PONTIFÍCIA UNIVERSIDADE CATÓLICA DE MINAS GERAIS
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O IMPACTO DOS INIBIDORES SELETIVOS DA RECAPTAÇÃO DE SEROTONINA E DO METILFENIDATO NA SAÚDE ÓSSEA: revisões sistemáticas da literatura

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Orientador: Prof. Dr. Rodrigo Villamarim Soares
Coorientador: Prof. Dr. Ildeu Andrade Júnior

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3- Prof. Dr. Murilo Fernando Neuppmann Feres – USP
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Prof. Dr. Rodrigo Villamarim Soares
Coordenador do Programa de Pós-graduação em Odontologia

À minha família,

por todo o amor, incentivo e exemplo.

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“Que os vossos esforços desafiem as impossibilidades, lembrai-vos de que as grandes coisas do homem foram conquistadas do que parecia impossível.”

(CHARLES CHAPLIN)

RESUMO

O aumento global no consumo de medicamentos, especialmente aqueles destinados ao tratamento de transtornos de saúde mental, motiva a investigação dos potenciais impactos dessas substâncias nas complexas vias metabólicas do tecido ósseo. Inibidores Seletivos da Recaptação de Serotonina (ISRS) e Metilfenidato (MF) são psicofármacos comumente utilizados para tratar depressão e Transtorno do Déficit de Atenção e Hiperatividade (TDAH), respectivamente. A literatura indica que tais substâncias também podem influenciar de maneira significativa a saúde óssea. Entretanto, a heterogeneidade metodológica, divergências nos resultados e variáveis de confusão presentes nos estudos clínicos dificultam a interpretação e comparação desses dados, assim como o maior entendimento de suas implicações clínicas. Com o objetivo de avaliar sistematicamente esses efeitos com base em evidências, foram conduzidas duas revisões sistemáticas da literatura em modelos animais, com foco na associação entre ISRS e MF com a saúde óssea, bem como em seus mecanismos biológicos subjacentes. As buscas bibliográficas foram realizadas nas bases de dados MEDLINE, Embase, Scopus e CINAHL, além de literatura cinzenta, até dezembro de 2023, sem restrições quanto a idioma ou data de publicação. Estratégias de busca específicas foram empregadas para cada tema. O processo de triagem de títulos e resumos seguiu critérios de elegibilidade predefinidos, com estudos selecionados submetidos à leitura em texto completo, seguida pela extração de dados e avaliação de risco de viés através da ferramenta SYRCLE. Cada etapa foi realizada por dois avaliadores independentes, com divergências resolvidas por um terceiro avaliador. Oito estudos sobre ISRS e cinco sobre MF atenderam aos critérios de elegibilidade, e seus resultados foram analisados e sintetizados qualitativamente. Os achados principais indicam predominantemente efeitos deletérios nos tecidos ósseos associados tanto aos ISRS quanto ao MF. Especificamente, os ISRS (escitalopram e fluoxetina) impactam negativamente o tecido ósseo de roedores de maneira dose-dependente e diretamente relacionada ao tempo de tratamento. Alterações foram constatadas principalmente na microarquitetura óssea, propriedades biomecânicas e no comportamento das células ósseas, com osteoblastos mostrando maior susceptibilidade do que osteoclastos. O MF demonstra uma propensão a exercer efeitos deletérios em vários parâmetros ósseos, especialmente em ossos longos, com uma correlação positiva observada com variações de dose e duração do tratamento.

Adicionalmente, o tratamento com MF tem o potencial de afetar diretamente o tecido ósseo por meio de ações em células ósseas específicas e também indiretamente por meio de efeitos correlacionados com o peso corporal. Novos estudos em ambas as classes medicamentosas devem abranger uma análise das relações tempo-dose, particularmente levando em consideração padrões hormonais e possíveis diferenças entre os sexos.

Palavras-chave: Inibidores seletivos de recaptação de serotonina. Metilfenidato. Efeitos fisiológicos de drogas. Remodelação óssea. Revisão sistemática.

ABSTRACT

The global increase in medication consumption, especially those intended for the treatment of mental health disorders, motivates the investigation of potential impacts of these substances on the intricate metabolic pathways of bone tissue. Selective Serotonin Reuptake Inhibitors (SSRIs) and Methylphenidate (MP) are commonly used psychopharmacological agents to treat depression and Attention Deficit Hyperactivity Disorder (ADHD), respectively. Literature indicates that such substances can significantly influence bone health. However, methodological heterogeneity, discrepancies in results, and confounding variables in clinical studies complicate the interpretation and comparison of these data, as well as the definition of their clinical implications. With the aim of systematically evaluating these effects based on evidence, two systematic literature reviews were conducted in animal models, focusing on the association between SSRIs and MP with bone health, as well as their underlying biological mechanisms. Literature searches were performed in the MEDLINE, Embase, Scopus, and CINAHL databases, along with grey literature, until December 2023, without restrictions regarding language or publication date. Specific search strategies were employed for each topic. The title and abstract screening process followed predefined eligibility criteria, with selected studies undergoing full-text reading, followed by data extraction and bias assessment using the SYRCLE tool. Each step was carried out by two independent evaluators, with discrepancies resolved by a third evaluator. Eight studies on SSRIs and five on MP met the eligibility criteria, and their results were qualitatively analyzed and synthesized. The main findings predominantly indicate deleterious effects on bone tissues associated with both SSRIs and MP. Specifically, SSRIs (escitalopram and fluoxetine) negatively impact rodent bone tissue in a time- and dose-dependent manner. Changes were mainly observed in bone microarchitecture, biomechanical properties, and the behavior of bone cells, with osteoblasts exhibiting greater susceptibility than osteoclasts. MP demonstrates a propensity to exert deleterious effects on various bone parameters, especially in long bones, with a positive correlation observed with dosage and treatment duration variations. Additionally, MP treatment has the potential to directly affect bone tissue through actions on specific bone cells and indirectly through effects correlated with body weight. Further studies in both drug classes should encompass an analysis of

time-dose relationships, particularly considering hormonal patterns and potential gender differences.

Keywords: Selective serotonin reuptake inhibitors. Methylphenidate. Physiological effects of drugs. Bone remodeling. Systematic review.

LISTA DE TABELAS

LISTA DE ABREVIATURAS E SIGLAS

5-HT	5-hidroxitriptamina (serotonina)
5-HTT	Transportador de 5-hidroxitriptamina
ADHD	Attention Deficit Hyperactivity Disorder
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CMO	Conteúdo mineral ósseo
CREB	Proteína ligadora do elemento responsivo ao AMP cíclico
DMO	Densidade mineral óssea
ISRS	Inibidores seletivos da receptação de serotonina
LRP5	Receptor de lipoproteína de baixa densidade 5
MF	Metilfenidato
MP	Methylphenidate
MUSC	Medical University of South Carolina
PDE	Fosfodiesterase
PDSE	Programa de Doutorado Sanduíche no Exterior
PKA	Fosfoquinase A
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PROSPERO	International Prospective Register of Systematic Reviews
PUC Minas	Pontifícia Universidade Católica de Minas Gerais
RS	Revisões sistemáticas da literatura
SNC	Sistema nervoso central
SSRIs	Selective Serotonin Reuptake Inhibitors
SYRCLE	SYstematic Review Centre for Laboratory animal Experimentation
TCAs	Antidepressivos tricíclicos
TCAs	Antidepressivos tricíclicos
TD	Transportadores de dopamina
TDAH	Transtorno de déficit de atenção e hiperatividade
TN	Transportador de noradrenalina
TRAP	Fosfatase ácida resistente ao tartarato
TSH	Hormônio estimulante da tireoide
UFMG	Universidade Federal de Minas Gerais

SUMÁRIO

1 INTRODUÇÃO

O uso de medicamentos prescritos tem aumentado significativamente em todo o mundo, particularmente devido a uma demanda crescente por tratamentos direcionados a saúde mental, doenças crônicas e relacionadas ao envelhecimento (Makrygiannakis; Kaklamanos; Athanasiou, 2018; OECD/EU, 2014). Além disso, essa tendência é afetada por outros fatores, tais como a cobertura expandida por meio de planos de saúde e publicidade direta ao consumidor, o que por vezes pode levar ao uso *off label* de várias classes medicamentosas (Mulcahy; Eibner; Finegold, 2016; OECD/EU, 2014; Wilkes; Bell; Kravitz, 2000). A possível influência de diferentes substâncias farmacêuticas na homeostase tecidual vem sendo amplamente estudada e várias alterações nas vias metabólicas que interferem na remodelação óssea vem sendo observadas (Bartzela *et al.*, 2009; Diravidamani; Sivalingam; Agarwal, 2012).

A remodelação óssea é um processo complexo e dinâmico que desempenha um papel vital na manutenção da integridade estrutural e funcional do sistema esquelético, fornecendo suporte mecânico para estatura e locomoção e protegendo órgãos vitais do indivíduo (Kim *et al.*, 2020a). O equilíbrio entre a formação e a reabsorção óssea é necessário para preservar funções biológicas vitais, evitando o acúmulo de danos ósseos, mantendo sua resistência mecânica e homeostase do cálcio (Clarke, 2008). Esse processo é resultado de uma complexa interação entre moléculas de sinalização que atuam tanto em células osteoblásticas, precursoras da formação óssea, quanto em células osteoclásticas, que reabsorvem tecido ósseo (Meikle, 2006; Proff; Römer, 2009). Esses eventos podem ser modulados por fatores genéticos, ambientais, nutricionais e pela administração de diversos tipos de medicamentos. As vias de ação dos fármacos podem contribuir para o aumento da produção local de fatores reguladores do metabolismo ósseo, estimulando sua síntese e/ou atuando como análogos sintéticos, ou, ao contrário, neutralizando o efeito desses fatores reguladores, inibindo seletivamente sua síntese ou bloqueando seus domínios ativos (Bartzela *et al.*, 2009; Makrygiannakis; Kaklamanos; Athanasiou, 2019).

Dentre as doenças crônicas mais presentes atualmente, a depressão tem sido considerada como um grande mal da humanidade, afetando cerca de 350 milhões de indivíduos (Smith, 2014). Estima-se que essa seja a segunda principal causa de incapacidade no mundo e é previsto que seja a primeira em 2030 (Ferrari *et al.*, 2013). As proporções aumentadas de distúrbios psicológicos multiplicaram a prescrição de

fármacos destinados ao manejo desta condição em todas as faixas etárias, e estudos prévios relatam potenciais variáveis de alteração das propriedades ósseas, dependendo do mecanismo de ação dessas substâncias (Bonnet *et al.*, 2007; Mercurio *et al.*, 2022). Os medicamentos inibidores seletivos da recaptação de serotonina (ISRS), por exemplo, foram considerados causadores de efeitos deletérios no osso, relacionados a redução da massa óssea, arquitetura alterada e propriedades mecânicas inferiores (Mercurio *et al.*, 2022; Rapp; Pfeiffer, 2019; Zhou *et al.*, 2018). Em contrapartida, efeitos esqueléticos mínimos foram observados com a administração de antidepressivos tricíclicos (TCAs) (Warden *et al.*, 2010). Já os inibidores da fosfodiesterase (PDE) demonstraram efeito benéfico na microarquitetura óssea trabecular, associado ao aumento dos níveis séricos de osteocalcina (Bonnet *et al.*, 2007).

Em contrapartida, o transtorno do déficit de atenção/hiperatividade (TDAH) é uma condição do neurodesenvolvimento caracterizada por um padrão persistente de desatenção, hiperatividade e impulsividade que resulta em vários graus de comprometimento funcional (Polanczyk *et al.*, 2014). Comumente diagnosticado em crianças, o TDAH afeta 1 em cada 11 americanos, com aproximadamente o dobro da prevalência nos homens em comparação com mulheres (Ramtekkar *et al.*, 2010). O metilfenidato (MF), medicamento estimulante do sistema nervoso central (SNC), é um dos medicamentos mais prescritos para o TDAH, com mais de 10 milhões de prescrições por ano e sua eficácia clínica corresponde a 65-75% (Castells *et al.*, 2011; Uddin *et al.*, 2018). O MF é geralmente bem tolerado e tem uma boa adesão pelos pacientes, mas foi relatado em alguns estudos que pode afetar adversamente o crescimento esquelético (MTA Cooperative Group, 2004; Swanson *et al.*, 2006). Além disso, os efeitos esqueléticos do MF não se limitam apenas ao crescimento, mas também demonstraram alterar a qualidade e a quantidade ósseas (Burns *et al.*, 2024; Komatsu *et al.*, 2012; Uddin *et al.*, 2018).

