the region of the neck of the femur (metaphysis) and cortical bone of femoral diaphysis. The following parameters were evaluated: cortical volume (Ct.BV), cortical thickness (Ct.Th), trabecular bone volume density (BV/TV), trabecular number (Tb.N), separation (Tb.Sp) and thickness (Tb.Th) (24,25). Data were expressed as mean±S.E.M.

2.5 Analysis of biomechanical properties

After radiographic, the same right femurs were used to three-point flexural strength biomechanical test that was done in a universal testing machine (3345, Instron®, Massachusetts, USA) (22). The test applied 500N load module with speed 3 mm. s^{-1} at the central point of the femoral diaphysis, continuously until the moment of the fracture. Values were selected every 0.02s and automatically saved. Based on the recorded load-deformation curves, the biomechanical parameters were calculated: maximum displacement, fracture load, stiffness, and energy absorption were obtained based on the load–deformation curve (23), using Bluehill 2 v2.6 (Instron Corp., USA). The data were expressed as mean±S.E.M.

2.6 Analysis of bone composition by micro-Raman spectroscopy

In another set of experiments, after euthanasia, bone samples of right femur were sectioned in the femoral neck (area of interest), measuring 4mm from the lesser trochanter, using a precision metallographic cutter (Isomet 4000, Buehler®, Uzwil,

Switzerland) and diamond disc, under irrigation with distilled water and at low speed (150RPM). The sample were immediately analyzed by using a micro-Raman spectrometer (Labram HR800, Horiba Jobin Yvon, Paris, France) coupled to a confocal microscope (model BX41, manufactured by Horiba Jobin Yvon) operating with an objective Olympus Mplan N 10x with 10x magnification with 0.25 numerical aperture and 10.6 mm of work distance. The Raman spectra were obtained with excitation at 785 nm in the range of 750 to 2000 cm^{-1} with 60 s of analysis and 3 accumulations per region, and the laser power on sample surface was lower than 5 mW.

For the standardization, the pieces were analyzed in three distinct points distant from each other by 500 μm using the spinal canal as a reference. The spatial distribution of the organic and inorganic components was determined by the relative intensities corresponding to the Raman peaks. The Raman bands selected for evaluation were phosphate hydroxyapatite (HA) (957-962 cm^{-1}) and carbonated HA (1050-1100 cm^{-1}) obtained from the inorganic component; and amide I (1620-1700 cm^{-1} and amide III (1215-1300 cm^{-1}) attributed to organic component of the matrix bone (26,27).

The data were obtained by using the LabSpec 5 software data acquisition command system (Horiba, Jobin Yvon, Paris, France). For a better understanding ratios of the band were calculated: 1) mineral/matrix ratio (MTMR) (phosphate HA/amide III) which is a measure of the amount of mineralization in bone and provides information on bone tissue compositions; 2) carbonated HA/phosphate HA ratio (CTPR) that quantify the extent of type B carbonate substitution, meaning the greater the carbonate replacement, the more mature the bone composition because increase of the phosphate to carbonate ratio indicate accelerated loss of carbonate (26,28); 3) mineral maturity/crystallinity (~1030 cm^{-1}/~1020 cm^{-1}) that is a complex outcome that reflects length and thickness of crystal and chemical composition and structural order (29), which is inversely correlated with carbonate content and increases with tissue age (30); 4) collagen maturity (pyridinoline/ dehydro-dihydroxylysinonorleucine; 1660 cm^{-1}/1690 cm^{-1}) that measures the secondary structure of the collagen fibers, represented by underlying subbands in the amide I peak (29); and, 5) HA carbonate/amide I (~1070 cm^{-1}/~1667 cm^{-1}) for remodeling evaluation (31). All spectra were acquired on the same day and under environmental conditions to avoid optical misalignment and changes in laser power. The data were expressed as mean±S.E.M.

2.7 Scanning electron microscopy (SEM) of bone surface

After Raman analysis, the same bone samples were fixed in Karnovisky for at least 6h and then kept in a cacodylate buffer. The femur was cut with a diamond blade cutter to obtain a femoral neck fragment (0.5 × 0.2 cm fragment and 0.5 mm thick). The fragment was placed in an Eppendorf tube and left in the desiccator for 24 h for drying. The fragments were assembled into stubs for metallization with gold powder (Quorum Metallist QT150ES, Quorum Technologies, Laughton, England) for scanning electron microscopy (MEV inspect-50, FEI, Hillsboro, OR, USA) (32). The image was obtained with a 400x magnification and the roughness was verified using an ImageJ® plugin Surface plot 3D (33).

2.8 Histopathological analysis

After euthanasia, the proximal epiphysis of the left femur from each animal was collected for histopathological evaluation. Each femur was fixed in 10% formaldehyde, demineralized with 10% EDTA in a period of six to eight weeks. Then, the proximal epiphysis of the left femur was cut at a 4 µm section through the longitudinal axis. Subsequently, the specimens the femurs were included in paraffin. Paraffin was cut into sections 5 µm thick and prepared for hematoxylin and eosin staining.

One examiner blinded to the groups performed the histopathological evaluations, and the averages of the counts were obtained. Three histological sections from each animal were analyzed in at least five fields of HE stained slide (n = 6), located in the femoral neck and were captured at ×20 and ×40 magnifications. The number of osteoblasts (N.Ob/B.Pm), osteoclasts (N.Oc/B.Pm) and osteocytes for bone area (N.Oct/B.Ar) were evaluated (34). The histological parameters were presented as mean ± S.E.M. The cell account was performed using the Image J® software (NIH, Bethesda, MD, USA) using the cell contain command.

2.9 Analysis of percentage of collagen bone filling by confocal microscopy

As collagen is a structural protein that presents a natural phenomenon of autofluorescence (35), the same slides used for the histopathological study were analyzed by confocal microscope LSM 710 (Zeiss, Jena, Thuringia, Germany) using the manufacturer's software (Zen 2.1 lite black, 64-bit version, 758 MB, Zeiss, Jena,

Thuringia, Germany). The images were obtained using a 488 nm wavelength laser and FITC-green fluorescence emission channel (32).

The image was obtained using a 20x magnification and the percentage of collagen bone filling in the femoral neck region was measured, using the image of the entire length of the slide. To measure the percentage of collagen bone filling, the ImageJ® Software was used (36). The data were expressed as mean±S.E.M.

2.10 Statistical analysis

To test data normality, the Shapiro Wilk test will be used. Parametric data was presented as means ± standard error of the mean (SEM), using the Analysis of Variance (ANOVA) tests followed by the Bonferroni test. The non-parametric data was presented as median (variation) using Kruskal-Wallis test followed by the Dunn's test. The t-Student Test was used for ascent time and maximum load lifted analyses. For all analyzes, $p < 0.05$ was considered statistically significant. Statistical analyzes were performed, and charts were constructed using the GraphPad Prism 6 program for Windows.

3 Results

3.1 Exercise and Kefir improved bone microarchitecture

At day 150, compared to normal animals, the fractal dimension of femurs was 35% lower in GC-treated animals (Figure 2A and H). GIO mainly affected the trabecular bone, reducing BV/TV by 35% (Figure 2D) as well as trabecular thickness (-33%) (Figure 2E). GC increased the space between trabecula (+21%) (Figure 2G) ($p<0.05$). GIO did not induce change on either cortical bone (Figure 2B and 2C) or trabecular number (Figure 1F). Animals submitted to GIO showed significant reduction on the mineral content of femur (-26%), without change on either mineral maturity/crystallinity or carbonated HA/phosphate HA ratio (CTPR) (Table 1).

After 16-week intervention, only the association of exercise+Kefir significantly increased the fractal dimension (+38%) compared to GIO (Figure 2A and 2H). Exercise+Kefir improved cortical volume (34%) compared to Ex group (Figure 2B), as well as BV/TV (34%) (Figure 1D), Tb.Th (33%) (Figure 2H) and Tb.N (9% compared to K) (Figure 2F) while reduced Tb.Sp (34%), when compared to GIO (Figure 2G), as

seen on micro CT analyses. Exercise and Kefir, individually, were not able to reverse the effects of GIO on trabecular bone (Figures 2B-H). Exercise+Kefir significantly increased mineral/matrix ratio ($p<0.05$) (Table 1). Neither GIO nor the treatments changed the macroscopic parameters of femur length or weight. Taken together, these results showed that Kefir and exercise, together, improved bone microarchitecture.

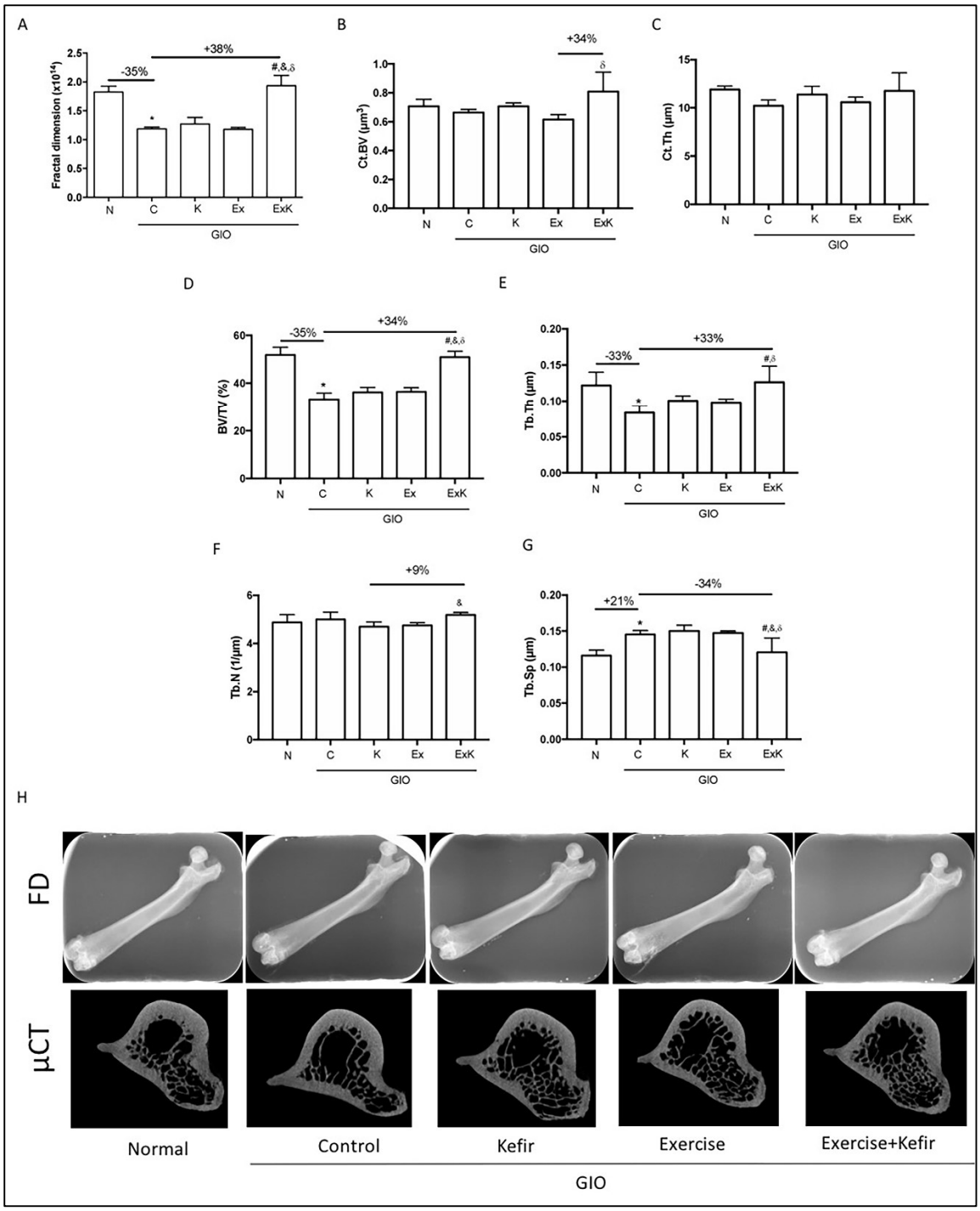


FIGURE 2 | Exercise and Kefir improved bone microarchitecture of rat femur with GIO. (A) fractal dimension; (B) cortical volume (Ct.BV); (C) cortical thickness (Ct.Th); (D) trabecular bone volume per total volume (BV/TV), (E) trabecular thickness (Tb.Th); (F) trabecular number (Tb.N); (G) trabecular separation (Tb.Sp) of femur; and (H) radiographic imagens of femur, representing the fractal dimension (FD) and representative reconstruction of trabecular bone of the region of the neck of the femur by micro-computed tomography (µCT). Data represent the mean±SEM of six animals per group. Statistical analyses were performed by ANOVA followed by the Bonferroni test. *P<0.05 vs. normal; $^{\#}$P<0.05 vs. control; $^{\&}$P<0.05 vs. Kefir; $^{\delta}$P<0.05 vs. exercise.