Embora as vias metabólicas de ação dos medicamentos antidepressivos e estimulantes do SNC e suas possíveis interações com a fisiologia óssea tenham sido amplamente estudadas, seus efeitos biológicos ainda permanecem inconclusivos. A considerável variedade de desenhos experimentais, tamanho e caracterização de amostras, doses e vias de administração dos medicamentos e possíveis fatores de confusão atrelados muitas vezes produz resultados divergentes entre os estudos. Nesse sentido, revisões sistemáticas da literatura (RS) localizam, avaliam, comparam

e sintetizam a evidência dos estudos científicos para então prover um direcionamento a essas questões, ao incluir um extenso resumo da evidência disponível (Page *et al.*, 2021).

Portanto, o presente estudo visa contribuir na redução de uma lacuna substancial na literatura ao analisar criticamente e sintetizar as evidências disponíveis acerca dos efeitos dos ISRS e do MF na saúde óssea, particularmente em modelos animais. A escolha específica desses fármacos para este estudo se justifica pela utilização amplamente difundida destes em todos os grupos etários, sendo ambos a primeira linha de escolha, dentro da classe de antidepressivos e psicoestimulantes, respectivamente. A compreensão aprofundada dessas interações poderá subsidiar uma avaliação mais abrangente dos riscos e benefícios associados ao uso dessas substâncias, fornecendo uma base sólida para futuros estudos, visando o esclarecimento de suas possíveis correlações com outros fatores, incluindo os efeitos intrínsecos de patologias associadas e possíveis interações medicamentosas.

2 REFERENCIAL TEÓRICO

2.1 Inter-relação entre Inibidores Seletivos da Recaptação de Serotonina e a saúde óssea

Os fármacos psicotrópicos agem dentro do SNC para alterar a função cerebral, resultando em mudanças temporárias na percepção, humor, consciência e comportamento (Warden *et al.*, 2010). A serotonina (5-hidroxitriptamina, 5-HT) é um neurotransmissor implicado na etiologia de muitas doenças mentais e seu transportador (5-HTT) é o alvo da principal classe de antidepressivos, os ISRS (Vaswani; Linda; Ramesh, 2003).

Os ISRS constituem o grupo mais utilizado de antidepressivos, tanto em crianças quanto em adolescentes e adultos, representando mais de 60% de todos os antidepressivos prescritos em todo o mundo (Bonnet *et al.*, 2007; Pirraglia; Stafford; Singer, 2003). Embora tradicionalmente associados a transtornos depressivos, os ISRS também são prescritos para diversas outras condições psiquiátricas, incluindo transtorno de estresse pós-traumático, transtorno de ansiedade generalizada, transtorno do pânico, transtorno disfórico pré-menstrual, e algumas condições não psiquiátricas, como dor crônica, fibromialgia e sintomas vasomotores pós-menopausa (ondas de calor e suores noturnos) (Haney; Warden; Bliziotes, 2010). Eles agem inibindo o transportador de 5-HT para bloquear sua recaptação e prolongar sua atividade extracelular, aumentando assim a sua disponibilidade pré-sináptica (Vaswani; Linda; Ramesh, 2003). Curiosamente, os receptores de 5-HT foram identificados em osteoblastos, osteoclastos e osteócitos (Hodge *et al.*, 2013), e por isso os medicamentos que antagonizam a recaptação da 5-HT podem influenciar a saúde óssea (Fernandes *et al.*, 2016).

A natureza bioquímica específica das vias serotoninérgicas e seus efeitos diretos e/ou indiretos no metabolismo ósseo ainda permanecem pouco claros. Estudos laboratoriais levantaram diversas hipóteses nesse tópico. Foi demonstrado que 95% da serotonina é sintetizada perifericamente, no intestino (Crowell, 2004). Essa serotonina circulante, ao se ligar ao receptor Htr1b expresso na superfície do osteoblasto, inibe a fosforilação da proteína ligadora do elemento responsivo ao AMP cíclico (CREB) pela fosfoquinase A (PKA), levando à diminuição da proliferação do osteoblasto e perda óssea (Ducy; Karsenty, 2010; Oury *et al.*, 2010).

Um papel muito importante nessa via é mediado pela sinalização Wnt β-catenina. Wnt é fundamentalmente uma glicoproteína que inibe a proteína relacionada ao receptor de lipoproteína de baixa densidade 5 (LRP5), inibindo assim a síntese de serotonina intestinal (Westendorf; Kahler; Schroeder, 2004). A sinalização Wnt β-catenina demonstrou ter um papel importante no controle da diferenciação, proliferação, sobrevivência do osteoblasto e formação óssea, regulando a transcrição de genes alvo de osteoprotegerina (Westendorf; Kahler; Schroeder, 2004). Portanto, a serotonina derivada do intestino, em associação com a sinalização Wnt, modula funcionalmente a proliferação do osteoblasto.

Por outro lado, a serotonina derivada do cérebro sinaliza para os neurônios ventromediais hipotalâmicos via receptores Htr2c, reduzindo a atividade simpática e esse menor fluxo simpático é transmitido aos receptores β2 adrenérgicos no osteoblasto, o que aumenta a formação óssea e reduz a reabsorção óssea por meio da via dependente do relógio molecular/ciclina D e da via dependente da PKA/fator de transcrição ativador 4 (Ducy; Karsenty, 2010). Adicionalmente, um novo mecanismo sugerido por Ortuño *et al.* (2016) apontaram que ISRS poderiam atuar na ativação dependente de calmodulina de Ca2+ dos osteoclastos, desencadeando a cascata c-Fos/NFATc1, resultando na diminuição da reabsorção óssea. Ao mesmo tempo, os ISRS agem na recaptação da serotonina derivada do cérebro, o que por sua vez resulta na dessensibilização do Htr2c, levando ao aumento na atividade simpática, o que, por sua vez, intensifica a reabsorção óssea e diminui o acúmulo ósseo (Ortuño *et al.*, 2016). Considerando a diversidade das hipóteses levantadas, mais esforços são necessários para determinar essas vias biológicas e os efeitos diferenciais da serotonina derivada do intestino e do cérebro na fisiopatologia óssea, bem como dos ISRS em suas ações em diferentes dosagens, períodos de tratamento, vias de administração e subtipos de drogas, em diferentes locais do esqueleto.

Além das investigações em animais, estudos clínicos têm destacado a relação entre o uso de ISRS e a saúde óssea. Primeiramente, evidenciou-se que a administração crônica de ISRS para o tratamento de condições psiquiátricas em humanos está associada à redução da densidade mineral óssea (DMO) e, consequentemente à osteoporose (Rizzoli *et al.*, 2012; Zhou *et al.*, 2018). Adicionalmente, uma revisão sistemática e meta-análise avaliou a associação entre o uso de ISRS e o risco de fraturas em estudos de caso-controle e coorte, indicando que os usuários de ISRS podem apresentar um aumento no risco de fraturas em

comparação com não usuários de ISRS (Kumar *et al.*, 2020). Portanto, a saúde óssea deve ser considerada ao prescrever essa classe de medicamentos, especialmente para aqueles com fatores de risco preexistentes.

Ao contrário dos estudos em animais, a pesquisa em humanos apresenta muitos fatores de confusão que precisam ser abordados. A depressão em si é um dos principais fatores que causam a perda de massa óssea em quase todas as faixas etárias em diferentes populações, mas observou-se que tanto a depressão quanto os ISRS agem de forma independente nos ossos por mecanismos diferentes, acelerando assim a perda óssea em pacientes depressivos que fazem uso de ISRS (Gebara *et al.*, 2014; Haney; Warden; Bliziotes, 2010; Rizzoli *et al.*, 2012). Outros fatores de confusão incluem tabagismo, consumo de álcool, idade, gênero, consumo de produtos lácteos, exposição ao sol, suplementos alimentares, condições de saúde, baixo índice de massa corporal, etnia, comorbidades, medicações concomitantes, e ainda não há estudos suficientes para discutir essas questões relacionadas a esses fatores de confusão (Feuer *et al.*, 2015; Rizzoli *et al.*, 2012; Zemel *et al.*, 2011).

2.2 Inter-relação entre Metilfenidato e a saúde óssea

O TDAH é uma desordem do neurodesenvolvimento caracterizada por desatenção, hiperatividade e impulsividade, causando comprometimento funcional em âmbitos acadêmicos e sociais (Mechler *et al.*, 2022). Sua prevalência é de aproximadamente 5% em crianças e adolescentes e de aproximadamente 2,5% em adultos, sem diferenças significativas entre países em todo o mundo (Faraone *et al.*, 2015; Polanczyk *et al.*, 2007; Polanczyk *et al.,* 2014). As diretrizes clínicas atuais recomendam uma abordagem de tratamento multimodal e multidisciplinar individualizada. Para cada paciente, deve ser estabelecido um quadro de intervenções farmacológicas e/ou não farmacológicas, que leve em conta a idade, a gravidade dos sintomas e as necessidades individuais (Kooij *et al.*, 2019).

O MF, entre outros estimulantes do SNC, é considerado um medicamento de primeira linha para o tratamento do TDAH em crianças e adultos (Mechler *et al.*, 2022). Seu principal mecanismo de ação ocorre através dos transportadores de dopamina (TD) noradrenalina (TN). Quando o medicamento se conecta aos transportadores correspondentes, esses são inibidos, resultando no aumento dos níveis de dopamina

e norepinefrina na fenda sináptica, gerando assim o seu efeito estimulante clássico no SNC, principalmente no córtex pré-frontal (Shellenberg *et al.*, 2020).

Embora os medicamentos psicoestimulantes tenham evidenciado eficácia na melhoria dos sintomas do TDAH, alguns estudos clínicos levantam preocupações acerca de seus potenciais efeitos na saúde óssea, principalmente relacionados à DMO e conteúdo mineral ósseo (CMO), bem como a susceptibilidade a fraturas e alterações no crescimento (Burns *et al.*, 2024; Carucci *et al.*, 2021; Lahat *et al.*, 2000; Zhang; Shen; Yan, 2021). Diversos mecanismos têm sido propostos para desempenhar um papel nesse contexto. Foi demonstrado que medicamentos psicoestimulantes podem retardar os picos de hormônio do crescimento em crianças com TDAH em comparação com aquelas sem essa condição (Feuer *et al.*, 2016). Além disso, Kim *et al.* (2020b) observaram que crianças que utilizam MF apresentam níveis mais baixos de hormônio estimulante da tireoide (TSH) em comparação com crianças sem TDAH. Da mesma forma, Holtkamp *et al.* (2002) relataram que MF pode causar um atraso significativo do crescimento, depletando os níveis de fatores de crescimento dependentes do hormônio do crescimento.

Alguns estudos afirmam, ainda, que a atividade intracerebral dos medicamentos psicoestimulantes estimula um efeito anorexígeno, que pode contribuir para múltiplas funções críticas do desenvolvimento esquelético (Bou Khalil *et al.*, 2017; Storebø *et al.*, 2023). Um estudo clínico revelou uma diminuição nas concentrações séricas de leptina após o início do tratamento com MF (Poulton *et al.*, 2012). Essa redução nos níveis de leptina correlacionou-se com uma perda significativa de massa gorda e óssea, indicativa de uma diminuição na formação óssea. A leptina, influenciando a homeostase óssea através da via adrenérgica β-2, pode estar implicada na redução do apetite observada com o uso de MF (Takeda *et al.*, 2002). Especula-se, ainda, que a noradrenalina exerça efeitos inibitórios na formação óssea, enquanto facilita simultaneamente a reabsorção óssea (Ma *et al.*, 2011). Esses efeitos podem ser mediados pelos osteoblastos, que possuem β-2 adrenorreceptores, cuja ativação libera o ativador do receptor nuclear do fator kappa beta (RANKL), promovendo subsequentemente a reabsorção óssea mediada por osteoclastos (Aitken *et al.*, 2009; Elefteriou, 2008; Elefteriou; Campbell; Ma, 2014). A interação do MF nessa via específica precisa ser investigada em maior profundidade.

É relevante observar que, devido às características comportamentais, crianças com TDAH apresentam um aumento no risco de fraturas em comparação com aquelas

sem esse diagnóstico (Chou *et al.*, 2014). Dessa forma, embora o tratamento dessa condição possa proteger contra esses riscos, a relação entre a medicação para o TDAH e o risco de fraturas pode ser complexa. Por um lado, a terapia medicamentosa pode reduzir o risco de fraturas controlando os sintomas do TDAH (Gao *et al.*, 2023). Por outro lado, os efeitos colaterais dos medicamentos podem aumentar o risco de fraturas, seja indiretamente, ao diminuir o apetite, o ganho de peso e aporte de nutrientes, ou diretamente, estimulando a formação de osteoclastos (Komatsu *et al.*, 2012).

Embora muitos estudos tenham mensurado os efeitos do tratamento clínico com MF sobre tecido ósseo, ainda não há um consenso claro quanto à relação entre as alterações observadas e o uso específico desse fármaco, ou se tais mudanças são atribuíveis a outras causas, como a própria condição de TDAH (Carucci *et al.*, 2021). Investigações em modelos animais têm sido conduzidas com o propósito de examinar mais detalhadamente os mecanismos biológicos pelos quais o MF pode influenciar a saúde óssea. Os estudos incorporados nesta revisão sistemática da literatura identificam distintas vias metabólicas que podem estar em operação, demandando investigações clínicas subsequentes para sua completa elucidação.

2.3 Revisões sistemáticas da literatura em modelos animais

As RS são uma prática comum em pesquisas clínicas, especialmente em ensaios clínicos randomizados, mas ainda não são amplamente conduzidas no campo da ciência de animais de laboratório. Apesar do fato de que a maioria dos experimentos com animais são realizados para informar a pesquisa clínica, a partir da construção do conhecimento básico que irá subsidiar futuras avaliações direcionadas ao ser humano, as RS de experimentos com animais ainda são bastante escassas. Menos de 250 revisões sistemáticas de estudos pré-clínicos em animais foram publicadas antes de 2010, em oposição às quase 6.000 Revisões Cochrane de estudos clínicos (Ritskes-Hoitinga *et al.*, 2014).

Dado que muitos estudos que utilizam animais de laboratório visam melhorar a saúde humana e os cuidados de saúde, parece razoável que a investigação que utiliza animais seja revista de forma semelhante e siga padrões de qualidade igualmente elevados (Hooijmans *et al.*, 2014). Alguns cientistas sugeriram que uma avaliação mais rigorosa dos resultados dos estudos em animais, sob a forma de uma revisão

sistemática, deveria ser um pré-requisito antes de iniciar estudos em pacientes (Sandercock; Roberts, 2002).

Em geral, as RS de estudos com animais permitem isolar variáveis específicas, fomentando a melhor compreensão dos dados avaliados, e podem também orientar o desenho de novos experimentos, demonstrando a extensão das evidências atuais no campo e fornecendo informações sobre quais questões precisam ser abordadas. Além disso, essas RS podem contribuir para melhorar a qualidade metodológica dos experimentos e evitar esforços repetidos (Hooijmans *et al.*, 2014). No entanto, não é simples ou adequado traduzir os resultados encontrados em animais de laboratório para pacientes em ensaios clínicos. Em muitos casos, o valor preditivo das experiências com animais é baixo (McGonigle; Ruggeri, 2014). É plausível que as diferenças marcantes nos resultados entre estudos em animais e humanos se devam, em parte, a diferenças biológicas/fisiológicas fundamentais entre humanos e outras espécies. No entanto, outros fatores evitáveis relacionados com a concepção, condução e relato de experimentos pré-clínicos com animais podem desempenhar um papel igualmente importante. Evitar a má qualidade metodológica, diferenças na concepção entre estudos experimentais em animais e ensaios clínicos, e viés de publicação podem melhorar significativamente a validade interna e externa e, portanto, o valor preditivo dos estudos com animais (Hooijmans; Ritskes-Hoitinga, 2013; Van Der Worp *et al.*, 2010).

3 OBJETIVOS

3.1 Objetivo geral

O objetivo desse trabalho é fazer um levantamento investigativo, sumarizar informações e avaliar a qualidade das evidências científicas disponíveis sobre a influência de ISRS e do MF na saúde óssea, por meio de revisões sistemáticas da literatura.

3.2 Objetivos específicos

a) analisar os possíveis mecanismos celulares e moleculares pelos quais os ISRS e o MF podem afetar a saúde óssea em animais;
b) comparar e sintetizar as evidências apresentadas por estudos experimentais em animais sobre o impacto dos ISRS e MF na saúde óssea;
c) avaliar a qualidade metodológica dos estudos incluídos por meio de ferramentas de avaliação específicas;
d) explorar potenciais fatores de confusão ou viés nas evidências disponíveis;
e) identificar lacunas de conhecimento e propor possíveis direções para futuras pesquisas nessa área.

4 MATERIAL E MÉTODOS

4.1 Protocolo e registro

Na elaboração deste estudo, foram adotadas as orientações e diretrizes determinadas pelo Prefered Reporting Itens for Systematic Reviews and Meta-Analyses: The PRISMA Statement (Moher *et al.*, 2009) e Cochrane Handbook for Systematic Reviews of Interventions (versão 5.3) – disponíveis no aplicativo Review Manager 5.3.

Um protocolo para cada uma das revisões sistemáticas foi submetido na base International Prospective Register of Systematic Reviews (PROSPERO), para obtenção do registro das pesquisas (CRD42023468466; CRD42021278529), a fim de agregar valor aos trabalhos realizados e evitar a realização de estudos similares (ANEXO A).

4.2 Formulação da pergunta

Para auxiliar na elaboração das perguntas de fundamentação dos estudos, foram definidos para cada um dos temas o acrônimo PECO: (P: population/população; E: exposure/exposição; C: control/controle; O: outcome/desfecho), conforme a estrutura descrita na Tabela 1.

Tabela 1. Pergunta estruturada para elaboração de Revisão Sistemática em animais

PECO	ISRS x saúde óssea	Metilfenidato x saúde óssea
P - População	Animais de todas as espécies, machos ou fêmeas.	Animais de todas as espécies, machos ou fêmeas.
E - Exposição	Administração de medicamentos antidepressivos ISRS.	Administração do medicamento estimulante do SNC Metilfenidato.
C – Controle	Grupo controle (placebo ou não fazer nada) ou sham.	Grupo controle (placebo ou não fazer nada) ou sham.

PECO	ISRS x saúde óssea	Metilfenidato x saúde óssea
O – Outcome – Desfecho	Alteração das características ósseas ou de suas vias fisiológicas.	Alteração das características ósseas ou de suas vias fisiológicas.
Pergunta estruturada	A utilização de medicamentos ISRS afeta a saúde óssea em animais?	A utilização do medicamento Metilfenidato afeta a saúde óssea em animais?

Fonte: Elaborado pela autora

4.3 Critérios de elegibilidade

Foram incluídos estudos que apresentaram as seguintes características:

a) Estudos experimentais em animais que incluam pelo menos um grupo experimental e um grupo controle ou sham;
b) Administração sistêmica ou local das classes de medicamentos determinadas para cada estudo (ISRS ou MF), que possam interferir nos processos fisiológicos do osso, ou que possam ter efeitos colaterais relacionados à fisiologia e remodelação óssea, além de alterações nas propriedades biomecânicas;
c) Estudos que relataram dados de fenótipo ósseo, concentração de marcadores de remodelação óssea, morfologia óssea ou propriedades mecânicas do osso entre usuários e controles dessas substâncias;
d) Descrições adequadas de dosagens, administração e regimes terapêuticos;

Foram excluídos os estudos que apresentaram as seguintes características:

a) Outros desenhos de estudo que não correlacionem os medicamentos avaliados (ISRS ou MF) a remodelação/fisiologia óssea. Estudos em humanos, revisões narrativas, artigos de opinião, e estudos in vitro foram excluídos;

b) Estudos em que o animal avaliado não recebeu a medicação diretamente (ex: quando animais gestantes foram tratados e apenas os filhotes foram avaliados);
c) Estudos com resultados parciais ou estudos pilotos.

4.4 Fontes de busca

Para identificar os estudos publicados, foi realizada uma pesquisa eletrônica computadorizada nas seguintes bases de dados: MEDLINE via PubMed (http://www.ncbi.nlm.nih.gov/PubMed), EMBASE (https://www.embase.com/search), Scopus (https://www.scopus.com/search), CINAHL (https://www.ebsco.com/products/research-databases/cinahl-database) e Google Scholar; sem restrição de idioma e sem limite de data. Além disso, a busca por estudos na literatura cinzenta foi estendida ao banco dados opengrey.eu – Grey Literature Database.

4.5 Estratégias de busca

Foram desenvolvidas estratégias de busca específicas para cada um dos tópicos abordados, utilizando-se as palavras chaves, termos indexados, expressões e as suas possíveis combinações obtidas através de um mapeamento conceitual, a fim de englobar o maior número de trabalhos relacionados com os objetivos propostos para este estudo. Após a obtenção da estratégia de busca principal para a base de dados Medline/Pubmed, a mesma foi adaptada, com termos equivalentes, para as outras bases de dados pesquisadas. As estratégias de busca das duas revisões sistemáticas da literatura propostas nesta tese podem ser encontradas nas tabelas suplementares dos artigos científicos.

4.6 Seleção dos estudos

Os estudos encontrados pelas ferramentas eletrônicas de busca foram reunidos em uma única base (Endnote) para tabulação e organização dos estudos recuperados. Em seguida, os arquivos foram exportados para o Covidence Systematic Review Software (Veritas Health Innovation, Melbourne, Australia) onde os títulos e resumos foram lidos de forma independente por dois revisores (NF e MF), e os artigos

que apresentarem características compatíveis aos critérios de elegibilidade desta pesquisa foram selecionados para a leitura do texto na íntegra. Da mesma forma, os estudos em texto completo foram criteriosamente avaliados e selecionados para a inclusão definitiva nas revisões sistemáticas. Nessa etapa, os motivos de exclusão foram identificados individualmente. Desacordos em quaisquer das etapas anteriores foram resolvidos por discussões ou avaliação de um terceiro pesquisador (IA).

4.7 Extração dos dados

Os dados de interesse foram extraídos dos estudos incluídos pelos revisores (NF e IA) de maneira independente, através de um formulário padronizado de extração de dados, que foi elaborado de acordo com os critérios de elegibilidade. Os dados incluem informações sobre características do estudo, população, intervenções, desfechos e resultados finais. Quaisquer desfechos relacionados a expressão de mediadores químicos relacionados ao metabolismo ósseo, quantidade e atividade das células ósseas, parâmetros de microarquitetura, morfológicos, ou propriedades biomecânicas foram coletados.

4.8 Avaliação da qualidade da evidência científica

A qualidade dos estudos é determinada pela capacidade em minimizar vieses e maximizar a validade interna e externa. Portanto, os estudos selecionados foram avaliados de acordo com o desenho do estudo, a condução e o relato dos dados. O método de acesso à qualidade metodológica varia de acordo com os tipos de estudos avaliados.

Como os estudos de intervenção em animais diferem dos ensaios clínicos randomizados em muitos aspectos, a metodologia para revisões sistemáticas dos ensaios clínicos precisa ser adaptada e otimizada para estudos de intervenção em animais. A ferramenta RoB para estudos de intervenção em animais (ferramenta RoB da SYRCLE [SYstematic Review Centre for Laboratory animal Experimentation]) é baseada na ferramenta Cochrane RoB e foi adaptada para aspectos de viés que desempenham um papel específico nos estudos de intervenção em animais.

Essa ferramenta é constituída por 10 domínios, relacionados a 6 tipos de viés (Tabela 2).

Tabela 2. Domínios da ferramenta SYRCLE para avaliar o risco de viés

Tipo de viés	Domínios
Viés de seleção	a) Geração de sequência
	b) Características base
	c) Ocultação de alocação
Viés de desempenho	d) Habitação aleatória
	e) Cegamento
Viés de detecção	f) Avaliação aleatória dos resultados
	g) Cegamento
Viés de atrito	h) Dados de resultados incompletos
Viés de relato	i) Relato seletivo dos resultados
Outros vieses	j) Outras fontes de viés

Fonte: Elaborado pela autora

Para cada um dos domínios, é atribuído "low" (indicando um baixo risco de viés); "high" (indicando alto risco de viés); ou "unclear" (indicando que detalhes relatados foram insuficientes para avaliar adequadamente o risco de viés). As avaliações foram feitas pelos dois pesquisadores independentes (NF e IA) e as divergências foram resolvidas por consenso ou consultando o terceiro avaliador (MF).

4.9 Síntese dos resultados e redação dos artigos científicos

Esforços foram feitos para padronizar para garantir consistência e comparabilidade entre os estudos. Os desfechos foram agrupados de acordo com similaridades entre modelos animais e tipos de avaliação para facilitar a análise. Uma análise qualitativa foi então conduzida para resumir os principais resultados de cada estudo. As discrepâncias, semelhanças e tendências foram identificadas e descritas sempre que possível. Considerando a grande heterogeneidade observada entre os estudos e a dificuldade em ter acesso aos dados brutos, a análise quantitativa (meta-análise) não foi recomendada.

4.10 Recursos

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001.