Table 1. Effect of exercise and Kefir in Raman analysis of rat femur with GIO

Parameters / Groups	N	C	K	Ex	ExK
MTMR	1.64±0.07	1.24±0.03*	1.31±0.03	1.42±0.03	1.46±0.02$^{\#}$
CTPR	0.84±0.01	0.69±0.04	0.77±0.59	0.88±0.61	0.91±0.81
Mineral Maturity	0.99±0.00	1.01±0.00	1.043±0.03	0.97±0.01	1.00±0.00
Collagen Maturity	0.74±0.17	0.39±0.12*	1.14±0.83	1.01±0.92	1.14±0.45$^{\#}$
Bone remodeling	1.16±0.01	1.69±0.16*	1.36±0.13	1.02±0.08$^{\#}$	1.15±0.87$^{\#}$

Data are reported as ratios of the band and represent the mean ± SEM of six animals per group. Statistical analyses were performed by ANOVA followed by the Bonferroni test. *P<0.05 vs. normal; $^{\#}$P<0.05 vs. control

MTMR= Matrix to Mineral Ratio; CTPR= Carbonate to Phosphate Ratio; N= Normal; C= Control; K= Kefir; Ex= Exercise; ExK= Exercise+Kefir

3.2 Exercise and Kefir ameliorated bone strength and quality

GIO significantly decreased the flexural strength, by 81%, as well as the fracture load in 80% (Figures 3A and 3B). The content of bone collagen was reduced by 56% (Figure 3C and 3D) and the remaining collagen showed less maturity (Table 1) after GIO ($p<0.05$). GCs also significantly reduced the number of osteocytes, by 84% (Figure 3G).

Both treatments used alone significantly enhanced bone biomechanical properties, but the associated therapy, using exercise+Kefir, showed a greater improvement, by 84% in flexural strength and 80% in fracture load (Figures 3A and 3B). Kefir and Exercise administered isolated or in association increased the percentage of collagen bone filling after GIO ($p<0.05$) (Figure 3C and 3D), but only exercise+Kefir improved collagen maturity ($p<0.05$) (Table 1). The exercise itself increased the number of osteocytes ($p<0.05$), also seen on Exercise+Kefir group (+88%) on this parameter (Figure 3G). Corroborating our data, it was found that the time spent for climbing decreased by 41% in the Ex group and by 40% in the ExK group, while the load lifted by the animals during a climb increased by 32% in the Ex group and 56% in the ExK group (Figures 3E and 3F). This demonstrates that even lifting progressively heavier loads the animals were able to perform the exercise in shorter time. Together, these findings suggest that the combined treatments contribute to bone strength and greater biomechanical properties.

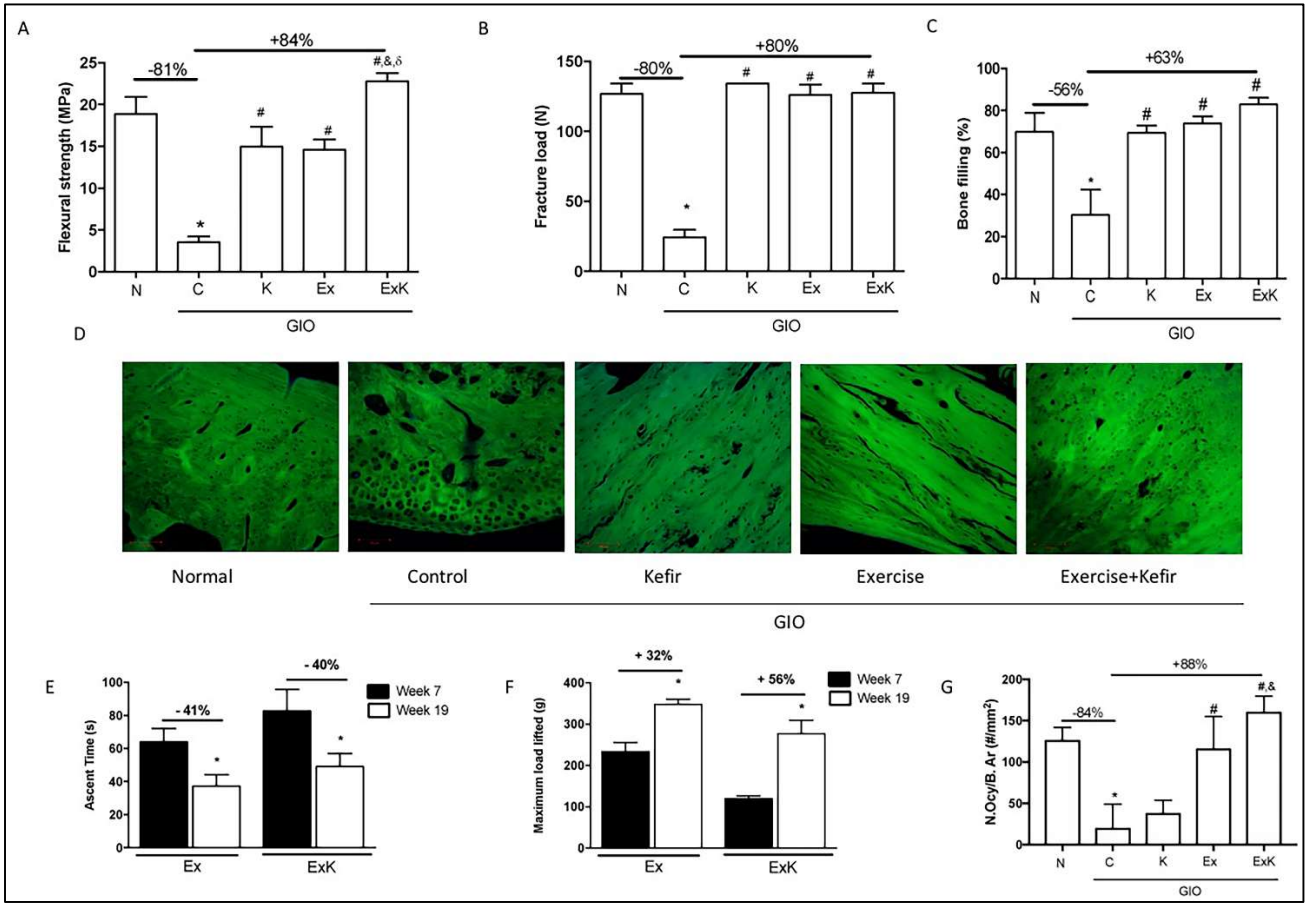


FIGURE 3 | Exercise and Kefir ameliorated bone strength and quality of rat femur with GIO. (A) flexural strength (Mpa); (B) fracture load (N); (C) the percentage of collagen bone filling; (D) representative imagens in Confocal of percentage of collagen bone filling in femur; (E) ascent time of climb exercise; (F) maximum load lifted during climb exercise; and (G) number of osteocytes (N.Ocy/B.Ar). Data represent the mean±SEM of six animals per group. Statistical analyses were performed by ANOVA followed by the Bonferroni test. *P < 0.05 vs. normal; $^{\#}$P<0.05 vs. control; $^{\&}$P<0.05 vs. Kefir; $^{\delta}$P < 0.05 vs. exercise. For ascent time and maximum load lifted analyses t-Student Test was used. *P < 0.05 week 7 vs. week 19.

3.3 Exercise and Kefir stimulated bone formation

Glucocorticoids directly affect osteoblast, as seen by the 5-fold reduction in the number of this cell per bone perimeter (Figure 4A and 4C). Meanwhile, GIO increased in 80% the number of osteoclasts (Figure 3B). SEM analysis revealed that GIO increased bone tissue roughness on femur (Figure 5). A significant increase in bone remodeling was given by HA carbonate/amide I ratio from Raman spectrometry (Table 1).

Exercise solo and exercise+Kefir were able to reverse the lower on osteoblast number (Figure 4A and 4C) and decreased significantly bone remodeling (Table 1). All treatments reduced the number of osteoclast ($p<0.05$) (Figure 4B). While Kefir and the association exercise+Kefir reduced bone roughness (Figure 5).

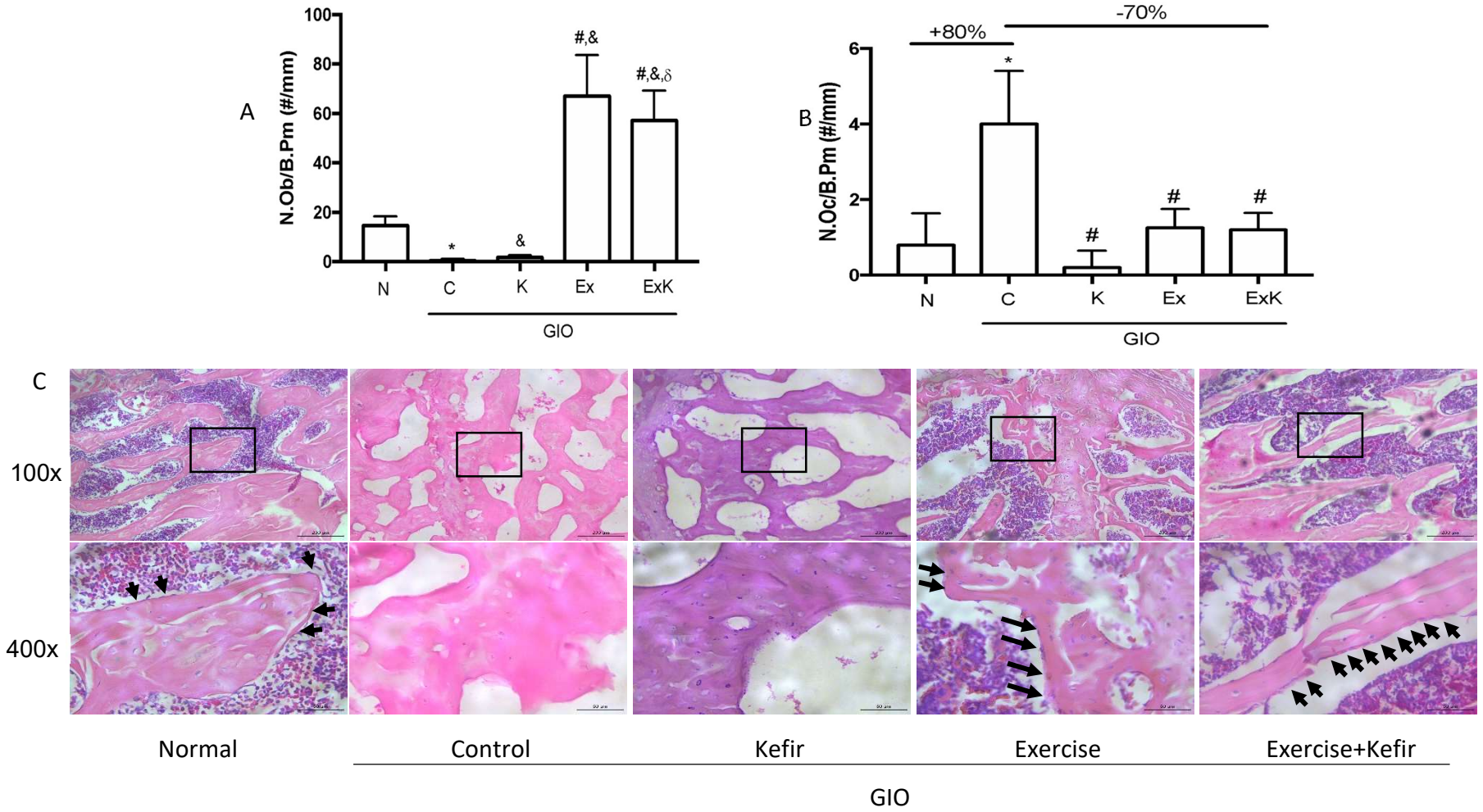


FIGURE 4 | Exercise and Kefir stimulated bone formation of rat femur with GIO. (A) number of osteoblasts (N.Ob./B.Pm.); (B) number of osteoclasts (N.Oc./B.Pm.), and (C) representative imagens in H&E staining of cell count in femur in 10x and 40x magnifications. Data represent the mean±SEM of six animals per group. Statistical analyses were performed by ANOVA followed by the Bonferroni test. *P<0.05 vs. normal; $^{\#}$P < 0.05 vs. control; $^{\&}$P<0.05 vs. Kefir; $^{\delta}$P<0.05 vs. exercise.