5 ARTIGO CIENTÍFICO 1

The role of selective serotonin reuptake inhibitors (SSRIs) in bone health: A systematic review of in vivo animal studies

Artigo formatado dentro das normas do periódico **Bone (Qualis Odontologia A1).**

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The role of selective serotonin reuptake inhibitors (SSRIs) in bone health: A systematic review of in vivo animal studies

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Abstract

Selective serotonin reuptake inhibitors (SSRIs) currently stand as the preferred therapeutic approach for depression, representing a significant proportion of prescriptions for individuals experiencing depressive symptoms. The emerging understanding of serotonin receptors in bone physiology prompts inquiries into the potential impact of serotonin reuptake blockade on bone health. This systematic review critically evaluated evidence concerning the association between SSRIs and bone health in animal models, as well as the conceivable underlying mechanisms. Registered at PROSPERO (CRD42021278529), this study adhered to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. A comprehensive search of the MEDLINE, Embase, Scopus, and CINAHL databases, along with grey literature, was conducted without imposing constraints on year or language, extending until December 2023. The focus was on studies administering SSRIs in vivo to animal models and investigating bone-related outcomes. From a pool of 1624 articles retrieved across all databases, a meticulous eligibility assessment of 69 full-text articles resulted in the inclusion of eight studies. The qualitative evaluation of these studies encompassed analysis and comparison of study design, sample characteristics, drug regimen, bone-related outcomes, and risk of bias. Overall findings suggest that the administration of escitalopram and fluoxetine negatively impacts rodent bone tissue in a dose- and time-dependent manner, influencing bone microarchitecture, biomechanical properties, and the behavior of bone cells, with osteoblasts exhibiting greater susceptibility than osteoclasts. However, it is paramount to acknowledge a critical risk of bias within the included studies, that requires caution in interpreting the conclusions regarding bone health. The existing literature highlights the necessity for ongoing investigation to elucidate plausible mechanisms of SSRIs effects on bone, encompassing an exploration of dose-response relationships and temporal effects across various SSRIs, with attention to both male and female subjects.

Keywords: Selective Serotonin Reuptake Inhibitors; Drug Evaluation, Preclinical; Physiological Effects of Drugs; Bone Remodeling; Bone Resorption; Bone Regeneration; Systematic Review.

Introduction

Depression represents a significant public health concern and a primary contributor to disability throughout the world [1]. According to the National Health and Nutrition Examination Survey, half of individuals experiencing moderate to severe depression undergo antidepressant treatment [2]. Currently, selective serotonin reuptake inhibitors (SSRIs) hold prominence as the preferred pharmacotherapy, constituting 65% of the total antidepressant prescriptions in primary care [3]. The primary mechanism of action of SSRIs entails the inhibition of presynaptic serotonin reuptake at the serotonin transporter (SERT), leading to an augmented concentration of serotonin (5-HT) at the postsynaptic membrane within the serotonergic synapse, stimulating postsynaptic receptors for a more extended period [4]. While this inhibition of the SERT underlies their therapeutic effects, it is noteworthy that the impact of SSRIs transcends this mechanism alone. The presence of additional mechanisms of action suggests a complex interplay that requires further exploration to fully elucidate the multifaceted effects of SSRIs in psychiatric treatment [5].

It has been reported that 5-HT, the main target related to SSRI action, exerts peripheral effects extending beyond the gastrointestinal tract and cardiovascular system, encompassing notable influences within the skeletal framework [6]. The presence of 5-HT receptors has been established in all major bone cell types, namely osteoblasts, osteocytes, and osteoclasts, where the stimulation of these receptors significantly modulates bone cell activities [7-10]. These insights underscore the existence of functional pathways within bone cells that both respond to and regulate the uptake of 5-HT. In vitro studies have substantiated the functionality of 5-HT signaling in bone cells, yielding diverse effects [6]. Some studies propose a direct stimulatory impact of 5-HT on bone formation pathways [8,9,11,12], while others report inhibitory effects [8,13]. This interaction emphasizes the multifaceted role of 5-HT, and consequently by SSRIs, in modulating various aspects of bone metabolism.

Multiple clinical investigations have underscored a positive correlation between the administration of SSRIs and a reduction in bone mineral density, along with an elevated susceptibility to fractures [14-16]. Despite this evidence, the potential mechanisms associated with this condition remain unclear. Animal studies have played a pivotal role in mitigating a critical confounding factor inherent in clinical analyses, precisely the prescription of SSRIs to individuals with depression, where depression itself may act as a potential instigator for bone loss [14,15,17]. Therefore, this

systematic review of pre-clinical studies aimed to explore, compare, and synthesize potential biological mechanisms underlying these observed effects, thereby providing insight into the intricate interplay between SSRIs and the skeletal system.

Material and methods

Protocol and registration

This systematic review was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines [18], and the protocol was registered in advance on PROSPERO (CRD42021278529).

Eligibility criteria

The criteria for this systematic review, encompassing Participants, Exposure, Comparison, and Outcomes (PECO), were initially established by the authors and are detailed in Table 1. Inclusion criteria comprised preclinical in vivo animal studies featuring, at a minimum, an experimental group and a control or sham group. Studies included systemic or local administration of SSRIs, with explicit descriptions of dosages, administration modalities, and therapeutic regimens. Additionally, eligible studies reported data on concentrations of bone remodeling markers, inflammatory mediators, bone cells, bone microarchitecture, phenotype, morphology, and mechanical properties. The exclusion criteria consisted of study designs that lacked a correlation between SSRIs and bone remodeling/physiology, partial or pilot studies, investigations where the drug was not directly administered to the assessed animals, human studies, and those with inaccessible or missing data, unavailable full text, or incomplete reporting. No restrictions were imposed concerning species, sex, age, study duration, language, or year of publication.

Table 1. Structured question for the development of a systematic review of animals

PECO	
P - Population	Animals of all species, male or female.
E - Exposure	Administration of selective serotonin reuptake inhibitor (SSRI) antidepressant medications.
C - Comparison	Control group (placebo or no intervention) or sham.
O - Outcome	Alteration in bone characteristics or its physiological pathways.
Structured question	Does the use of SSRI medications affects bone health in animals?

Information sources and search strategy

The search strategy was developed in collaboration with the Medical University of South Carolina library services. MEDLINE, Embase, Scopus, and CINAHL databases were searched without year or language restriction until December 2023 for studies that administered SSRI in vivo within animal models, and investigated bone-related outcomes. Supplementary sources were also accessed by consulting the Google Scholar (https://scholar.google.com.br) and System for Information on Grey Literature in Europe (SIGLE) through the OpenGrey (www.opengrey.eu) database. The electronic search strategy incorporated paramount descriptors in the field, as identified in precedent literature, integrating Medical Subject Heading (MeSH) terms, text words (tw.), Emtree terms, and additional free terms. These terms were systematically grouped using boolean operators "OR" and "AND." A detailed presentation of the systematic search strategy employed across databases is provided in Supplementary Table 1. Additional manual searching was performed on the references listed in the selected studies.

Study Selection

The studies identified through electronic search tools were compiled in a single database (Endnote) to facilitate the systematic organization of retrieved studies and duplicate removal. Subsequently, the files were exported to the Covidence Systematic Review Software (Veritas Health Innovation, Melbourne, Australia), where titles and abstracts were independently reviewed by two reviewers (NF and MF). Articles displaying characteristics compatible with the eligibility criteria of this research were then selected for full-text reading. In parallel, the full-text studies were rigorously

evaluated, and those aligning with the predefined criteria were definitively incorporated into the systematic review. At this stage, reasons for exclusion were individually identified. Disagreements at any of the previous stages were resolved through discussions or the evaluation of a third researcher (IA).

Data Extraction

The relevant data were independently extracted from the included studies by a pair of reviewers (NF and IA) using a standardized data extraction form developed by the eligibility criteria. The extracted data encompassed details regarding study characteristics, population, interventions, outcomes, and final results. Specifically, outcomes pertaining to the expression of chemical mediators associated with bone metabolism, the quantity and activity of bone cells, microarchitectural parameters, morphological attributes, and biomechanical properties were systematically collected. Any discrepancies or disagreements between the reviewers were resolved through discussion or, if necessary, consultation with a third researcher (MF), ensuring methodological rigor throughout the data extraction phase.

Risk of bias assessment in individual studies

The risk of bias in the included study was independently assessed by two reviewers (NF and IA) according to the Systematic Review Center for Laboratory Animal Experimentation (SYRCLE) risk of bias tool [19]. This tool consists of 10 items to identify different types of potential bias, including selection (sequence generation, baseline characteristics, allocation concealment), performance (random housing, blinding), detection (random outcome assessment, blinding), attrition (incomplete outcome data), reporting (selective outcome reporting), and other (other sources) bias in studies. Each of the ten domains was classified as “low risk of bias,” “high risk of bias,” or “unclear risk of bias” when there was insufficient information to evaluate. Regarding the “baseline characteristics,” the weight, sex, and age of the animals were considered relevant for the evaluated outcomes. Studies that partially reported this information were classified as "unclear," while those that mentioned all of this data were classified as having a "low” risk of bias in this domain.

Summary measures and synthesis of results

When conducting a systematic review, a meta-analysis should be considered if the studies have enough similarities in terms of population, intervention methods, and reported outcomes to provide a cohesive summary. In this research, there was a great deal of variability regarding study design and animal species, different doses, duration of treatment, and routes of administration of SSRIs, as well as some variations in time between intervention and outcome assessment. Given the heterogeneity among the selected studies, a meta-analysis was not recommended. Alternatively, qualitative descriptions were provided to integrate the current evidence.

Results

Study Selection

The search strategy returned 1830 studies for assessment from all the databases. After excluding 206 duplicates and following title and abstract screening, 69 records were selected for full-text appraisal. At this stage, 61 studies were excluded: 23 comprised other interventions in addition to medication; 13 for not using SSRI as an antidepressant drug; 13 were meeting abstracts with no published paper; 7 had different study designs; and five because the animals that received the drug were not evaluated, but only the newborns. Therefore, eight studies that met the inclusion criteria were included. The identification, screening, eligibility, and inclusion process is summarized in Figure 1 (PRISMA flowchart).

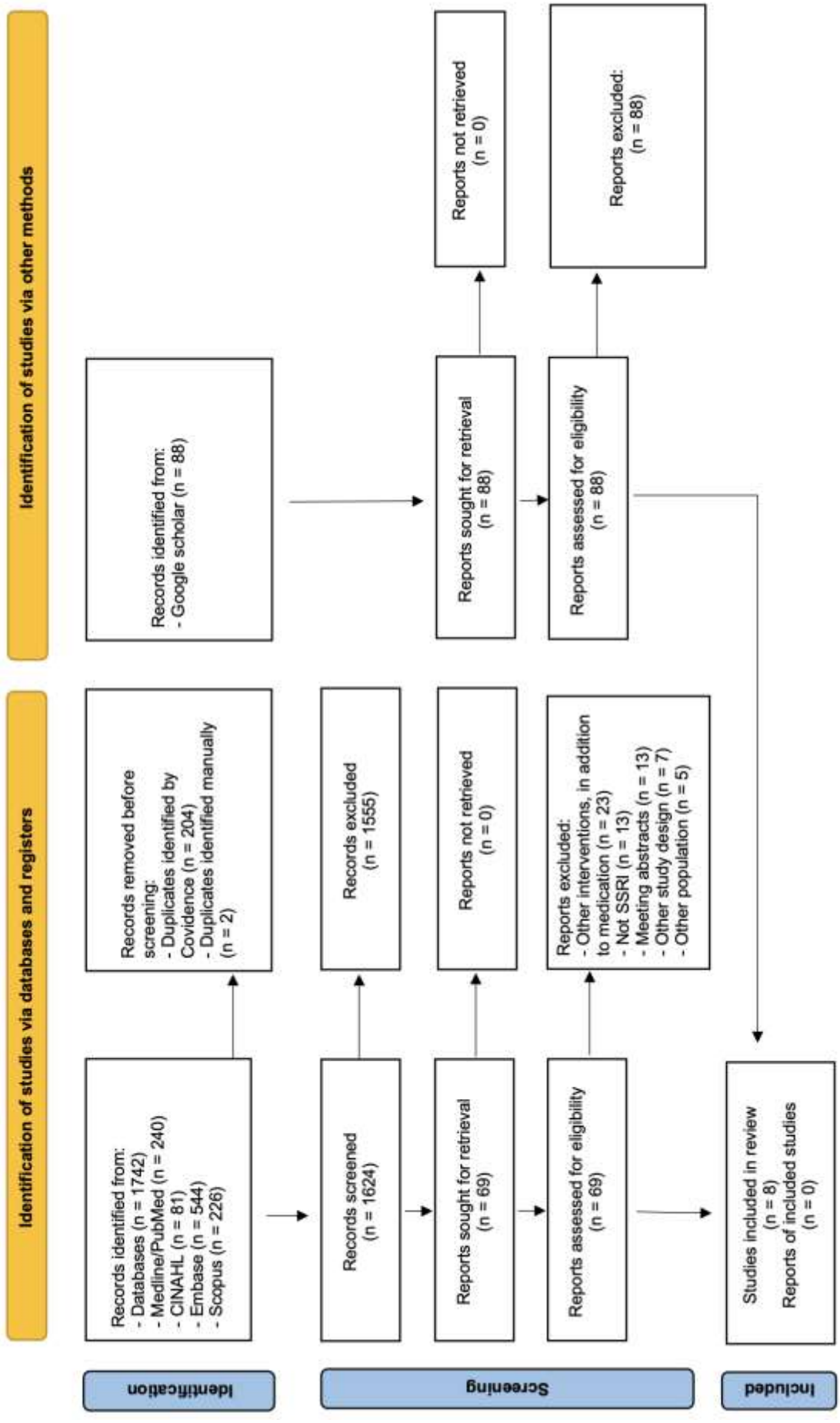

Figure 1. The flow diagram of articles selected for evaluating SSRIs in bone tissue was adapted from the PRISMA statement.

Study Characteristics

This systematic review incorporated eight in vivo animal studies, spanning the publication years from 2005 to 2023 (Table 2). All experimental procedures were exclusively conducted on rats or mice. Among the included studies, fluoxetine emerged as the predominantly employed SSRI, administered at dosages ranging from 5 mg/kg/day to 20 mg/kg/day. Additionally, the effects of escitalopram (2 mg/kg/day) were investigated. The mode of administration for these pharmacological agents encompassed subcutaneous injection, oral delivery, intraperitoneal injection, and intragastric gavage, with study durations extending from 21 days to 6 months. Different assessment approaches were employed for outcome evaluations, including molecular analysis, histomorphometry, microcomputed tomography (MicroCT), caliper measurements, dual-energy X-ray absorptiometry (DXA), and mechanical testing. Notably, three studies exclusively utilized female animals, four studies focused on male animals, and one study reported outcomes for both sexes.

Table 2. Descriptive characteristics of studies evaluating the effects of SSRI on bone parameters.

Reference	Characteristics of animals	Groups (n/group)	Drug regimen	Method of evaluation	Evaluated parameters	Main findings
Warden et al. (2005)	C57BL6J mice Female 4 weeks old Weight: NR	Fluoxetine low-dose (n = 10) Fluoxetine high-dose (n = 10) Control (saline) (n = 10)	**Fluoxetine low-dose** **DS:** Fluoxetine **TD:** 4 weeks **TOA:** 4 weeks **D:** 5 mg/kg/day **WA:** intraperitoneal injection **Fluoxetine high-dose** **DS:** Fluoxetine **TD:** 4 weeks **TOA:** 4 weeks **D:** 20 mg/kg/day **WA:** intraperitoneal injection	DXA in vivo (whole-body minus tail, cranium, and whole-right-hindlimb [femur, tibia, and foot]) DXA ex vivo (cranium, vertebrae L2-L5, femur) Peripheral quantitative computed tomography (femur) Histomorphometry (femur, vertebra L5, cranium)	BMC (% change from week 0) BMC (mg) Localized femoral volumetric BMD and BMC (%) BFRs (NR), MS/BS (NR), MAR (NR), BFR/BS ($\mu m^3/\mu m2$/year) OcS/BS (NR) and ES/BS (NR)	↓ BMC (high-dose, whole-body and hindlimb) ↓ BMC (high-dose, vertebrae) ↓ BMC (high-dose, femurs, cancellous and cortical) ↓ Volumetric BMD (high-dose, femurs, cortical) ↓ Endosteal BFR/BS (high-dose, femur, cortical) ↓ Periosteal BFR/BS (high-dose, femur, cortical) ↓ BFR/BS (high-dose, femur, cancellous + cortical) ↓ MAR (high-dose, femur, cancellous + cortical)
Galindo (2006)	Wistar rats Male 1 day old 6-8.5 g	30-day-old + fluoxetine (n = 15) 30-day-old control (saline) (n = 15) 90-day-old + fluoxetine (n = 15) 90-day-old control (saline) (n = 15)	**30-day-old fluoxetine** **DS:** Fluoxetine **TD:** 21 days **TOA:** 30 days **D:** 10 mg/kg/day **WA:** Subcutaneous injection **90-day-old fluoxetine** **DS:** Fluoxetine **TD:** 21 days **TOA:** 90 days **D:** 10 mg/kg/day **WA:** Subcutaneous injection	Caliper measurement (femur) Photometry and colorimetric testing (serum)	Femur length (mm) Alkaline phosphatase (U/L)	↓ Femur length (30-day-old) ↑ Alkaline phosphatase (30-day-old)
Bonnet et al. (2007)	C57BL6J mice Female 10 weeks old	Fluoxetine (n = 12) Control (saline) (n = 12)	**DS:** Fluoxetine **TD:** 4 weeks **TOA:** 4 weeks **D:** 20 mg/kg/5 times per week	Micro CT (femur)	BV/TV (%),Tb.N, Tb.Th (µm), SMI, DA, Length (mm), ML diameter (mm), AP diameter (mm), Cortical area (mm^2), Cortical width (mm)	↓ Tb.Th (cancellous) ↑ SMI (cancellous) ↓ Cortical width (cortical)

	Weight: NR		**WA:** Subcutaneous injection	Bone mechanical testing (femur)	Ultimate force (N), Extrinsic energy (N mm), Ultimate displacement (mm^2), Stiffness (N/mm), Young's modulus (MPa)	↓ Stiffness ↓ Young's modulus
				ELISA (plasma)	Osteocalcin (ng/ml), CTX (ng/ml)	↓ Osteocalcin
Westbroek et al. (2007)	Sprague-Dawley rats Female 8 weeks old 200 g	Fluoxetine (n = 11) Control (water) (n = 10)	**DS:** Fluoxetine **TD:** 6 months **TOA:** 6 months **D:** 5 mg/kg/day **WA:** Gastric intubation	DXA in vivo	Femur BMD (g/cm^2), Total BMD (g/cm^2)	No difference
				Micro CT (femur)	Cortical volume (mm^3) Cortical thickness (mm) Trabecular bone volume (mm^3) Endocortical bone volume (mm^3) BV/TV (N/A), Tb.Th (mm) Conn. D (N/A) SMI (N/A), Perimeter (mm)	↓ Tb.Th (femur metaphysis) ↑ Endocortical bone volume (femur metaphysis)
				Bone mechanical testing (femur)	Maximum force (N), Strain (%), Ultimate stress (N/mm^2), Young's modulus (N/mm^2)	↓ Ultimate stress ↓ Young's modulus
				Histomorphometry (femur)	Osteoclasts number, unmineralized matrix	No difference
Ortuño et al. (2016)	C57BL6J mice Male and female 14 weeks old Weight: NR	Fluoxetine long treatment (n = 5-16*) *n* variable by experiment Fluoxetine short treatment (n = 5-16*) *n* variable by experiment Control (vehicle) (n = 5-16*) *n* variable by experiment	**Fluoxetine long treatment** **DS:** Fluoxetine **TD:** 6 weeks **TOA:** 6 weeks **D:** 20 mg/kg/day **WA:** Drinking water **Fluoxetine short treatment** **DS:** Fluoxetine **TD:** 3 weeks **TOA:** 3 weeks **D:** 20 mg/kg/day **WA:** Drinking water	ELISA (serum and urine) (male and female)	Osteocalcin (ng/ml), Dpd (nmol/mmol Cr)	↓ Osteocalcin (long treatment, serum, female and male) ↓Dpd (short treatment, urine, female)
				Micro CT (vertebra and tibia) (male and female)	BV/TV (%)	↓ BV/TV (long treatment, tibia and vertebra, cancellous, female and male) ↑ BV/TV (short treatment, vertebra, cancellous, female)
				Histomorphometric analyses (vertebrae L3-L4) (female only)	BFR/BF (µm3/µm2/year), Oc.S/BS (%), Ob.S/BS (%)	↓ Oc.S/BS (short treatment, female) ↓ Ob.S/BS (long treatment, female)
				Real-time PCR (qPCR) (long bones) (male and female)	OPG/RANKL ratio, OPG (NR), RANKL (NR)	↓ OPG/RANKL ratio (long treatment, female and male) ↓ RANKL (short treatment, female)

Kumar et al. (2018)	Wistar rats Male 6-8 weeks old 150-200 g	Fluoxetine (n = 6) Escitalopram (n = 6) Control (n = 6)	**Fluoxetine** **DS:** Fluoxetine **TD:** 40 days **TOA:** 40 days **D:** 8.2 mg/kg/day **WA:** Oral **Escitalopram** **DS:** Escitalopram **TD:** 40 days **TOA:** 40 days **D:** 2 mg/kg/day **WA:** Oral	ELISA (serum) Micro CT (vertebrae L2-L4 and tibia)	P1NP (NR), β-CTX (NR), pCREB (NR) BMD, BV/TV (%),Tb. Th (mm), Tb.Sp (mm), Tb. N (1/mm), Tb. Pf, SMI, Conn. D, (1/mm^3)	↓ P1NP (fluoxetine) ↓ pCREB (fluoxetine* and escitalopram) *The reduction was more significant in fluoxetine group ↓ BV/TV (fluoxetine, tibia, cancellous) ↓ Tb.Th (fluoxetine, tibia, cancellous) ↓Tb.N (fluoxetine, tibia, cancellous) ↑Tb.Sp (fluoxetine, tibia, cancellous) ↑ Tb.Pf (fluoxetine, tibia, cancellous) ↑ Conn.D (fluoxetine, tibia, cancellous)
Wadhwa et al. (2019)	Wistar rats Male 6-8 weeks old 150-200 g	Escitalopram (n = 6) Control (saline) (n = 6)	**DS:** Escitalopram **TD:** 40 days **TOA:** 40 days **D:** 2 mg/kg/day **WA:** Oral	ELISA (femur) ELISA (serum) Measurement of proteins with the Folin phenol reagent (femur) Method of Tennis-wood et al. (1976) (femur) Micro CT (vertebrae L2-L4)	DDK-1 (ng/ml), Sclerostin (pg/ml) RANKL (pmol/L) Alkaline phosphatase (µmol PNP/h/µg protein) TRAP5b activity BMD, BV/TV (%), Tb.Th (mm), Tb.Sp (mm), Tb.N (1/mm), Tb.Pf, SMI, Conn. D (1/mm^3)	↑ DKK-1 ↑ Sclerostin ↑ RANKL ↓ Alkaline phosphatase ↑ TRAP5b ↓ BMD (cancellous) ↓ BV/TV (cancellous) ↓ Tb.Th (cancellous) ↓ Tb.N (cancellous) ↑ Tb.Sp (cancellous)
Chirokikh et al. (2023)	Sprague-Dawley rats Male 4 weeks old ≅ 55g (extracted from graph)	Fluoxetine (n = 10) Control (water) (n = 10)	**DS:** Fluoxetine **TD:** 4 weeks **TOA:** 4 weeks **D:** 20 mg/kg/day **WA:** Drinking water	Caliper measurement (femur, tibia) Histomorphometry (tibia) Micro CT (tibia)	Length (mm), AP diameter (mm) (mid-diaphysis), ML diameter (mm) (mid-diaphysis) Growth plate height TV (mm^3), BV (mm^3), BV/TV (N/A), Tb.N (1/mm), Tb.Th (mm), Tb.Sp (mm), vBMD (mgHA/cm^3), Ct.V (mm^3), Ps.V (mm^3), Ec.V (mm^3), Ct.Th (mm), TMD (mgHA/cm^3), pMOI (mm^4)	No difference No difference ↓ TV (cancellous) ** **After adjusting for body weight, no significant difference was identified ↑ Ct.V (cortical)*** *** Adjusted for body weight

	Bone mechanical testing (femur)	Yield force (N), Ultimate force (N), Stiffness (N/mm), Energy to failure (mJ)	↑ Ultimate force*** *** Adjusted for body weight

Description of acronyms in alphabetical order: **AP:** Anteroposterior; **BFR/BS:** Bone-formation rate/Bone surface; **BMC:** Bone mineral content; **BMD:** Bone mineral density; **BV:** Bone volume; **BV/TV:** Trabecular bone volume; **Conn. D:** Connection density; **Ct.Th:** Cortical thickness; **Ct.V:** Cortical volume; **CTX:** C-terminal collagen crosslinks; **D:** Dosage; **DA:** Degree of Anisotropy; **DDK-1:** Dickkopf-1 (an inhibitor of the Wnt pathway that can act as a biomarker for reduced bone formation); **Dpd:** Deoxypyridinoline cross-links; **DS:** Drug subtype; **DXA:** Dual-energy x-ray absorptiometry; **Ec.V:** Endocortical volume; **ES/BS:** Eroded surface/bone surface; **MAR:** Mineral apposition rate; **ML:** Mediolateral; **MS/BS:** Mineralizing Surface/Bone Surface; **N/A:** not applicable; **NR:** not reported; **Ob.S/BS:** Osteoblast surface/Bone surface; **Oc.S/BS:** Osteoclast surface/Bone surface; **OPG:** Osteoprotegerin; **P1NP:** Type 1 procollagen amino terminal peptide; **pCREB:** Phosphorylated transcription factor cAMP-response element-binding protein; **pMOI:** polar moment of inertia; **Ps.V:** Periosteal volume; **RANKL:** Receptor activator of nuclear factor κB ligand; **SMI:** Structure Model Index; **SSRI:** Selective Serotonin Reuptake Inhibitor; **Tb.N:** Trabecular number**; Tb.Pf:** trabecular pattern factor; **Tb.Sp:** Trabecular separation; **Tb.Th:** Trabecular thickness; **TD:** Treatment duration; **TMD:** Tissue mineral density; **TOA:** Time of outcome assessment; **TV:** Total volume; **vBMD:** Volumetric bone mineral density; **WA:** Way of administration; **↑↓:** Statistically significant increase (↑) or decrease (↓) of the parameter compared to the control group. The "evaluated parameters" not elucidated in the "main findings" exhibited no statistically significant difference when compared to the control group.