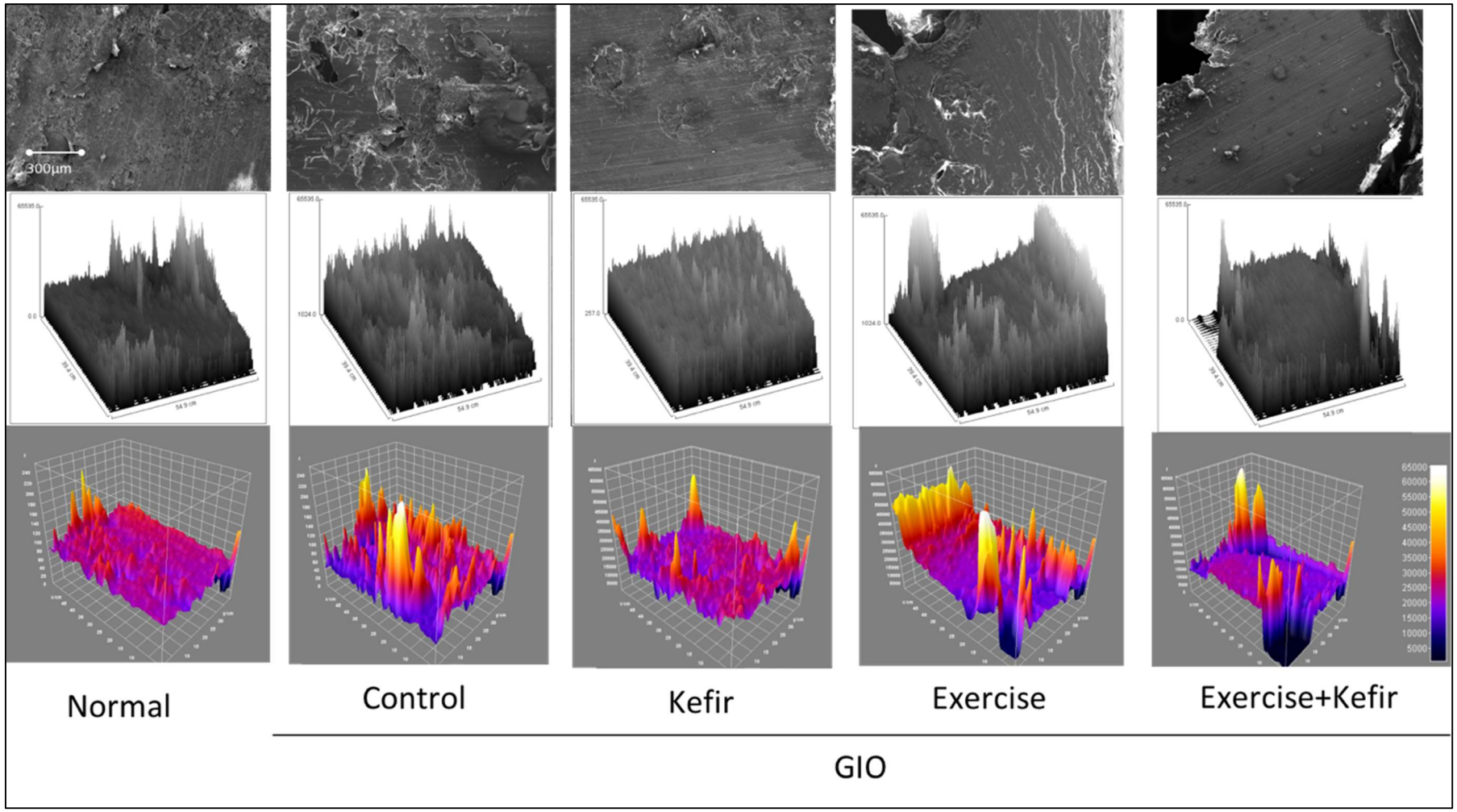


FIGURE 5 | Exercise and Kefir reduced bone roughness of rat femur with GIO. The image was obtained with a 400x magnification and the roughness was verified using an ImageJ® plugin Surface plot 3D. Microtopographic images that allow the visualization of recesses and degree of relief of femur samples. Images elaborated both in gray scale and in varied tones that allow the visualization of details of the bone surface. The greater the color variation in the images, the more irregular the surface is, and the more homogeneous, the greater the smoothness of the surface.

4 Discussion

In this study, we demonstrate that the GIO model caused a negative impact on bone tissue, mainly in trabecular bone. GCs changed bone microarchitecture, decreasing its mineral and collagen content, its biomechanical properties, as well as the number of osteocytes and osteoblasts. In addition, it was seen an increase on bone roughness and on osteoclast number. Together, these findings indicate that GIO model corroborate other pre-clinical studies and can reproduce the main aspects of glucocorticoid-induced osteoporosis in human (37–39). Meanwhile, the combined treatment using exercise+Kefir reversed many of the deleterious effects of GC on bone. The association ExK improved microarchitecture and quality of femur, ameliorated bone strength and biomechanical properties while stimulated bone formation when compared to GIO or the treatment used alone. To the best of the authors' knowledge, this is the first time that an *in vivo* evaluation of exercise+Kefir on bone tissue of rats with GIO has been performed.

Our findings indicated that GCs altered bone microarchitecture with a negative impact, especially on cancellous bone. Bone changes were marked by reduction of the mineral content. These conclusions are in agreement with the literature that has already reported that treatment with GCs alter bone metabolism affecting mainly trabecular bone (40). On the other hand, we demonstrated that the associated treatment with Exercise+Kefir prevented bone microarchitecture deterioration induced by GCs. Physical exercise is an important and well-known stimulus for osteoporosis prevention and treatment (41). Resistance training has been proven to be beneficial for bone microarchitecture, improving bone density and bone strength, besides it provokes a generation of stimuli capable of promoting a bone osteogenic response (42) along with mineral apposition (43). Meanwhile, despite the lack of studies in GIO, it was demonstrated that Kefir could prevent the progress of bone loss both in model of ovariectomy in animals (12) and clinically, in osteoporotic patients (16). The osteo-protective potential of Kefir peptides (KPs), remain unclear. However, some studies have pointed the positive effect of casein and whey proteins (two major proteins of milk) on bone metabolism. Casein phosphopeptides (CPPs) may promote calcium absorption and increase the bioavailability of calcium ions by other tissues, such as bone, while whey proteins have shown osteo-protective properties due to the ability to

suppress the inflammatory status (15). Therefore, this may explain the potentiated bone anabolic properties caused by Kefir on exercise.

Glucocorticoids also induced important reduction on flexural strength and fracture load. Several factors may contribute to this finding. In this study, the amount of bone collagen, as well as its maturity, was lowered after GC use. Type I collagen plays an important role in maintaining bone structure and function, having greater effects on bone strength than stiffness (44). Collagen composite improves the elastic properties of bone along with hydroxyapatite, which provides the support for the collagen matrix giving bone its strength and mechanical resistance, make bone a strong yet flexible structure (45). Thus, damage on bone collagen decreases bone strength (42), demonstrating the close relationship between changes in bone material and biomechanical properties. Conversely, the treatment with Exercise+Kefir was able to reverse the amount and quality of collagen observed after GIO, with consequent increase in bone biomechanical properties. It was suggested that exercise-mediated improvements in bone quality and mechanical properties are primarily driven by collagen (46). In addition, the treatment with Kefir, which is rich in calcium (51), rebounds positively in the treatment of osteoporosis, mitigating the lowered mechanical properties of bone (12). There is evidence that exercise and calcium may not work independently and demonstrates that high calcium intake can increase the effect of exercise (52–54). Thus, the greatest improvement of exercise+Kefir in bone biomechanical properties can also be related to the additional availability of calcium promoted by Kefir, which, in association with exercise, increased bone mineral content, as found in this study.

GIO was also marked by a significant reduction on osteocytes, which can also explain the reduction on biomechanical properties. GCs may alter the metabolism and function of the osteocyte as well as inducing cell death (47), or autophagy (48). Osteocyte death and bone strength seems to be linked to bone hydration status. The canalicular system surrounding the osteocyte body and its dendritic projections is responsible to store much of the bone water, responsible for the hydraulic properties in hard tissues (49). Death of osteocytes and disruption of the peri-cellular matrix may therefore affect hydration status, compromising, then, bone strength (50). Resistance exercise significantly reversed the reduction in osteocyte number induced by GIO. Osteocytes are highly sensitive to mechanical stimulation (51). It has been

reported that loading can reverse the decrease on the number of apoptotic osteocytes (52) due to an increased expression of anti-apoptotic genes and activation of integrins (53), prostaglandin receptors and subsequent signaling through protein kinase A or beta-catenin (54). Mechanical stimulation through exercise training seems therefore an interesting strategy to counteract the increase in osteocyte apoptosis associated with glucocorticoids to increase bone resistance.

Another contributor to bone strength is the muscle activity. We did not analyze the deleterious effects of GCs on muscle mass, even though it is known that GCs can induce myopathy due to an effect on muscle mass and muscle strength, which is one of the determinants of the risk of falls and fractures (39). On the other hand, when GIO animals were submitted to exercise, they not only became more adapted to the exercise model but also showed an improvement in muscle strength levels, since they did the climbing exercise in progressively shorter times even when lifting progressively larger loads. It is known that the mechanical load promoted by muscle strength and ground reaction force act on the bones increase bone density and strength. This seems to be one of the main reasons that exercise can improve bone health (58) and is indicated for the treatment of osteoporosis (10).

Further, we observed that GC unbalanced bone remodeling marked by reduction on osteoblast number. A number of mechanisms have been proposed to explain the deleterious effect of GCs on osteoblast. The Wnt/β-catenin pathway seems to be an important target, as previously demonstrated by our study group (17), since GCs upregulates Dkk-1 (55), Sost (56) and sFRP-1, all Wnt antagonists (57), while reduce phosphorylation of GSK3β and the amount of nuclear β-catenin (48). Inhibition of Wnt signaling, in turn, favors adipocyte differentiation in bone marrow stromal cells, rather than osteoblasts (53). Inhibition of bone morphogenetic proteins (BMPs), Runx2, and reduction of canopy cells, which are responsible for initiation of bone formation, may also contribute to the lower on bone formation after GC therapy (48). Exercise, in the other hand, was capable to stimulate the increase on osteoblast number and function.