Risk of bias within studies

Table 3 provides a comprehensive overview of the results stemming from the risk of bias assessment conducted on the eligible in vivo animal studies. A low risk of bias was found for all studies in “selective outcome reporting” (100%) and for some of them in “baseline characteristics” (62.5%). Only one study [20] explicitly affirmed that all outcome analyses were executed by researchers blinded to group allocation, thus earning a "low" classification in domain #7. Curiously, this study disclosed that its experimental design necessitated the awareness of experimental groups by the animal handler, leading to a corresponding "high" classification in item #5. The great majority of the studies scored "unclear" in eight out of the ten items of the SYRCLE tool [19]. A summary score for each individual study is not recommended for the SYRCLE tool, given the complexities involved in assigning and justifying specific “weights” to individual components [19].

Table 3. Summary of risk of bias assessment of studies evaluating the effects of SSRI on bone parameters

STUDIES	Signaling questions									
	1	2	3	4	5	6	7	8	9	10
Warden et al., 2005	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Unclear
Galindo, 2006	Unclear	Low	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Unclear
Bonnet et al., 2007	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Unclear
Westbroek et al., 2007	Unclear	Low	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Unclear
Ortuño et al., 2016	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Unclear
Kumar et al., 2018	Unclear	Low	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Unclear
Wadhwa et al., 2019	Unclear	Low	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Unclear
Chirokikh et al., 2023	Unclear	Low	Unclear	Unclear	High	Unclear	Low	Unclear	Low	Unclear

SYRCLE tool bias evaluation
1) Was the allocation sequence adequately generated and applied?; 2) Were the groups similar at baseline, or were they adjusted for confounders in the analysis?; 3) Was the allocation adequately concealed?; 4) Were the animals randomly housed during the experiment?; 5) Were the caregivers and/or investigators blinded by the knowledge which intervention each animal received during the experiment?; 6) Were animals selected at random for outcome assessment?; 7) Was the outcome assessor-blinded?; 8) Were incomplete outcome data adequately addressed?; 9) Are reports of the study free of selective outcome reporting?; 10) Was the study apparently free of other problems that could result in a high risk of bias?
Classification of bias: high risk, unclear risk, low risk.

Results of individual studies

1. Escitalopram

Upon oral administration of escitalopram to male rats at a dosage of 2 mg/kg/day over a 40-day interval, contrasting outcomes surfaced in two distinct studies. Kumar et al. [21] exclusively identified a reduction in circulating pCREB (responsible for osteogenic differentiation and mineralization) as a medication-related secondary effect related to bone, with no discernible microarchitectural alterations noted in the vertebrae or tibia. In contrast, Wadhwa et al. [22] reported a spectrum of harmful effects on various parameters related to bone tissue. Primarily, a reduction in alkaline phosphatase (ALP) and an increase in DDK-1, sclerostin, and TRAP5b were noted in the femur, along with elevated serum levels of RANKL. Furthermore, a significant compromise in vertebral bone microarchitecture was detected, evidenced by decreased BMD, BV/TV, and Tb. Th, and Tb. N, coupled with an increase in Tb. Sp.

2. Fluoxetine

Notable methodological variability was observed in studies investigating the use of fluoxetine. MicroCT analyses of female C57BL6J mice treated with 20 mg/kg/day for 3-6 weeks revealed harmful effects in the femur, characterized by decreased Tb.Th and cortical width and an increase in SMI [23]. In another study, a reduction in BV/TV in the tibia and vertebra was observed after six weeks of treatment, while an increase in the vertebra was noted after a shorter 3-week treatment [24]. In alignment with these findings, mechanical properties were compromised, as evidenced by reductions in stiffness and Young's modulus [23]. Levels of specific biological markers were also altered in this condition. Experimental animals exhibited decreased serum levels of osteocalcin at 4 [23] and 6 [24] weeks of treatment, reduced urinary Dpd levels (a marker of bone resorption) at three weeks of treatment [24], and a diminished OPG/RANKL ratio after six weeks, despite a decrease in RANKL observed at three weeks [24].

Histomorphometric analyses of vertebrae revealed a reduction in Oc.S/BS after short-term treatment (3 weeks) and a decrease in Ob.S/BS after six weeks [24]. Meanwhile, after 6 weeks of treatment, femur histomorphometry detected a reduction in endosteal and periosteal cortical BFR/BS, along with decreases in cortical and trabecular BFR/BS and MAR [25]. Lastly, DXA detected reductions in whole-body,

hindlimb, and vertebrae BMC [25]. Peripheral quantitative computed tomography revealed reduced volumetric BMD in the femoral cortex [25].

When 5 mg/kg/day was administered to female rodents, no bone-related alterations were discerned in mice after 4-week treatment [25]. In contrast, rats treated for six months exhibited a decrease in Tb. Th and an increase in endocortical bone volume in the femur [26]. Additionally, both ultimate stress and Young's modulus showed a significant reduction [26].

The effects of fluoxetine were also investigated in male rodents at various dosages. Administration of 20 mg/kg/day in mice for six weeks resulted in a spectrum of deleterious effects, marked by a reduction in serum osteocalcin concentration, decreased BV/TV in the tibia and vertebra, and a diminished OPG/RANKL ratio in long bones [24]. Conversely, the exact dosage administered to male rats for four weeks positively affected bone. Under these conditions, there was an increase in Ct.V and ultimate force in long bones [20]. Although a reduction in TV was also observed in the tibia, this difference did not prove significant when results were adjusted for body weight [20].

Interestingly, predominantly deleterious effects were observed when lower dosages (8.2-10 mg/kg/day) of this medication were administered to male rats for 30-40 days. While Galindo [27] reported a decrease in femur length and an increase in serum alkaline phosphatase, Kumar et al. [21] documented a reduction in circulating levels of P1NP and pCREB, along with a significant compromise in the bone microarchitecture in the tibia region, evidenced by reduced BV/TV, Tb.Th, and Tb.N, and an increase in Tb.Sp. Conversely, an increase in Tb.Pf and Conn.D were also observed under the same conditions.

Discussion

SSRIs emerged as a significant therapeutic advance in psychopharmacology and were extensively studied for their effects on the central nervous system and brain function [28]. Despite clinical studies documenting the impact of chronic usage on bone turnover and the association with bone disorders such as osteoporosis and an elevated risk of fracture [29,30], the precise biological mechanisms governing these effects remain inadequately established. This systematic review pioneers the assessment of whether the administration of SSRIs affects bone health in animal models. The findings of this study suggest that SSRIs administration appears to negatively influence bone

tissue, impacting various cellular and molecular pathways that regulate bone homeostasis.

While SSRIs as a class have similar mechanisms of action, each subtype exhibits distinctive pharmacokinetics, pharmacodynamics, chemical structure, side effect profiles, and efficacy, rendering them more or less suitable use for specific clinical niches [5]. Escitalopram, in particular, features an S-enantiomer, which becomes the focal compound in depression treatment due to its highest specificity for the SERT among the SSRIs [31]. Two of the included studies delved into the effects of escitalopram on rat bone parameters under experimentally analogous conditions [21,22]. Despite this experimental congruence, the outcomes manifested notable disparities. Kumar et al. [21] underscored that, despite a slight reduction in serum pCREB levels attributed to escitalopram, potentially indicative of diminished osteoblastic proliferation, such alterations proved insufficient to induce changes in bone formation. Consequently, no significant effects were discerned on P1NP levels or the vertebrae or tibia microarchitectural parameters. These observations led them to posit that, in the context of prescribing antidepressants to individuals predisposed to bone loss, escitalopram might be a more advantageous option.

In contrast, Wadhwa et al. [22] reported an elevation in DDK-1, sclerostin, and TRAP5b and a decrease in alkaline phosphatase in the femur, along with a rise in serum RANKL levels following escitalopram treatment. Some investigations [32,33] have underscored the pivotal role of the Wnt/β-catenin pathway in osteoblast proliferation. Sclerostin and DKK-1, inhibitors of the Wnt pathway, exhibited increased bone levels, suggesting a probable detrimental impact on bone accrual. Furthermore, the reduction in bone-specific alkaline phosphatase, a recognized marker for bone formation, correlates directly with decreased osteoblastic activity. The heightened RANKL activity in the bloodstream and increased femoral TRAP5b levels may also be associated with elevated bone resorption. These findings align with the deterioration observed in all microarchitectural parameters assessed in vertebrae. Consequently, escitalopram adversely impacts bone health through reduced bone formation and enhanced bone resorption mechanisms. There is a notable absence of studies involving female animals, which are crucial for elucidating the role of hormonal variations in this context.

Fluoxetine, the oldest and most extensively studied SSRIs, displays minimal specific binding to SERT [31]. Higher doses can elevate synaptic norepinephrine and

dopamine levels [34]. Comparing female rodents administered an equivalent fluoxetine dosage (20 mg/kg/day) revealed intriguing findings. Two included studies [23,25] followed a 4-week treatment protocol in mice, unveiling various detrimental indicators for bone tissue. Overall, femoral microarchitectural parameters exhibited compromised states, reduced BMC and BMD at different sites, and deteriorated biomechanical properties. This impairment appears strongly associated with bone formation disruption, as indicated by Bonnet et al. [23], who reported reduced serum osteocalcin levels. In line with this, Warden et al. [25] observed decreased endosteal and periosteal bone formation rates at the femoral mid-shaft, along with a reduced distal metaphysis bone formation rate and mineral apposition rate, indicating alterations in cortical and trabecular bone related to an osteoblastic phenotype.

When evaluating the same dosage over different treatment durations (3 or 6 weeks), some discrepancies emerged. Short-term treatment (3 weeks) showed slight improvement in specific bone parameters, such as increased BV/TV and decreased Oc.S/BS in the vertebra, decreased Dpd in urine, and RANKL expression in long bones [24]. Conversely, long-term treatment (6 weeks) decreased serum osteocalcin, reduced BV/TV in the tibia and vertebra, decreased Ob.S/BS in the vertebra, and decreased OPG/RANKL in long bones [24]. In vitro experiments conducted by the same authors supported and explained these findings. This study demonstrates that fluoxetine affects bone mass accrual in mice through two distinct mechanisms. Initially, it directly disrupts Ca2+ calmodulin signaling in osteoclasts, resulting in reduced phosphorylation of CREB, as well as diminished expression of the transcription factor Fos and nuclear factor of activated T cells (Nfatc1) - pivotal factors governing osteoclast biology. This interference hampers the maturation and activity of osteoclasts. This mechanism corresponds with the observed decline in bone resorption after a 3-week treatment period, independent of the 5-HTT transporter. Nevertheless, prolonged treatment (6 weeks) counteracts this direct inhibitory impact on osteoclasts, leading to reduced bone formation and subsequent bone loss. This secondary effect is linked to attenuated serotonin signaling in the hypothalamus and an augmented output from the sympathetic nervous system (SNS) [24].

Brain serotonin, operating through an Htr2c–CREB cascade, positively regulates bone mass accrual by suppressing sympathetic output [35,36]. In this study, mice treated with fluoxetine for 3 and 6 weeks exhibited progressively reduced levels of hypothalamic Htr2c, negatively impacting bone remodeling over time [24]. The

central SNS-mediated detrimental effect on bone remodeling appears delayed compared to fluoxetine's direct anti-resorptive action. Therefore, the temporal discrepancy observed may be attributed to the requirement of a prolonged duration for the drug to induce an elevation in inter-synaptic brain serotonin levels. This elevation is necessary to desensitize the Htr2c receptor and reduce its expression sufficiently. This process takes longer than the drug's ability to reach osteoclasts peripherally and directly disrupt their function [24].

The impact of low-dose fluoxetine (5 mg/kg/day) has been examined by two studies [25,26], which found a substantial disparity in treatment duration. Mice treated for four weeks exhibited no differences in BMC, BMD, BFR, and MAR in the vertebra, long bones, or skull [25]. However, rats treated with the exact dosage for six months displayed subtle deleterious alterations characterized by a decrease in femur Tb.Th coupled with a deterioration of its biomechanical properties. Nevertheless, the vast majority of other parameters assessed through DXA, MicroCT, mechanical testing, and histomorphometry remained unchanged, suggesting that the potential effects of fluoxetine on bone are dose-dependent [26].