Exercise has been shown to induce mesenchymal stem cells (MSCs) to differentiate towards osteoblasts instead of adipocytes, and increase in the activities of ALP and the levels of osteopontin (OPN) (58). Specifically in osteoblasts, exercise

has been shown to up-regulate the expression of osteogenic markers such as OCL, Runx2, Osx, BAP, BMP2, and type I collagen in osteoblasts, also increasing the serum levels of BALP and OCL, markers for bone formation (59). The beneficial effect of exercise on osteoblast might involve the Wnt/b-Catenin signaling pathway, since fluid shear stress induces nuclear translocation of b-Catenin in osteoblasts (60).

Finally, along with the decrease on osteoblast it was seen an increase on osteoclast count and on eroded surfaces, as in accordance to previous studies (37,47,61–65), due to both increased generation of new osteoclasts and prolongation of the lifespan of preexisting osteoclast (6,66,67). Resorbed surfaces or surfaces in the process of resorption are characterized by excavations resulting from the action of osteoclasts (68). Thus, the increased roughness analyzed by scanning electron microscopy reinforces our findings. GCs are known to induce gene transcriptions associated with changes in osteoclast cytoskeleton organization which stimulate their resorbing capacity (69). In addition, it has been shown that GIO is accompanied by increased bone marrow adiposity (6,37,61). Large amounts of adipoccines are secreted by these marrow adipocytes, such as adiponectin and IL-6, some of which induce osteoclastogenesis (70), inflammation (71) and can interrupt hematopoiesis (72), aggravating bone loss (73). Adiponectin, for example, which is an adipokine highly expressed by marrow adipocytes (74), stimulates RANKL expression in mature osteoclasts and is associated with low BMD in elderly patients (75,76). IL-6, in turn, induces the expression of RANKL in osteoclasts and their precursors, which increases the recruitment of hematopoietic macrophage precursors to the osteoclast lineage, increasing bone resorption (77). Thus, the increased number and activity of osteoclasts may be partially responsible for the reduction in trabecular bone volume observed with excess GCs (37), as found in this study.

On the other hand, exercise and Kefir, isolated or associated, decreased the number of osteoclasts, reversing the effects caused by GCs. As seen in MEV analyses bone surface was more preserved with less roughness (68), suggesting less osteoclast activity. Mechanical stress inhibits osteoclast differentiation and function (78). It has been reported that treadmill and vibration stimulation training downregulated RANKL expression and up-regulated OPG expression in bone cells in a rat model with glucocorticoid-induced osteoporosis. Meanwhile, mice are fed with probiotics showed significant reduction in TNFa and RANKL in both the intestine and bone marrow

(79,80). In clinical study, it was observed that Kefir-treatment decreased of β C-terminal telopeptide of type I collagen (β-CTX) (16), indicator of bone resorption (81), demonstrating that Kefir increases bone mass initially by decreasing bone resorption first (16), as verified in this study.

In summary, the present suggests that the association of the oral probiotic Kefir and resistance exercise improved bone microarchitecture, quality, strength as well as its biomechanical properties through stimulation of bone formation. Thus, this association may be an interesting choice in order to support the treatment of patients with glucocorticoid-induced osteoporosis.

5 Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

6 Author Contributions

RFV: Design and conduct of the study, data analysis, and elaboration of manuscript. VC: Analysis of scanning electron microscopy. BA and APA: Raman analysis. TACM and RD: Conduction of the study as undergraduate student. LV: Kefir administration and analysis HS, BC and DT: assistance in inducing GIO and monitoring of treatment. FWGC and LMK: digital radiographic analysis. RFCL: Critical review of the manuscript. DG: Data analysis. KMAP: Histopathological analysis. PG: Supervision and data analysis and elaboration of manuscript.

7 Acknowledgments

We are grateful to the Nucleus of Study in Microscopy and Image Processing (NEMPI) from the Faculty of Medicine of Federal University of Ceará (UFC) for all histology and digital imaging services and the Analytical Center from the UFC for confocal analysis and scanning microscopy analysis.

8 Ethics statement

This animal study was reviewed and approved by the Committee on the Ethics of Animal Experiments of the Federal University of Ceará (Permit Number: 137/17).

9 Data availability statement

All datasets generated for this study are included in the article/supplementary material.

References

1. Henneicke H, Herrmann M, Kalak R, Brennan-speranza TC, Heinevetter U, Bertollo N, et al. Corticosterone selectively targets endo-cortical surfaces by an osteoblast-dependent mechanism ☆. Bone [Internet]. 2011;49(4):733–42. Available from: http://dx.doi.org/10.1016/j.bone.2011.06.013

2. Sarinho C, Pinho M, Melo P. Doença óssea induzida pelos glicocorticoides: mecanismos e importância na prática pediátrica. Rev Paul Pediatr. 2017;35(2):207–15.

3. Lane NE. Glucocorticoid-Induced Osteoporosis: New Insights into the Pathophysiology and Treatments. Curr Osteoporos Rep. 2019;17(1):1–7.

4. Briot K, Roux C. Glucocorticoid-induced osteoporosis. Rheum e Musculoskelet Dis. 2015;

5. Chotiyarnwong P, McCloskey E V. Pathogenesis of glucocorticoid-induced osteoporosis and options for treatment. Nat Rev Endocrinol. 2020; 16:473–447.

6. Weinstein RS. Glucocorticoid-Induced Osteoporosis and Osteonecrosis. Endocrinol Metab Clin North Am. 2012;41(3):595–611.

7. Staa TP Van, Laan RF, Barton IP, Cohen S, Reid DM, Cooper C. Bone Density Threshold and Other Predictors of Vertebral Fracture in Patients Receiving Oral Glucocorticoid Therapy. ARTHRITIS Rheum. 2003;48(11):3224–9.

8. Ahmed Z, Wang Y, Ahmad A, Khan ST, Nisa M, Ahmad H, et al. Kefir and Health: A Contemporary Perspective. Crit Rev Food Sci Nutr. 2013; Pages 422-434.

9. Compston J. Bone quality: What is it and how is it measured? Arq Bras Endocrinol Metabol. 2006;50(4):579–85.

10. Buckley L, Guyatt G, Fink HA, Cannon M, Grossman J, Hansen KE, et al. 2017 American College of Rheumatology Guideline for the Prevention and Treatment of Glucocorticoid-Induced Osteoporosis. 2017;69(8):1521–37.

11. Cho D, Kim K, Jeon Y. A synergistic bone sparing effect of curcumin and alendronate in ovariectomized rat. Acta Neurochir. 2012; 154:2215–23.

12. Chen H, Tung Y, Chuang C, Tu M. Kefir improves bone mass and microarchitecture in an ovariectomized rat model of postmenopausal osteoporosis. Osteoporos Int. 2015; 26:589–99.

13. Marchiori RC. Caracterização do kefir e propriedades probióticas: uma revisão. Rev Inst Lat Cândido Tostes, 2007; v.62:p.21-31.

14. Weschenfelder S, Pereira GM, Carvalho HHC, Wiest JM. Physicolchemical and sensorial characteristic of traditional kefir and derivatives. Arq Bras Med Veterinária e Zootec. 2011;63(2):473–80.

15. Tu M, Han K, Chang GR, Lai G. Bone Loss and Modulate the Structure of the Gut. 2020;1–16.

16. Tu MY, Chen HL, Tung YT, Kao CC, Hu FC, Chen CM. Short-Term Effects of Kefir-Fermented Milk Consumption on Bone Mineral Density and Bone Metabolism in a Randomized Clinical Trial of Osteoporotic Patients. PLoS One. 2015;10(12):1–17.

17. Sousa LH, Linhares EVM, Alexandre JT, Lisboa MR, Furlaneto F, Freitas R, et al. Effects of Atorvastatin on Periodontitis of Rats Subjected to Glucocorticoid-Induced Osteoporosis. J Periodontol. 2016;87(10):1206–16.

18. Sousa LHT, Moura EV, Queiroz AL, Val D, Chaves H, Lisboa M, et al. Effects of glucocorticoid-induced osteoporosis on bone tissue of rats with experimental periodontitis. Arch Oral Biol [Internet]. 2017; 77:55–61. Available from: http://dx.doi.org/10.1016/j.archoralbio.2017.01.014

19. Stringhetta-Garcia CT, Singulani MP, Santos LF, Louzada MJQ, Nakamune ACS, Chaves-Neto AH, et al. The effects of strength training and raloxifene on bone health in aging ovariectomized rats. Bone [Internet]. 2016; 85:45–54. Available from: http://dx.doi.org/10.1016/j.bone.2015.11.023

20. Rosa DD, Peluzio M do CG, Bueno TP, Cañizares EV, Miranda LS, Dorbignyi BM, et al. Evaluación de la toxicidad subcrónica del kéfir por administración oral en ratas Wistar. Nutr Hosp. 2014;29(6):1352–9.

21. Pornprasertsuk S, Ludlow J, Webber R, Tyndall D, Yamauchi M. Analysis of fractal dimensions of rat bones from film and digital images. Dento,axillofacial Radiol. 2001; 30:179–83.

22. Trebacz H, Zdunek A. Three-point bending and acoustic emission study of adult rat femora after immobilization and free remobilization. 2006; 39:237–45.

23. Shiguemoto GE, Prestes J, Leite RD, Pereira GB, Pontes CLS, D'A´vila F V., et al. Effects of resistance training on matrix metalloproteinase-2 activity and biomechanical and physical properties of bone in ovariectomized and intact rats. 2012;607–17.

24. Dempster DW, Compston JE, Drezner MK, Glorieux FH, Kanis JA, Malluche H, et al. Standardized Nomenclature, Symbols, and Units for Bone Histomorphometry: A 2012 Update of the Report of the ASBMR Histomorphometry Nomenclature Committee. 2014;28(1):2–17.

25. Bouxsein ML, Boyd SK, Christiansen BA, Guldberg RE, Jepsen KJ, Mu R. Guidelines for Assessment of Bone Microstructure in Rodents Using Micro – Computed Tomography. J Bone Miner Res. 2010;25(7):1468–86.

26. Akkus O, Adar F, Schaffler MB. Age-related changes in physicochemical properties of mineral crystals are related to impaired mechanical function of cortical bone. Bone. 2004;34(3):443–53.

27. Morris MD, Mandair GS. Raman assessment of bone quality. Clin Orthop Relat Res. 2011;469(8):2160–9.

28. Shen J, Fan L, Yang J, Shen A, Hu J. A longitudinal Raman microspectroscopic study of osteoporosis induced by spinal cord injury. Osteoporos Int. 2010; 21:81–7.

29. Paschalis EP, Gamsjaeger S, Klaushofer K. Vibrational spectroscopic techniques to assess bone quality. 2017;2275–91.

30. Farlay D, Panczer G, Rey C, Delmas P, Boivin G. Maturidade mineral e índice de cristalinidade são características distintas do mineral ósseo. 2021;1–22.

31. Mandair GS, Akhter MP, Esmonde-White FWL, Lappe JM, Bare SP, Lloyd WR, et al. Altered collagen chemical compositional structure in osteopenic women with past fractures: A case-control Raman spectroscopic study. Bone [Internet]. 2021;148(March):115962. Available from: https://doi.org/10.1016/j.bone.2021.115962

32. Lima M dos R, Lopes AP, Martins C, Brito GAC, Carneiro VC, Goes P. The effect of Calendula officinalis on oxidative stress and bone loss in experimental periodontitis. Front Physiol. 2017;8(JUN):1–9.

33. Tawse-Smith A, Atieh M, Tompkins G, Duncan W, Reid M, Stirling C. The effect of piezoelectric ultrasonic instrumentation on titanium discs: a microscopy and trace elemental analysis in vitro study. Int J Dent Hyg. 2015;14(3):191–201.

34. Sousa Ferreira VC, Lopes AP, Alves NM, Sousa FRN, Pereira KMA, Gondim DV, et al. Bisphosphonate-related osteonecrosis induced change in alveolar bone architecture in rats with participation of Wnt signaling. Clin Oral Investig. 2021;25(2):673–82.

35. Monici M. Cell and tissue autofluorescence research and diagnostic applications. Biotechnol Annu Rev. 2005; 11:227–56.