Male mice subjected to a high fluoxetine dose (20 mg/kg/day) were assessed at two distinct time points [24]. After a 3-week treatment period, no significant changes were observed in bone markers or bone microarchitecture in the vertebra or tibia. However, upon extending the treatment to 6 weeks, deleterious skeletal effects were evident, and characterized by reduced serum osteocalcin levels, diminished BV/TV in the vertebra and tibia, as well as decreased OPG/RANKL in long bones. These outcomes are consistent with findings in female mice exposed to the same experimental model, as previously reported. Similar to the female mice, this male phenotype was associated with a tendency for higher SNS output. The authors hypothesized that brain serotonin, signaling through an Htr2c–CREB cascade, plays a positive role in bone mass accrual by suppressing sympathetic output. Prior studies [37-39] demonstrated that elevated serotonin concentrations can desensitize Htr2c. Accordingly, after six weeks of treatment, Ortuño et al. [24] reported diminished hypothalamic levels of pCREB, a downstream mediator. Furthermore, mice treated for 3 and 6 weeks exhibited progressively reduced hypothalamic Htr2c levels. Over time, fluoxetine diminishes serotonin signaling in Htr2c neurons, negatively impacting bone remodeling akin to the absence of brain serotonin [35,36].

In the most recent study in this systematic review [20], the exact dosage was administered to male rats for four weeks. In this condition, contrary to the previously reported results, fluoxetine exhibited some skeletal benefits, evident in the increased tibial cortical volume and ultimate force. In contrast, most of the assessed bone parameters did not show significant differences. A distinctive aspect of this study was that animals treated with fluoxetine gained significantly less weight than those in the control group during the experimental period. Consequently, the outcomes were compared directly and after adjusting for body weight using linear regression. Further investigations are essential to elucidate the intricate relationship between fluoxetine, body weight, and skeletal development.

Finally, two investigations explored lower doses (8.2 [21] and 10 [27] mg/kg/day) in Wistar male rats. The animals treated with fluoxetine (10 mg/kg/day) from the first to the 21st day of life exhibited reduced femur length at 30 days old, accompanied by an elevated serum concentration of ALP. However, these differences ceased to be evident at 90 days old, suggesting the reversible nature of the skeletal effects of fluoxetine upon cessation of its administration [27]. No consistent mechanism has been correlated with these findings, necessitating further in-depth exploration of this condition's underlying pathways.

The treatment with 8.2 mg/kg/day for 40 days [21] was associated with reduced serum P1NP and pCREB levels and the deterioration of most microarchitectural parameters in the tibia. As observed in previous investigations involving escitalopram, P1NP serves as a metabolite resulting from procollagen-I cleavage before incorporating type 1 collagen into the bone matrix [40]. Consequently, the diminished levels of circulating fragments reflect a decreased rate of collagen synthesis, subsequently impacting bone formation. Additionally, the authors postulated that fluoxetine, through the enhancement of gut 5HT and subsequent activation of Htr1b receptors on osteoblasts, contributes to reduced CREB phosphorylation, leading to decreased CREB levels and, in turn, observable reductions in osteoblastic proliferation, as indicated by lowered P1NP. Another marker, the C-telopeptide cross-link for type 1 collagen (βCTX), is released into the circulation during the degradation of type 1 collagen by osteoclasts, indicating bone resorption [21]. Interestingly, the administration of fluoxetine did not induce alterations in this bone marker, suggesting that the skeletal effects of this medication are primarily associated with changes in

osteoblastic activities. In contrast, only limited evidence regarding its impact on osteoclastic activity is available.

Establishing a causative association across studies proves to be challenging due to various critical experimental factors that may yield different effects on bone parameters. These factors include, but are not limited to, the specific SSRIs subtype, dosage protocol, mode of administration, duration of treatment, animal species, investigative model utilized, and other confounding factors. Investigations into the effects of different SSRIs protocols and other variables, such as induced disease models, are essential to understand better how SSRIs works in bone tissue. The heterogeneity of bone parameters and protocols could have improved the comparison among studies. An effort was made to establish a cause-and-effect correlation between SSRIs subtypes, treatment time, and dosage, but there has yet to be an apparent homogeneity of results.

The limitations of the present research protocol predominantly arise from the heterogeneity and quality of the eligible studies. Systematic literature reviews assess the methodological quality of included studies through the risk of bias evaluation tools [19]. In most evaluated domains, a status of "unclear" was assigned. While some studies acknowledged a random component in sequence generation and outcome assessment, a notable limitation surfaced due to the need for explicit methodology detailing the randomization process. Furthermore, comprehensive information regarding allocation concealment, random housing, blinding of investigators, raters, and caregivers, and the presentation of "incomplete outcome data" remained inadequately elucidated. This is a significant shortcoming since studies with inadequate sample size and poor method description could provide false-negative results, leaving potential findings undetected, besides subjective outcome measurements and the implementation of measurement bias. The absence of some information may be attributed to word count limitations required by scientific journal submission guidelines. Nevertheless, numerous journals are adopting the ARRIVE guidelines [41] to enhance the reporting quality of research publications, a measure expected to mitigate this issue in the future. Furthermore, encouraging the publishing of supplementary materials with more meticulous details is advisable.

Conclusions

This systematic review suggests that the utilization of SSRIs, particularly fluoxetine and escitalopram, may exert a predominantly negative impact on the bone health of rodents. Most of these effects appear to be leading to compromised bone formation and, to a lesser extent, to an increase in bone resorption. However, it is crucial to note that the included studies exhibited a critical risk of bias. Consequently, the conclusions regarding bone health should be interpreted within the context of potential bias. The available literature underscores the imperative for continued investigation into elucidating plausible mechanisms of SSRIs on bone, encompassing exploration of the dose-response relationship and temporal effects across various SSRIs in both male and female subjects. Therefore, in the clinical context, it is prudent to incorporate considerations for bone health when prescribing this class of drugs, especially for individuals with pre-existing risk factors for bone-related issues.

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Supplementary Table 1. Search Strategy According to Database

DATABASE	SEARCH STRATEGY	RESULTS
PUBMED	("Selective Serotonin Reuptake Inhibitors"[Mesh] OR "Selective Serotonin Reuptake Inhibitors"[Pharmacological Action] OR "Selective Serotonin Reuptake Inhibitor*" OR "Adrenergic Uptake Inhibitors"[Mesh] OR "Adrenergic Uptake Inhibitors"[Pharmacological Action] OR "Amitriptyline"[Mesh] OR "Amoxapine"[Mesh] OR "Amphetamine"[Mesh] OR "Atomoxetine Hydrochloride"[Mesh] OR "Benzphetamine"[Mesh] OR "Desipramine"[Mesh] OR "dibutylone"[Supplementary Concept] OR "Dothiepin"[Mesh] OR "ethylphenidate"[Supplementary Concept] OR "Imipramine"[Mesh] OR "Maprotiline"[Mesh] OR "Mazindol"[Mesh] OR "Methamphetamine"[Mesh] OR "Nortriptyline"[Mesh] OR "Opipramol"[Mesh] OR "Protriptyline"[Mesh] OR "Reboxetine"[Mesh] OR "Reserpine"[Mesh] OR "Tapentadol"[Mesh] OR "Tetrabenazine"[Mesh] OR "Trimipramine"[Mesh] OR "Tyramine"[Mesh] OR "Viloxazine"[Mesh] OR "Antidepressive Agents"[Mesh] OR "Antidepressive Agents"[Pharmacological Action] OR Antidepressive-Agent*[tiab] OR Antidepressant*[tiab] OR Antidepressant-Drug[tiab] OR Antidepressant-Drugs[tiab]OR Thymoleptic*[tiab] OR Thymoanaleptic*[tiab] OR "Antidepressive Agents, Second-Generation"[Mesh] OR "Antidepressive Agents, Second-Generation"[Pharmacological Action] OR Second-Generation-Antidepressive-Agent*[tiab] OR Atypical-Antidepressant*[tiab] OR "Benzphetamine"[Mesh] OR "Desipramine"[Mesh] OR ethylphenidate[tiab] OR "Amisulpride"[Mesh] OR "Bupropion"[Mesh] OR "Citalopram"[Mesh] OR "Fluoxetine"[Mesh] OR "Fluvoxamine"[Mesh] OR "Mianserin"[Mesh] OR "nefazodone"[Supplementary Concept] OR "Paroxetine"[Mesh] OR "Quipazine"[Mesh] OR "Ritanserin"[Mesh] OR "Sulpiride"[Mesh] OR "Trazodone"[Mesh] OR "Tryptophan"[Mesh] OR "Venlafaxine-Hydrochloride"[Mesh] OR "Serotonin Antagonists"[Mesh] OR "Serotonin Antagonists"[Pharmacological Action] OR Antiserotonergic-Agent*[tiab] OR Serotonin-Block*[tiab] OR Serotonin-Receptor-Antagonist*[tiab] OR Serotonin-Receptor-Block*[tiab] OR Serotonin-Antagonist*[tiab] OR Selective-Serotonin-Reuptake-Inhibitor*[tiab] OR Serotonin-Reuptake-Inhibitor*[tiab] OR Serotonin-Uptake-Inhibitor*[tiab] OR "alaproclate"[Supplementary Concept] OR "Citalopram"[Mesh] OR "Clomipramine"[Mesh] OR "femoxetine"[Supplementary Concept] OR "Fenfluramine"[Mesh] OR "indalpine"[Supplementary Concept] OR "Norfenfluramine"[Mesh] OR "Olanzapine"[Mesh] OR "Sertraline"[Mesh] OR "Vilazodone Hydrochloride"[Mesh] OR "Vortioxetine"[Mesh] OR "Zimeldine"[Mesh] OR "Serotonin and Noradrenaline Reuptake Inhibitors"[Mesh] OR "Serotonin and Noradrenaline Reuptake Inhibitors"[Pharmacological Action] OR "Serotonin-and-Noradrenaline-Uptake-Inhibitor*"[tiab] OR "Serotonin-and-Norepinephrine-Reuptake-Inhibitor*" [tiab] OR SNRI[tiab] OR SNRIs[tiab] OR SSRI OR SSRIs OR nantenine OR paliperidone OR palonosetron OR palonosetron [Mesh] OR pancopride OR paracetamol plus tramadol OR pelanserin OR "pelanserin" [Supplementary Concept] OR perospirone OR Piboserod OR pirenperone OR pirepemat OR pizotifen OR "pizotifen maleate" OR pruvanserin OR quetiapine OR r53434 Ramosetron OR renzapride OR ricasetron OR risperidone OR ritanserin OR robalzotan OR roluperidone OR sarpogrelate OR sb773812 OR seganserin OR sequifenadine OR sergolexole OR serotonin 1 antagonist OR serotonin 1A antagonist OR serotonin 1B antagonist OR serotonin 1D antagonist OR serotonin 2 antagonist) AND ("Bone Remodeling"[Mesh] OR Bone-Turnover*[tiab] OR "Bone Regeneration"[Mesh] OR Bone-Regenerat*[tiab] OR Osteoconduct*[tiab] OR "Bone Resorption"[Mesh] OR Bone-Resorpt*[tiab] OR Osteoclastic-Bone-Loss[tiab] OR "Osteoclasts"[Mesh] OR Cementoclast*[tiab] OR Odontoclast*[tiab] OR Osteoclast*[tiab] OR "Osteoblasts"[Mesh] OR Osteoblast*[tiab] OR "Osteolysis"[Mesh] OR Osteolyses*[tiab] OR "Tooth Movement Techniques"[Mesh] OR Tooth-Movement*[tiab] OR Tooth-Intrusion*[tiab] OR Tooth-Depression*[tiab] OR "Tooth Migration"[Mesh] OR Tooth-Drift*[tiab] OR osteopenia[tiab] OR "Bone Diseases, Metabolic"[Mesh] OR "Osteoporosis"[Mesh] OR "bone mass"[tiab] OR "bone defect"[tiab] OR Osteogenesis OR "bone mineral density" OR osteodensit* OR osseointegrat* OR osteoprogenitor* OR "skeletal development" [TW]) AND (animal* OR equine OR mouse OR mice OR rodent* OR oryctolagus OR danio OR brachydanio OR caenorhabditis OR murine OR rat OR rats OR dogs OR dog OR horse OR feline OR canine* OR bovine OR cricetus OR cow OR cows OR cattle OR pig OR pigs OR "miniature pig" OR "miniature pigs")	240
SCOPUS	ABS("adrenergic uptake inhibitor*" OR amphetamine OR {Atomoxetine Hydrochloride} OR benzphetamine OR desipramine OR dibutylone OR dothiepin OR ethylphenidate OR imipramine OR maprotiline OR mazindol OR methamphetamine OR nortriptyline OR opipramol OR protriptyline OR reboxetine OR reserpine OR tapentadol OR tetrabenazine OR trimipramine OR tyramine OR viloxazine OR "antidepressive agent*" OR "second generation antidepressive agent*" OR amisulpride OR bupropion OR citalopram OR fluoxetine OR fluvoxamine OR maprotiline OR mianserin OR nefazodone OR paroxetine OR quipazine OR ritanserin OR sulpiride OR trazodone OR tryptophan OR {Venlafaxine-Hydrochloride} OR {Serotonin Antagonists} OR "Serotonin Uptake Inhibitor*" OR alaproclate OR amoxapine OR citalopram OR clomipramine OR femoxetine OR fenfluramine OR fluoxetine OR fluvoxamine OR indalpine OR norfenfluramine OR olanzapine OR paroxetine OR sertraline OR trazodone OR {Vilazodone Hydrochloride} OR vortioxetine OR zimeldine OR "Noradrenaline Reuptake Inhibitor*") OR TITLE-ABS ("Antidepressive Agent*" OR "antidepressant*" OR "antidepressant drug*" OR thymoleptic* OR thymoanaleptic* OR "atypical-antidepressant*" OR "Second Generation Antidepressive Drug*" OR "Antiserotonergic Agent*" OR "Serotonin Block*" OR "Serotonin Receptor Antagonist*" OR "Serotonin Receptor Block*" OR "Serotonin Antagonist*" OR "Selective Serotonin Reuptake Inhibitor*" OR "Serotonin W/2 Inhibitor*" OR "Noradrenaline Uptake Inhibitor*" OR "Norepinephrine Reuptake Inhibitor*" OR snri* OR nri* OR ssri*) AND ABS({bone remodeling} OR {bone regeneration} OR {bone resorption} OR osteoclast* OR osteoblast* OR osteolysis OR "tooth movement technique*" OR {tooth migration} OR "metabolic bone diseases" OR osteoporosis) OR TITLE-ABS ("bone turnover*" OR "bone regenerat*" OR osteoconduct* OR "osteoclastic bone loss*" OR cementoclast* OR odontoclast* OR osteolysis* OR "tooth movement" OR "tooth intrusion" OR "tooth depression" OR "tooth drift*" OR osteopenia OR osseointegrat* OR "bone defect*" OR osteogenesis OR "bone mineral density" OR "bone density" OR "bone mass" OR "bone metabolism" OR osteodensit* OR osteoprogenitor* OR	226