36. Gonçalves DC, Evangelista RC, Silva RR da, Maria JS Santos, Francisco S Silva J, Aragão KS, Brito GA, et al. Infliximab attenuates inflammatory osteolysis in a model of periodontitis in Wistar rats. Exp Biol Med. 2014;239(4).

37. Wei Yao, Cheng Z, Busse C, Pham A, Nakamura MC, Lane NE. Glucocorticoid-induced bone loss can be reversed by the actions of PTH and

Risedronate on different pathways for bone formation and mineralization. Arthritis Rheum [Internet]. 2008;58(11):3485–3497. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3624763/pdf/nihms412728.pdf

38. Mohan G, Lay EY-A, Berka H, Ringwood L, Kot A, Chen H, et al. A novel hybrid compound LLP2A-Ale both prevented and rescued the osteoporotic phenotype in a mouse model of glucocorticoid-induced. Calcif Tissue Int. 2017;100(1):67–79.

39. Dubrovsky AM, Nyman JS, Uppuganti S, Chmiel KJ, Kimmel DB, Lane NE. Bone Strength/Bone Mass Discrepancy in Glucocorticoid-Treated Adult Mice. JBMR Plus. 2021;5(3):1–10.

40. Canalis E, Mazziotti G, Giustina A, Bilezikian JP. Glucocorticoid-induced osteoporosis: Pathophysiology and therapy. Osteoporos Int. 2007;18(10):1319–28.

41. Fernandes Moreira LD, de Oliveira ML, Lirani-Galvão AP, Marin-Mio RV, dos Santos RN, Lazaretti-Castro M. Exercício físico e osteoporose: Efeitos de diferentes tipos de exercícios sobre o osso e a função física de mulheres pós-menopausadas. Arq Bras Endocrinol Metabol. 2014;58(5):514–22.

42. Turner CH, Robling AG. Exercises for improving bone strength. Br J Sports Med. 2005;39(4):188–9.

43. Menkes A, Mazel S, Redmond RA, Koffler K, Libanati CR, Gundberg CM, et al. Strength training increases regional bone mineral density and bone remodeling in middle-aged and older men. J Appl Physiol. 1985;74(5):2478–84.

44. Burr DB. The Contribution of the Organic Matrix to Bone's Material Properties. Bone [Internet]. 2002;31(1):8–11. Available from: http://dx.doi.org/10.1016/S8756-3282(02)00815-3

45. Kjaer M, Jørgensen NR, Heinemeier K, Magnusson SP. Chapter Eleven - Exercise and Regulation of Bone and Collagen Tissue Biology. Prog Mol Biol Transl Sci. 2015; 135:259–91.

46. Wallace JM, Ron MS, Kohn DH. Short-term exercise in mice increases tibial post-yield mechanical properties while two weeks of latency following exercise increases tissue-level strength. Calcif Tissue Int. 2009;84(4):297–304.

47. Nancy E Lane, Yao W, Balooch M, Nalla RK, Balooch G, Habelitz S, et al. Glucocorticoid-Treated Mice Have Localized Changes in Trabecular Bone Material Properties and Osteocyte Lacunar Size That Are Not Observed in Placebo-Treated or Estrogen-Deficient Mice. J Bone Min Res [Internet]. 2006;21(3):466–76. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3624763/pdf/nihms412728.pdf

48. Komori T. Glucocorticoid Signaling and Bone Biology. Horm Metab Res. 2016;48(11):755–63.

49. Wilson EE, Awonusi A, Morris MD, Kohn DH, Tecklenburg MMJ, Beck LW. Three structural roles for water in bone observed by solid-state NMR. Biophys J. 2006;90(10):3722–31.

50. Weinstein RS, Wan C, Liu Q, Wang Y, Almeida M, O'Brien CA, et al. Endogenous glucocorticoids decrease skeletal angiogenesis, vascularity, hydration, and strength in aged mice. Aging Cell. 2010;9(2):147–61.

51. Fonseca H, Moreira-Gonçalves D, Esteves JLS, Viriato N, Vaz M, Mota MP, et al. Voluntary exercise has long-term in vivo protective effects on osteocyte viability and bone strength following ovariectomy. Calcif Tissue Int. 2011; 88(6):443–54.

52. Noble BS, Peet N, Stevens HY, Brabbs A, Mosley JR, Reilly GC, et al. Mechanical loading: Biphasic osteocyte survival and targeting of osteoclasts for bone destruction in rat cortical bone. Am J Physiol - Cell Physiol. 2003; 284(4 53-4):934–43.

53. Plotkin LI, Mathov I, Aguirre JI, Parfitt AM, Manolagas SC, Bellido T. Mechanical stimulation prevents osteocyte apoptosis: Requirement of integrins, Src kinases, and ERKs. Am J Physiol - Cell Physiol. 2005; 289(3 58-3):633–43.

54. Kitase Y, Barragan L, Qing H, Kondoh S, Jiang JX, Johnson ML, et al. Mechanical induction of PGE2 in osteocytes blocks glucocorticoid-induced apoptosis

through both the β-catenin and PKA pathways. J Bone Miner Res. 2010;25(12):2657–68.

55. Almeida M, Han L, Ambrogini E, Weinstein RS, Manolagas SC. Glucocorticoids and tumor necrosis factor α increase oxidative stress and suppress Wnt protein signaling in osteoblasts. J Biol Chem. 2011;286(52):44326–35.

56. Sato AY, Cregor M, Delgado-Calle J, Condon KW, Allen MR, Peacock M, et al. Protection From Glucocorticoid-Induced Osteoporosis by Anti-Catabolic Signaling in the Absence of Sost/Sclerostin. J Bone Miner Res. 2016;31(10):1787–90.

57. Hayashi K, Yamaguchi T, Yano S, Kanazawa I, Yamauchi M, Yamamoto M, et al. BMP/Wnt antagonists are upregulated by dexamethasone in osteoblasts and reversed by alendronate and PTH: Potential therapeutic targets for glucocorticoid-induced osteoporosis. Biochem Biophys Res Commun [Internet]. 2009;379(2):261–6. Available from: http://dx.doi.org/10.1016/j.bbrc.2008.12.035

58. Marędziak M, ̄mieszek A, Chrząstek K, Basinska K, Marycz K. Physical Activity Increases the Total Number of Bone-Marrow-Derived Mesenchymal Stem Cells, Enhances Their Osteogenic Potential, and Inhibits Their Adipogenic Properties. Stem Cells Int. 2015;2015.

59. Lester ME, Urso ML, Evans RK, Pierce JR, Spiering BA, Maresh CM, et al. Influence of exercise mode and osteogenic index on bone biomarker responses during short-term physical training. Bone. 2009;45(4):768–76.

60. Norvell SM, Alvarez M, Bidwell JP, Pavalko FM. Fluid shear stress induces β-catenin signaling in osteoblasts. Calcif Tissue Int. 2004;75(5):396–404.

61. Li J, Zhang N, Huang X, Xu J, Fernandes JC, Dai K, et al. Dexamethasone shifts bone marrow stromal cells from osteoblasts to adipocytes by C/EBPαlpha promoter methylation. Cell Death Dis. 2013;4(10):1–11.

62. Weinstein RS, Nicholas RW, Manolagas SC. Apoptosis of osteocytes in glucocorticoid-induced osteonecrosis of the hip. J Clin Endocrinol Metab. 2000;85(8):2907–12.

63. Yao W, Dai W, Jiang JX, Lane NE. Glucocorticoids and Osteocyte Autophagy. Bone [Internet]. 2013;54(2):279–284. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3624763/pdf/nihms412728.pdf

64. Wang FS, Lin CL, Chen YJ, Wang CJ, Yang KD, Huang YT, et al. Secreted frizzled-related protein 1 modulates glucocorticoid attenuation of osteogenic activities and bone mass. Endocrinology. 2005;146(5):2415–23.

65. Reid IR. Glucocorticoid osteoporosis--mechanisms and management. Eur J Endocrinol. 1997;137(3):209–17.

66. JIA D, O´BRIEN CA, STEWART SA, MANOLAGAS S., WEINSTEIN RS. NIH Public Access. Endocrinol Metab Clin N Am. 2006;147(12):5592–9.

67. Hofbauer LC, Zeitz U, Schoppet M, Skalicky M, Schüler C, Stolina M, et al. Prevention of glucocorticoid-induced bone loss in mice by inhibition of RANKL. Arthritis Rheum. 2009;60(5):1427–37.

68. BROMAGE T. Interpretation of scanning electron microscopic images of abraded forming bone surfaces. Am J Phys Anthr. 1984; 64:2275–91.

69. Zou W, Kitaura H, Reeve J, Long F, Tybulewicz VLJ, Shattil SJ, et al. Syk, c-Src, the αvβ3 integrin, and ITAM immunoreceptors, in concert, regulate osteoclastic bone resorption Wei. J Cell Biol. 2007;176(6):877–88.

70. Xue Y, Jiang L, Cheng Q, Chen H, Yu Y, Lin Y, et al. Adipokines in Psoriatic Arthritis Patients: The Correlations with Osteoclast Precursors and Bone Erosions. 2012;7(10):1–11.

71. Fantuzzi G. Molecular mechanisms in allergy and clinical immunology. J ALLERGY CLIN IMMUNOL. 2005;115(5):911–9.

72. Patel VS, Chan ME, Rubin J, Rubin CT. MARROW ADIPOSITY AND HEMATOPOIESIS IN AGING AND OBESITY: EXERCISE AS AN INTERVENTION. Curr Osteoporos Rep. 2018;16(2):105–15.

73. Neumann E, Junker S, Schett G, Frommer K, Müller-Ladner U. Adipokines in bone disease. Nat Rev Rheumatol. 2016; 12:296–302.

74. Cawthorn WP, Scheller EL, Learman BS, Parlee SD, Simon BR, Mori H, et al. Bone marrow adipose tissue is an endocrine organ that contributes to increased circulating adiponectin during caloric restriction. Cell Metab. 2014;20(2):368–75.

75. Barbour KE, Zmuda JM, Boudreau R, Elsa S, Horwitz MJ, Evans RW, et al. The Effects of Adiponectin and Leptin on Changes in Bone Mineral Density. Osteoporos Int2. 2012;23(6):1699–710.

76. Lourdes Basurto, Galva´n R, Cordova N, Saucedo R, Vargas C, Campos S, et al. Adiponectin is associated with low bone mineral density in elderly men. Eur J Endocrinol. 2009; 160:289–93.

77. Kudo O, Sabokbar A, Pocock A, Itonaga I, Fujikawa Y, Athanasou NA. Interleukin-6 and interleukin-11 support human osteoclast formation by a RANKL-independent mechanism. 2003; 32:1–7.

78. Suzuki N, Yoshimura Y, Deyama Y, Suzuki K, Kitagawa Y. Mechanical stress directly suppresses osteoclast differentiation in RAW264.7 cells. Int J Mol Med. 2008;21(3):291-6.

79. Li J, Chassaing B, Tyagi AM, Vaccaro C, Luo T, Adams J, et al. Sex steroid deficiency – associated bone loss is microbiota dependent and prevented by probiotics. J Clin Invest. 2016;126(6):2049–63.

80. Britton RA, Irwin R, Quach D, Schaefer L, Zhang J, Parameswaran N, et al. Probiotic L. reuteri treatment prevents bone loss in a menopausal ovariectomized mouse model. J Cell Physiol. 2014;229(11):1822–30.

81. Bauer D, Krege J, Lane N, Leary E, Libanati C, Miller P, et al. National Bone Health Alliance Bone Turnover Marker Project: current practices and the need for US harmonization, standardization, and common reference ranges. Osteoporos Int. 2012;23(10):2425–33.

Conclusão Geral

4. CONCLUSÃO GERAL

Em suma, podemos concluir que o exercício físico de força pode reduzir o grau de progressão da perda óssea, mostrando os efeitos benéficos do tratamento com exercícios, o que ajuda fundamentar a escolha do exercício resistido como parte do tratamento da osteoporose. O Kefir, por sua vez, potencializou os efeitos benéficos do exercício sobre o tecido ósseo. A associação de exercício com Kefir reverteu os efeitos deletérios dos GCs no fêmur melhorando a microarquitetura, a qualidade do fêmur e as propriedades biomecânicas, ao mesmo tempo que estimulou a formação óssea. Assim, o tratamento com Kefir e exercícios físicos apresentam-se como possíveis ferramentas adjuvantes para melhorar o quadro de perda óssea provocados pela OIG.

Referências

REFERÊNCIAS

ADAMI, G.; SAAG, K. G. Glucocorticoid-induced osteoporosis: 2019 concise clinical review. **Osteoporosis International**, vol. 30, no. 6, p. 1145–1156, 2019. https://doi.org/10.1007/s00198-019-04906-x.

AHMED, Zaheer; WANG, Yanping; AHMAD, Asif; KHAN, Salman Tariq; NISA, Mehrun; AHMAD, Hajra; AFREEN, Asma. Kefir and Health: A Contemporary Perspective. **Critical Reviews in Food Science and Nutrition**, , p. Pages 422-434, 2013. .

ALMEIDA, Maria; LAURENT, Michaël R.; DUBOIS, Vanessa; CLAESSENS, Frank; O'BRIEN, Charles A.; BOUILLON, Roger; VANDERSCHUEREN, Dirk; MANOLAGAS, Stavros C. Estrogens and androgens in skeletal physiology and pathophysiology. **Physiological Reviews**, vol. 97, no. 1, p. 135–187, 2017. https://doi.org/10.1152/physrev.00033.2015.

AMICHE, M. Amine; ABTAHI, Shahab; DRIESSEN, Johanna H.M.; VESTERGAARD, Peter; DE VRIES, Frank; CADARETTE, Suzanne M.; BURDEN, Andrea M. Impact of cumulative exposure to high-dose oral glucocorticoids on fracture risk in Denmark: a population-based case-control study. **Archives of Osteoporosis**, vol. 13, no. 1, 2018. https://doi.org/10.1007/s11657-018-0424-x.

ANDIA, Denise Carleto; CERRI, Paulo Sérgio; SPOLIDORIO, Luis Carlos. Tecido ósseo: aspectos morfológicos e histofisiológicos T. vol. 35, no. 2, p. 191–198, 2006. Available at: https://www.researchgate.net/profile/Denise_Andia/publication/268358609_Tecido_osseo_aspectos_morfologicos_e_histofisiologicos/links/54aa6a5f0cf274dc0b5c2693/Tecido-osseo-aspectos-morfologicos-e-histofisiologicos.pdf.

ANDRADE, Altair Dantas; MARINHO, Carlos Frederico; BARCELOS, Marcio; ZORZAL, Marlei Bonella; CONZ, Marcio Baltazar. Biologia óssea : uma revisão da literatura. **REVISTA IMPLANTNEWS**, vol. 4, no. 6, p. 659–662, 2007. .

ARMAS, Laura A. G.; RECKER, Robert R. Pathophysiology of Osteoporosis: New Mechanistic Insights. **Endocrinol Metab Clin N Am**, vol. 41, p. 475–486, 2012. .

BALASUBRAMANIAN, A.; WADE, S. W.; ADLER, R. A.; LIN, C. J.F.; MARICIC, M.; O'MALLEY, C. D.; SAAG, K.; CURTIS, J. R. Glucocorticoid exposure and fracture risk in patients with new-onset rheumatoid arthritis. **Osteoporosis International**, vol. 27, no. 11, p. 3239–3249, 2016. DOI 10.1007/s00198-016-3646-z. Available at: http://dx.doi.org/10.1007/s00198-016-3646-z.

BORBA, Victória Zeghbi C.; LAZARETTI-CASTRO, Marise. Osteoporose Induzida por Glicocorticóide. **Arquivos Brasileiros de Endocrinologia & Metabologia**, vol. 43, p. 452–456, 1999. .

BRIOT, Karine; ROUX, Christian. Glucocorticoid-induced osteoporosis. **Rheumatic e Musculoskeletal Diseases**, 2015. https://doi.org/10.1136/rmdopen-2014-000014.

BUCKLEY, Lenore; GUYATT, Gordon; FINK, Howard A; CANNON, Michael; GROSSMAN, Jennifer; HANSEN, Karen E; HUMPHREY, Mary Beth; LANE, Nancy E; MAGREY, Marina; MILLER, Marc. 2017 American College of Rheumatology Guideline for the Prevention and Treatment of Glucocorticoid-Induced Osteoporosis. vol. 69, no. 8, p. 1521–1537, 2017. https://doi.org/10.1002/art.40137.

CANALIS, E.; MAZZIOTTI, G.; GIUSTINA, A.; BILEZIKIAN, J. P. Glucocorticoid-induced osteoporosis: Pathophysiology and therapy. **Osteoporosis International**, vol. 18, no. 10, p. 1319–1328, 2007. https://doi.org/10.1007/s00198-007-0394-0.

CHAKHTOURA, Marlene; DAGHER, H; SHARARA, S; AJJOUR, S; CAULEY, JA; MAHFOUD, Z; BOUDREAU, R; FULEIHAN, Ghada El Hajj. Systematic review of major osteoporotic fracture to hip fracture incidence rate ratios worldwide: Implications to FRAX derived estimates. **J Bone Miner Res**, 2021. .

CHE, Chun Tao; WONG, Man Sau; LAM, Christopher Wai Kei; MCPHEE, Derek J. Natural products from Chinese medicines with potential benefits to bone health. **Molecules**, vol. 21, no. 3, p. 1–52, 2016. https://doi.org/10.3390/molecules21030239.

CHEN, H; TUNG, Y; CHUANG, C; TU, M. Kefir improves bone mass and microarchitecture in an ovariectomized rat model of postmenopausal osteoporosis. **Osteoporos Int**, vol. 26, p. 589–599, 2015. https://doi.org/10.1007/s00198-014-2908-x.

CHO, Dae-chul; KIM, Kyoung-tae; JEON, Younghoon. A synergistic bone sparing effect of curcumin and alendronate in ovariectomized rat. **Acta Neurochir**, vol. 154, p. 2215–2223, 2012. https://doi.org/10.1007/s00701-012-1516-9.

CLARKE, Bart. Normal bone anatomy and physiology. **Clinical journal of the American Society of Nephrology : CJASN**, vol. 3 Suppl 3, p. 131–139, 2008. https://doi.org/10.2215/CJN.04151206.

COHEN, A. J.; ROE, F. J.C. Review of risk factors for osteoporosis with particular reference to a possible aetiological role of dietary salt. **Food and Chemical Toxicology**, vol. 38, no. 2–3, p. 237–253, 2000. https://doi.org/10.1016/S0278-6915(99)00145-3.

COOPER, C; FERRARI, S. IOF Compendium of Osteoporosis. **International Osteoporosis Foundation**, vol. 2nd Editio, p. 1–76, 2019. Available at: https://www.iofbonehealth.org/compendium-of-osteoporosis.

CORDEIRO, Leonardo P.; LINHARES, Eduarda O. N. N.; NOGUEIRA, Fernanda G. O.; MOREIRA-SILVA, Daniel; MEDEIROS-LIMA, Daniel J. M. Perspectives on glucocorticoid treatment for COVID - 19 : a systematic review. **Pharmacological Reports**, 2021. DOI 10.1007/s43440-021-00225-3. Available at: https://doi.org/10.1007/s43440-021-00225-3.

CORONADO-ZARCO, Roberto; OLASCOAGA-G, Andrea; MACÍAS-HERN, Salvador Israel. Osteoporosis and Sarcopenia Nonpharmacological interventions for osteoporosis treatment : Systematic review of clinical practice guidelines. **Osteoporosis and Sarcopenia**, vol. 5, p. 69–77, 2019.

https://doi.org/10.1016/j.afos.2019.09.005.

CRUEL, M.; GRANKE, M.; BOSSER, C.; AUDRAN, M.; HOC, T. L'abus d'alcool chronique chez les hommes modifie les propriétés mécaniques de l'os en affectant à la fois la qualité du tissu et les paramètres micro-architecturaux. **Morphologie**, vol. 101, no. 333, p. 88–96, 2017. DOI 10.1016/j.morpho.2017.03.001. Available at: http://dx.doi.org/10.1016/j.morpho.2017.03.001.

DA SILVA, Raíssa Gomes; PIPPA, Maria Guadalupe Barbosa; ZERBIN, Cristiano Augusto De Freitas. Evaluation of body composition and bone mineral density in women with rheumatoid arthritis. **Revista da Associacao Medica Brasileira**, vol. 53, no. 2, p. 135–141, 2007. https://doi.org/10.1590/s0104-42302007000200018.

DALMOLIN, Fabïola; PINTO FILHO, Saulo Tadeu Lemos; CORTES, Andrieli Machado; BRUN, Maurïcio Veloso; CAUDURO, Carlos Roberto; SCHOSSLER, Jöo Eduardo Wallau. Biomecânica óssea e ensaios biomecânicos - Fundamentos teóricos. **Ciencia Rural**, vol. 43, no. 9, p. 1675–1682, 2013. https://doi.org/10.1590/S0103-84782013000900022.

DOVIO, Andrea; PERAZZOLO, Laura; SABA, Laura; TERMINE, Angela; CAPOBIANCO, Marco; BERTOLOTTO, Antonio; ANGELI, Alberto. High-dose glucocorticoids increase serum levels of soluble IL-6 receptor a and its ratio to soluble gp130 : an additional mechanism for early increased bone resorption. **European Journal of Endocrinology**, , p. 745–751, 2006. https://doi.org/10.1530/eje.1.02147.

FENG, Xu; MCDONALD, Jay M. Disorders of Bone Remodeling. **Annu Rev Pathol.**, vol. 6, p. 121–145, 2011. https://doi.org/10.3109/9780203508954-27.

FINA, Brenda L; BRUN, Lucas R; RIGALLI, Alfredo. Increase of calcium and reduction of lactose concentration in milk by treatment with kefir grains and eggshell. vol. 67, no. 2, p. 133–140, 2016. .

FONTES, Tereza Maria P; ARAÚJO, Luiz Felipe B; SOARES, Paulo Roberto G. Osteoporose no climatério I : epidemiologia , definição , rastreio e diagnóstico. **Revista FEMINA**, vol. 40, no. 2, p. 109–116, 2012. .

GIANOUDIS, Jenny; BAILEY, Christine A.; EBELING, Peter R.; NOWSON, Caryl A.; SANDERS, Kerrie M.; HILL, Keith; DALY, Robin M. Effects of a targeted multimodal exercise program incorporating high-speed power training on falls and fracture risk factors in older adults: A community-based randomized controlled trial. **Journal of Bone and Mineral Research**, vol. 29, no. 1, p. 182–191, 2014. https://doi.org/10.1002/jbmr.2014.

GUZEL-SEYDIM, Zeynep B.; G¨OKIRMAKLI, Ça˘glar; GREENE, Annel K. A comparison of milk kefir and water kefir: Physical, chemical, microbiological and functional properties. **Trends in Food Science & Technology**, vol. 113, no. May, p. 42–53, 2021. https://doi.org/10.1016/j.tifs.2021.04.041.

HARTMANN, Kerstin; KOENEN, Mascha; SCHAUER, Sebastian; WITTIG-BLAICH, Stephanie; AHMAD, Mubashir; BASCHANT, Ulrike; TUCKERMANN, Jan P. Molecular Actions of Glucocorticoids in Cartilage and Bone During Health, Disease,

and Steroid Therapy. **Physiol Rev**, vol. 96, no. 2, p. 409–47, 2016. .

HENNEICKE, Holger; HERRMANN, Markus; KALAK, Robert; BRENNAN-SPERANZA, Tara C; HEINEVETTER, Uta; BERTOLLO, Nicky; DAY, Robert E; HUSCHER, Dörte; BUTTGEREIT, Frank; DUNSTAN, Colin R; SEIBEL, Markus J; ZHOU, Hong. Corticosterone selectively targets endo-cortical surfaces by an osteoblast-dependent mechanism ☆. **Bone**, vol. 49, no. 4, p. 733–742, 2011. DOI 10.1016/j.bone.2011.06.013. Available at: http://dx.doi.org/10.1016/j.bone.2011.06.013.

HSU, W. B.; HSU, W. H.; HUNG, J. S.; SHEN, W. J.; HSU, R. W.W. Transcriptome analysis of osteoblasts in an ovariectomized mouse model in response to physical exercise. **Bone and Joint Research**, vol. 7, no. 11, p. 601–608, 2018. https://doi.org/10.1302/2046-3758.711.BJR-2018-0075.R2.

JIA, D.; O´BRIEN, C.A.; STEWART, S.A.; MANOLAGAS, S.C; WEINSTEIN, R.S. NIH Public Access. **Endocrinol Metab Clin N Am**, vol. 147, no. 12, p. 5592–5599, 2006. .

KAJI, Hiroshi; YAMAUCHI, Mika; CHIHARA, Kazuo; SUGIMOTO, Toshitsugu. The threshold of bone mineral density for vertebral fracture in female patients with glucocorticoid-induced osteoporosis. **Endocrine Journal**, vol. 53, no. 1, p. 27–34, 2006. https://doi.org/10.1507/endocrj.53.27.

KANAZAWA, Masanori; MATSUMOTO, Yasuharu; TAKAHASHI, Kikuyo; SUZUKI, Hideaki; UZUKA, Hironori; NISHIMIYA, Kensuke; SHIMOKAWA, Hiroaki. Treadmill exercise prevents reduction of bone mineral density after myocardial infarction in apolipoprotein E-deficient mice. **European Journal of Preventive Cardiology**, vol. 27, no. 1, p. 28–35, 2020. https://doi.org/10.1177/2047487319834399.

KANIS, J. A.; BURLET, N.; COOPER, C.; DELMAS, P. D.; REGINSTER, J. Y.; BORGSTROM, F.; RIZZOLI, R. European guidance for the diagnosis and management of osteoporosis in postmenopausal women. **Osteoporosis International**, vol. 19, no. 4, p. 399–428, 2008. https://doi.org/10.1007/s00198-008-0560-z.

KANIS, J. A.; JOHANSSON, H.; ODEN, A.; MCCLOSKEY, E. V. Guidance for the adjustment of FRAX according to the dose of glucocorticoids. **Osteoporosis International**, vol. 22, no. 3, p. 809–816, 2011. https://doi.org/10.1007/s00198-010-1524-7.

KÄRNSUND, Sofia; LO, Bobby; BENDTSEN, Flemming; BURISCH, Johan; HOLM, Jakob. Systematic review of the prevalence and development of osteoporosis or low bone mineral density and its risk factors in patients with inflammatory bowel disease. **World Journal of Gastroenterology**, vol. 26, no. 35, p. 5362–5374, 2020. https://doi.org/10.3748/WJG.V26.I35.5362.

KOMORI, T. Glucocorticoid Signaling and Bone Biology. **Hormone and Metabolic Research**, vol. 48, no. 11, p. 755–763, 2016. https://doi.org/10.1055/s-0042-110571.

LANE, J. M.; NYDICK, M. Osteoporosis: current modes of prevention and treatment.

The Journal of the American Academy of Orthopaedic Surgeons, vol. 7, no. 1, p. 19–31, 1999. https://doi.org/10.5435/00124635-199901000-00003.

LANE, Nancy E. Glucocorticoid-Induced Osteoporosis: New Insights into the Pathophysiology and Treatments. **Current Osteoporosis Reports**, vol. 17, no. 1, p. 1–7, 2019. https://doi.org/10.1007/s11914-019-00498-x.

LERNER, Ulf H. Osteoclast formation and resorption. **Matrix Biology**, vol. 19, no. 2, p. 107–120, 2000. https://doi.org/10.1016/S0945-053X(00)00052-4.

LOURES, Marco Antônio R.; ZERBINI, Cristiano Augusto F.; DANOWSKI, Jaime S.; PEREIRA, Rosa Maria R.; MOREIRA, Caio; PAULA, Ana Patrícia de; CASTRO, Charlles Heldan M.; SZEJNFELD, Vera Lúcia; MENDONÇA, Laura Maria C.; RADOMINISKI, Sebastião C.; BEZERRA, Mailze C.; SIMÕES, Ricardo; BERNARDO, Wanderley M. Diretrizes da Sociedade Brasileira de Reumatologia para diagnóstico e tratamento da osteoporose em homens. **Revista Brasileira de Reumatologia**, vol. 57, no. S 2, p. 497–514, 2017. https://doi.org/10.1016/j.rbr.2017.06.002.

MARCHIORI, R.C. Caracterização do kefir e propriedades probióticas: uma revisão. **Rev. Inst. Lat. Cândido Tostes,** vol. v.62, p. p.21-31, 2007. .

MARIE, Pierre J. Strontium ranelate: A dual mode of action rebalancing bone turnover in favour of bone formation. **Current Opinion in Rheumatology**, vol. 18, no. SUPPL. 1, p. 11–15, 2006. https://doi.org/10.1097/01.bor.0000229522.89546.7b.

MESSINA, Osvaldo D.; VIDAL, Luis Fernando; VIDAL, Maritza Vidal; BULTINK, Irene E.M.; RATERMAN, Hennie G.; LEMS, William. Management of glucocorticoid-induced osteoporosis. **Aging Clinical and Experimental Research**, vol. 33, no. 4, p. 793–804, 2021. DOI 10.1007/s40520-021-01823-0. Available at: https://doi.org/10.1007/s40520-021-01823-0.

MILLER, Walter L; AUCHUS, Richard J. The Molecular Biology , Biochemistry , and Physiology of Human Steroidogenesis and Its Disorders. vol. 32, no. 1, p. 81–151, 2011. https://doi.org/10.1210/er.2010-0013.

MIRZA, Faryal; CANALIS, Ernesto. Secondary osteoporosis: Pathophysiology and management. **European Journal of Endocrinology**, vol. 173, no. 3, p. R131–R151, 2015. https://doi.org/10.1530/EJE-15-0118.

MORAES, Luci Fabiane Scheffer; DA SILVA, Everton Nunes; SILVA, Diego Augusto Santos; DE PAULA, Patrícia Ana. Gastos com o tratamento da osteoporose em idosos do Brasil (2008 – 2010): Análise dos fatores associados. **Revista Brasileira de Epidemiologia**, vol. 17, no. 3, p. 719–734, 2014. https://doi.org/10.1590/1809-4503201400030012.

NEWMAN, Christopher L.; CREECY, Amy; GRANKE, Mathilde; NYMAN, Jeffry S.; TIAN, Nannan; HAMMOND, Max A.; WALLACE, Joseph M.; BROWN, Drew M.; CHEN, Neal; MOE, Sharon M.; ALLEN, Matthew R. Raloxifene improves skeletal properties in an animal model of cystic chronic kidney disease. **Kidney Int.**, vol. 89, no. 1, p. 95–104, 2016. https://doi.org/10.1038/ki.2015.315.Raloxifene.

OVERMAN, Robert A; YEH, Jun-yen; DEAL, Chad L. Prevalence of Oral Glucocorticoid Usage in the United States : A General Population Perspective. **Arthritis Care & Research**, vol. 65, no. 2, p. 294–298, 2013. https://doi.org/10.1002/acr.21796.

PEREIRA, Rosa Maria Rodrigues; CARVALHO, Jozélio Freire de; PAULA, Ana Patrícia; ZERBINI, Cristiano; DOMICIANO, Diogo S.; GONÇALVES, Helenice; DANOWSKI, Jaime S.; NETO, João F. Marques; MENDONÇA, Laura M. C.; BEZERRA, Mailze C.; TERRERI, Maria Teresa; IMAMURA, Marta; WEINGRILL, Pedro; PLAPLER, Perola G.; RADOMINSKI, Sebastião; TOURINHO, Tatiana; SZEJNFELD, Vera L.; ANDRADA, Nathalia C. Diretrizes para prevenção e tratamento da osteoporose induzida por glicocorticoide. **Revista Brasileira de Reumatologia**, vol. 52, no. 4, p. 569–593, 2012. .

PICHLER, Karin; LORETO, Carla; LEONARDI, Rosalia; REUBER, Tobias; WEINBERG, Annelie Martina; MUSUMECI, Giuseppe. RANKL is downregulated in bone cells by physical activity (treadmill and vibration stimulation training) in rat with glucocorticoid-induced osteoporosis. **Histology and Histopathology**, vol. 28, p. 1185–1196, 2013. https://doi.org/10.14670/HH-28.1185.

PINTO, Aimée dos Santos; ZERBINI, Cristiano A F. Osteoporose secundária a drogas. **RBM Mar**, vol. 67, no. 3, 2010. .

PORFIRIO, ELISÂNGELA; FANARO, GUSTAVO BERNARDES. Suplementação com colágeno como terapia complementar na prevenção e tratamento de osteoporose e osteoartrite : uma revisão sistemática. **Revista Brasileira de Geriatria e Gerontologia**, vol. 19, no. 1, p. 153–164, 2016. .

POZZI, Samantha; MARCHESELLI, Raffaella; SACCHI, Stefano; BALDINI, Luca; ANGRILLI, Francesco; PENNESE, Elsa; QUARTA, Giovanni; STELITANO, Caterina; CAPAROTTI, Giuseppe; LUMINARI, Stefano; MUSTO, Pellegrino; NATALE, Donato; BROGLIA, Chiara; CUOGHI, Angela; DINI, Daniele; TONNO, Paolo Di; LEONARDI, Giovanna; PIANEZZE, Graziano; PITINI, Vincenzo; … POLLASTRI, Giuseppe. Bisphosphonate-associated osteonecrosis of the jaw: A review of 35 cases and an evaluation of its frequency in multiple myeloma patients. **Leukemia and Lymphoma**, vol. 48, no. 1, p. 56–64, 2007. https://doi.org/10.1080/10428190600977690.

RAJGOPAL, Raghav; BEAR, MacKenzie; BUTCHER, Martin K.; SHAUGHNESSY, Stephen G. The effects of heparin and low molecular weight heparins on bone. **Thrombosis research**, vol. 122, no. 3, p. 293–298, 2008. .

RALSTON, Stuart H. Bone structure and metabolism. **Medicine (United Kingdom)**, vol. 41, no. 10, p. 581–585, 2013. DOI 10.1016/j.mpmed.2013.07.007. Available at: http://dx.doi.org/10.1016/j.mpmed.2013.07.007.

RATERMAN, Hennie G.; BULTINK, Irene E. M.; LEMS, Willem F. Current Treatments and New Developments in the Management of Glucocorticoid-induced Osteoporosis. **Drugs volume**, vol. 79, p. 1065–1087, 2019. .

RIGGS, B. Lawrence; MELTON, L. Joseph. Evidence for two distinct syndromes of involutional osteoporosis. **The American Journal of Medicine**, vol. 75, no. 6, p.

899–901, 1983. https://doi.org/10.1016/0002-9343(83)90860-4.

ROCHWERG, Bram; AGARWAL, Arnav; SIEMIENIUK, Reed A C; AGORITSAS, Thomas; ZENG, Linan; AMIN, Wagdy; BURHAN, Erlina; BAUSCH, Jacquerioz; CALFEE, Carolyn S. A living WHO guideline on drugs for covid-19. 2020. https://doi.org/10.1136/bmj.m3379.

RODRIGUES, K. L.; CARVALHO, J. C.T.; SCHNEEDORF, J. M. Anti-inflammatory properties of kefir and its polysaccharide extract. **Inflammopharmacology**, vol. 13, no. 5–6, p. 485–492, 2005. https://doi.org/10.1163/156856005774649395.

RUBIN, Mishaela R; BILEZIKIAN, John P. The Role of Parathyroid Hormone in the Pathogenesis of Glucocorticoid-Induced Osteoporosis : A Re-Examination of the Evidence. **The Journal of Clinical Endocrinology & Metabolism**, vol. 87, no. 9, p. 4033–4041, 2002. https://doi.org/10.1210/jc.2002-012101.

SARINHO, Cavalcanti; PINHO, Maria; MELO, Pessoa. Doença óssea induzida pelos glicocorticoides: mecanismos e importância na prática pediátrica. **Rev Paul Pediatr.**, vol. 35, no. 2, p. 207–215, 2017. .

SHAH, Meghnaa; APPUSWAMY, Anusha; RAO, Veeravanallura; D.B, Sudhaker; DHALIWAL, Rubana. Treatment of bone fragility in patients with diabetes: antiresorptive versus anabolic? **Current Opinion in Endocrinology, Diabetes and Obesity**, vol. 28, no. 4, p. 377–382, 2021. .

SILVA-CUTINI, Mirian A; ALMEIDA, Simone A; NASCIMENTO, Andrews M; ABREU, Gláucia R; BISSOLI, Nazaré S; LENZ, Dominik; ENDRINGER, Denise C; BRASIL, Girlandia A; LIMA, Ewelyne M; BIANCARDI, Vinicia C; ANDRADE, Tadeu U. ScienceDirect Long-term treatment with kefir probiotics ameliorates cardiac function in spontaneously hypertensive rats. **The Journal of Nutritional Biochemistry**, vol. 66, p. 79–85, 2019. DOI 10.1016/j.jnutbio.2019.01.006. Available at: https://doi.org/10.1016/j.jnutbio.2019.01.006.

SOPHER, Aviva B.; FENNOY, Ilene; OBERFIELD, Sharon E. An update on childhood bone health: Mineral accrual, assessment and treatment. **Current Opinion in Endocrinology, Diabetes and Obesity**, vol. 22, no. 1, p. 35–40, 2015. https://doi.org/10.1097/MED.0000000000000124.

SOUSA FERREIRA, Vanessa Costa; LOPES, Amanda Pimentel; ALVES, Nicholas Militão; SOUSA, Fatima Regina Nunes; PEREIRA, Karuza Maria Alves; GONDIM, Delane Viana; GIRÃO, Vírginia Claúdia Carneiro; LEITÃO, Renata Ferreira Carvalho; GOES, Paula. Bisphosphonate-related osteonecrosis induced change in alveolar bone architecture in rats with participation of Wnt signaling. **Clinical Oral Investigations**, vol. 25, no. 2, p. 673–682, 2021. https://doi.org/10.1007/s00784-020-03551-7.

SOUZA, Mady Crusoé de; ASSEMANY, Faez Sobral; LIMA, Ana Tereza Cerqueira; SOUZA, Rodrigo Fernandes. Glicocorticoides e osteoporose – artigo de revisão. **Revista de Ciências Médicas e Biológicas**, vol. 9, no. 1, p. 57, 2010. https://doi.org/10.9771/cmbio.v9i1.4734.

STAA, T P Van; LAAN, R F; BARTON, I P; COHEN, S; REID, D M; COOPER, C.

Bone Density Threshold and Other Predictors of Vertebral Fracture in Patients Receiving Oral Glucocorticoid Therapy. **ARTHRITIS & RHEUMATISM**, vol. 48, no. 11, p. 3224–3229, 2003. https://doi.org/10.1002/art.11283.

SZULC, Pawel; BECK, Tom J; MARCHAND, François; DELMAS, Pierre D. Low Skeletal Muscle Mass Is Associated With Poor Structural Parameters of Bone and Impaired Balance in Elderly Men—The MINOS Study. **Journal of Bone and Mineral Research**, vol. 20, no. 5, 2005. https://doi.org/10.1359/JBMR.041230.

TU, Min-yu; HAN, Kuei-yang; CHANG, Gary Ro-lin; LAI, Guan-da. Bone Loss and Modulate the Structure of the Gut. , p. 1–16, 2020. .

TU, Min Yu; CHEN, Hsiao Ling; TUNG, Yu Tang; KAO, Chao Chih; HU, Fu Chang; CHEN, Chuan Mu. Short-Term Effects of Kefir-Fermented Milk Consumption on Bone Mineral Density and Bone Metabolism in a Randomized Clinical Trial of Osteoporotic Patients. **PLoS ONE**, vol. 10, no. 12, p. 1–17, 2015. https://doi.org/10.1371/journal.pone.0144231.

VAN STAA, Tjeerd Pieter; BISHOP, Nick; LEUFKENS, Hubert G.M.; COOPER, Cyrus. Are inhaled corticosteroids associated with an increased risk of fracture in children? **Osteoporosis International**, vol. 15, no. 10, p. 785–791, 2004. https://doi.org/10.1007/s00198-004-1606-5.

VASCONCELOS, Lorena; MACEDO, Luane; SOUSA, De; ALLANE, Thays; MAIA, Cordeiro; GOES, Paula; MARIA, Karuza; PEREIRA, Alves; NOGUEIRA, Jonas; MACIEL, Ferreira; MIYAJIMA, Fabio; VIANA, Delane. Biomedicine & Pharmacotherapy Milk Kefir therapy reduces inflammation and alveolar bone loss on periodontitis in rats. vol. 139, 2021. https://doi.org/10.1016/j.biopha.2021.111677.

VIANA, Joana U.; SILVA, Silvia L.A.; TORRES, Juliana L.; DIAS, João M.D.; PEREIRA, Leani S.M.; DIAS, Rosângela C. Influence of sarcopenia and functionality indicators on the frailty profile of community-dwelling elderly subjects: A cross-sectional study. **Brazilian Journal of Physical Therapy**, vol. 17, no. 4, p. 373–381, 2013. https://doi.org/10.1590/S1413-35552013005000102.

VOLPON, José B.; CECIM, Paulo Eugênio Santos; MIYASE, Cátia Isumi; GAVA, Nelson Fabrício. O alendronato de sódio na prevenção da osteopenia secundária à imobilização gessada, em ratas: avaliação histomorfométrica. **Revista Brasileira de Ortopedia**, vol. 43, no. 10, p. 442–451, 2008. https://doi.org/10.1590/s0102-36162008001000004.

WALSH, L. J.; WONG, C. A.; OBORNE, J.; COOPER, S.; LEWIS, S. A.; PRINGLE, M.; HUBBARD, R.; TATTERSFIELD, A. E. Adverse effects of oral corticosteroids in relation to dose in patients with lung disease. **Thorax**, vol. 56, no. 4, p. 279–284, 2001. https://doi.org/10.1136/thorax.56.4.279.

WANG, Tiantian; YU, Xijie; HE, Chengqi. Pro-inflammatory Cytokines: Cellular and Molecular Drug Targets for Glucocorticoid-induced-osteoporosis. **CURRENT DRUG TARGETS**, vol. 20, p. 1–15, 2019. https://doi.org/10.2174/1389450119666180405094046.

WEI YAO; CHENG, Zhiqiang; BUSSE, Cheryl; PHAM, Aaron; NAKAMURA, Mary C.;

LANE, Nancy E. Glucocorticoid-induced bone loss can be reversed by the actions of PTH and Risedronate on different pathways for bone formation and mineralization. **Arthritis Rheum**, vol. 58, no. 11, p. 3485–3497, 2008. DOI 10.1002/art.23454.Glucocorticoid. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3624763/pdf/nihms412728.pdf.

WEINSTEIN, Robert S. Glucocorticoid-Induced Osteoporosis and Osteonecrosis. **Endocrinology and Metabolism Clinics of North America**, vol. 41, no. 3, p. 595–611, 2012. https://doi.org/10.1016/j.ecl.2012.04.004.

YAO, Wei; DAI, Weiwei; JIANG, Jean X.; LANE, Nancy E. Glucocorticoids and Osteocyte Autophagy. **Bone**, vol. 54, no. 2, p. 279–284, 2013. DOI 10.1016/j.bone.2013.01.034.Glucocorticoids. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3624763/pdf/nihms412728.pdf.

YOON, V.; MAALOUF, N. M.; SAKHAEE, K. The effects of smoking on bone metabolism. **Osteoporosis International**, vol. 23, no. 8, p. 2081–2092, 2012. https://doi.org/10.1007/s00198-012-1940-y.

YUAN, Yu; CHEN, Xi; ZHANG, Lingli; WU, Juanni; GUO, Jianming; ZOU, Dongchen; CHEN, Binglin; SUN, Zhongguang; SHEN, Chao; ZOU, Jun. The roles of exercise in bone remodeling and in prevention and treatment of osteoporosis. **Progress in Biophysics and Molecular Biology**, vol. 122, no. 2, p. 122–130, 2016. DOI 10.1016/j.pbiomolbio.2015.11.005. Available at: http://dx.doi.org/10.1016/j.pbiomolbio.2015.11.005.

ZHANG, Jin; VALVERDE, Paloma; ZHU, Xiaofang; MURRAY, Dana; WU, Yuwei; YU, Liming; JIANG, Hua; DARD, Michel M.; HUANG, Jin; XU, Zhiwei; TU, Qisheng; CHEN, Jake. Exercise-induced irisin in bone and systemic irisin administration reveal new regulatory mechanisms of bone metabolism. **Bone Research**, vol. 5, no. November 2016, 2017. https://doi.org/10.1038/boneres.2016.56.

ZHAO, Xiong; WU, Zi Xiang; ZHANG, Yang; YAN, Ya Bo; HE, Qiang; CAO, Peng Chong; LEI, Wei. Anti-osteoporosis activity of Cibotium barometz extract on ovariectomy-induced bone loss in rats. **Journal of Ethnopharmacology**, vol. 137, no. 3, p. 1083–1088, 2011. DOI 10.1016/j.jep.2011.07.017. Available at: http://dx.doi.org/10.1016/j.jep.2011.07.017.

ZHOU, MANRU; WU, JINGKAI; YU, YONGJIE; YANG, YAJUN; LI, JIN; CUI, LIAO; YAO, WEIMIN; LIU, YUYU. Polygonum multiflorm alleviates glucocorticoid - induced osteoporosis and Wnt signaling pathway. vol. 17, p. 970–978, 2018. https://doi.org/10.3892/mmr.2017.7997.

ZIEGLER, Reinhard; KASPERK, Christian. Glucocorticoid-induced osteoporosis : Prevention and treatment. no. 98, p. 344–348, 1998.

ANEXO

Anexo A - Certificado de aprovação na Comissão de Ética no Uso de Animais (CEUA) da Universidade Federal do Ceara (UFC)

UNIVERSIDADE FEDERAL DO CEARÁ

Comissão de Ética no Uso de Animais – CEUA
Rua: Coronel Nunes de Melo, 1127 – Rodolfo Teófilo
Cep: 60430970 Fortaleza – CE

CERTIFICADO

Certificamos que o projeto intitulado **"Efeito do kefir no metabolismo ósseo de ratos com osteoporose induzida por glicocorticoide e submetidos ao exercício resistido"**, protocolo 137/17, sob responsabilidade da Profa. Dra. Paula Goes Pinheiro Dutra, que envolve a produção, manutenção e/ou utilização, pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica, encontra-se de acordo com os preceitos da Lei nº 11.794, de nº 8 de outubro de 2008, do Decreto 6899 de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA) e foi aprovado pela Comissão de Ética no Uso de Animais (CEUA – UFC) da Universidade Federal do Ceará, em reunião em 26 de abril de 2018.

Vigência do projeto	01/01/2018 – 01/01/2021
Espécie/Linhagem	Rato *Wistar*
Nº de Animais	60
Peso	200 – 250 g
Sexo	Macho
Origem	Biotério Central da UFC

Fortaleza, 08 de maio de 2018.

Alexandre Havt Bindá

Prof. Dr. Alexandre Havt Bindá
Coordenador da CEUA - UFC

UNIVERSIDADE FEDERAL DO CEARÁ
ALEXANDRE HAVT BINDÁ
COORDENADOR DA COMISSÃO DE ÉTICA E DO USO COM ANIMAIS - CEUA/UFC - MATRÍCULA SIAPE: 1666982