	osteocalcin OR "skeletal development") AND ABS((animal* OR equine OR mouse OR mice OR rodent* OR oryctolagus OR danio OR brachydanio OR caenorhabditis OR murine OR rat OR dog OR horse OR feline OR canine* OR bovine OR cricetus OR cow* OR cattle OR pig* OR "miniature pig*")))	
EMBASE	('adrenergic receptor affecting agent'/exp OR 'adrenergic reuptake inhibitor*' OR '3,4 methylenedioxyamphetamine'/exp OR '3,4 methylenedioxyethamphetamine' OR '3,4 methylenedioxypyrovalerone' OR 'amitriptyline'/exp OR 'amphetamine'/exp OR 'atomoxetine'/exp OR 'benzphetamine'/exp OR 'desipramine'/exp OR dibutylone OR 'dosulepin'/exp OR ethylphenidate OR 'imipramine'/exp OR 'mazindol'/exp OR 'methamphetamine'/exp OR 'midomafetamine'/exp OR 'nortriptyline'/exp OR 'opipramol'/exp OR 'protriptyline'/exp OR 'reboxetine'/exp OR 'reserpine'/exp OR 'tapentadol'/exp OR 'tetrabenazine'/exp OR 'trimipramine'/exp OR 'tyramine'/exp OR 'antidepressant agent'/exp OR 'antidepressive agent*':ab,ti OR antidepressant*:ab,ti OR 'antidepressant drug*':ab,ti OR thymoleptic*:ab,ti OR thymoanaleptic*:ab,ti OR 'second generation antidepressive agent*':ab,ti OR 'atypical antidepressant*':ab,ti OR 'second generation antidepressive drug*':ab,ti OR '5 hydroxytryptophan'/exp OR 'amisulpride'/exp OR 'amfebutamone'/exp OR 'maprotiline'/exp OR 'mianserin'/exp OR 'nefazodone'/exp OR 'quipazine'/exp OR 'ritanserin'/exp OR 'sulpiride'/exp OR 'tryptophan'/exp OR 'venlafaxine'/exp OR 'viloxazine'/exp OR 'serotonin antagonist'/exp OR '5 hydroxytryptamine antagonist*':ab,ti OR '5 ht antagonist*':ab,ti OR 'antiserotonergic agent*':ab,ti OR 'serotonin block*':ab,ti OR 'serotonin receptor antagonist*':ab,ti OR 'serotonin receptor block*':ab,ti OR 'serotonin antagonist*':ab,ti OR 'serotonin uptake inhibitor'/exp OR '5 ht uptake inhibitor*':ab,ti OR '5 hydroxytryptamine uptake inhibitor*':ab,ti OR 'selective serotonin reuptake inhibitor*':ab,ti OR 'serotonin reuptake inhibitor*':ab,ti OR 'serotonin uptake inhibitor*':ab,ti OR alaproclate OR 'amoxapine'/exp OR 'citalopram'/exp OR 'clomipramine'/exp OR femoxetine OR 'fenfluramin'/exp OR 'fluoxetine'/exp OR 'fluvoxamine'/exp OR indalpine OR 'norfenfluramine'/exp OR 'olanzapine'/exp OR 'paroxetine'/exp OR 'sertraline'/exp OR 'trazodone'/exp OR 'vilazodone'/exp OR 'vortioxetine'/exp OR 'zimeldine'/exp OR 'serotonin noradrenalin reuptake inhibitor'/exp OR 'nris and ssri*':ab,ti OR 'serotonin and noradrenaline uptake inhibitor*':ab,ti OR 'serotonin and norepinephrine reuptake inhibitor*':ab,ti OR snri*:ab,ti OR 'ssris and nri*':ab,ti) AND ('bone remodeling'/exp OR 'bone turnover*':ab,ti OR 'bone regeneration'/exp OR 'bone regenerat*':ab,ti OR 'bone conduction'/exp OR 'bone conduct*':ab,ti OR 'alveolar bone loss*':ab,ti OR 'osteoclast'/exp OR cementoclast*:ab,ti OR odontoclast*:ab,ti OR osteoclast*:ab,ti OR 'osteoblast'/exp OR osteoblast*:ab,ti OR 'osteolysis'/exp OR osteolyses*:ab,ti OR 'orthodontic tooth movement'/exp OR 'tooth movement*':ab,ti OR 'tooth intrusion*':ab,ti OR 'tooth depression*':ab,ti OR 'periodontal disease'/exp OR 'tooth migration':ab,ti OR 'tooth drift*':ab,ti OR osteporosis:ab,ti OR 'metabolic bone disease'/exp OR 'osteoporosis'/exp OR 'experimental bone loss'/exp) AND [embase]/lim NOT [medline]/lim	544
CINAHL	("Selective Serotonin Reuptake Inhibitors" OR "Adrenergic Uptake Inhibitors" OR "Amitriptyline" OR "Amoxapine" OR "Amphetamine" OR "Atomoxetine Hydrochloride" OR "Benzphetamine" OR "Desipramine" OR "dibutylone" OR "Dothiepin" OR "ethylphenidate" OR "Imipramine" OR "Maprotiline" OR "Mazindol" OR "Methamphetamine" OR "Nortriptyline" OR "Opipramol" OR "Protriptyline" OR "Reboxetine" OR "Reserpine" OR "Tapentadol" OR "Tetrabenazine" OR "Trimipramine" OR "Tyramine" OR "Viloxazine" OR "Antidepressive Agents" OR "Thymoleptic*" OR "Antidepressive Agents, Second-Generation" OR "Benzphetamine" OR "Desipramine" OR "Amisulpride" OR "Bupropion" OR "Citalopram" OR "Fluoxetine" OR "Fluvoxamine" OR "Mianserin" OR "nefazodone" OR "Paroxetine" OR "Quipazine" OR "Ritanserin" OR "Sulpiride" OR "Trazodone" OR "Tryptophan" OR "Venlafaxine-Hydrochloride" OR "Serotonin Antagonists" OR "alaproclate" OR "Citalopram" OR "Clomipramine" OR "femoxetine" OR "Fenfluramine" OR "indalpine" OR "Norfenfluramine" OR "Olanzapine" OR "Sertraline" OR "Vilazodone Hydrochloride" OR "Vortioxetine" OR "Zimeldine" OR "Serotonin and Noradrenaline Reuptake Inhibitors" OR "Serotonin-and-Noradrenaline-Uptake-Inhibitor*" OR "Serotonin-and-Norepinephrine-Reuptake-Inhibitor*" OR "SNRI" OR "SNRIs" OR "SSRI" OR "SSRIs" OR "nantenine" OR "paliperidone" OR "palonosetron" OR "pancopride" OR "paracetamol plus tramadol" OR "pelanserin" OR "perospirone" OR "Piboserod" OR "pirenperone" OR "pirepemat" OR "pizotifen" OR "pizotifen maleate" OR "pruvanserin" OR "quetiapine" OR "Ramosetron" OR "renzapride" OR "ricasetron" OR "risperidone" OR "ritanserin" OR "robalzotan" OR "roluperidone" OR "sarpogrelate" OR "sb773812" OR "seganserin" OR "sequifenadine" OR "sergolexole" OR "serotonin 1 antagonist" OR "serotonin 1A antagonist" OR "serotonin 1B antagonist" OR "serotonin 1D antagonist" OR "serotonin 2 antagonist") AND ("Bone Remodeling" OR "Bone-Turnover*" OR "Bone Regeneration" OR "Bone-Regenerat*" OR "Osteoconduct*" OR "Bone Resorption" OR "Bone-Resorpt*" OR "Osteoclastic-Bone-Loss" OR "Osteoclasts" OR "Cementoclast*" OR "Odontoclast*" OR "Osteoclast*" OR "Osteoblasts" OR "Osteoblast*" OR "Osteolysis" OR "Osteolyses*" OR "Tooth Movement Techniques" OR "Tooth-Movement*" OR "Tooth-Intrusion*" OR "Tooth-Depression*" OR "Tooth Migration" OR "Tooth-Drift*" OR "osteopenia" OR "Bone Diseases, Metabolic" OR "Osteoporosis" OR "bone mass" OR "bone defect" OR "Osteogenesis" OR "bone mineral density" OR "osteodensit*" OR "osseointegrat*" OR "osteoprogenitor*" OR "skeletal development") AND ("animal*" OR "equine" OR "mouse" OR "mice" OR "rodent*" OR "oryctolagus" OR "danio" OR "brachydanio" OR "caenorhabditis" OR "murine" OR "rat" OR "rats" OR "dogs" OR "dog" OR "horse" OR "feline" OR "canine*" OR "bovine" OR "cricetus" OR "cow" OR "cows" OR "cattle" OR "pig" OR "pigs" OR "miniature pig" OR "miniature pigs")	81

6 ARTIGO CIENTÍFICO 2

Does the use of Methylphenidate affect bone health? A systematic review of preclinical studies

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Does the use of Methylphenidate affect bone health? A systematic review of preclinical studies

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Abstract

Among psychostimulants, methylphenidate (MP), commonly known as Ritalin, has emerged as a prominent pharmacological intervention for attention-deficit/hyperactivity disorder (ADHD) in both pediatric and adult populations. Previous investigations have implicated potential associations between MP use and variations in bone mineral density, fracture risk, and growth modifications. Nonetheless, a persistent controversy surrounds the intricate relationship between MP administration and the mechanisms by which this medication may impact bone health. This systematic literature review was conceived to address a focused query: does the administration of MP influence bone health in animal models? In adherence to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines, registration with PROSPERO was undertaken (CRD42023468466). Subsequently, a comprehensive literature search encompassing MEDLINE, Embase, Scopus, and CINAHL databases, along with grey literature, was conducted to identify in vivo animal studies reporting bone-related parameters after MP treatment. No constraints were imposed regarding the year of publication or language, extending the search until December 2023. Among the 517 articles retrieved across databases, a meticulous eligibility assessment included five studies from 17 full-text articles. The qualitative synthesis was presented based on the in-depth analysis and comparison of study design, sample characteristics, drug regimen, and outcomes related to bone health in these studies. Overall, the investigations compared the potential effects of both low and high doses of MP on bone morphology, biomechanical properties, microstructural parameters, and histomorphometrics in both axial and appendicular skeletons of rats. A prevailing trend emerged, indicating that MP exhibits a propensity to exert deleterious effects on various bone parameters, particularly in long bones, with a positive correlation noted with dosage and treatment duration variations. Additionally, MP treatment potentially affects bone tissue directly through actions on specific bone cells and indirectly through effects correlated with body weight. However, it is imperative to underscore that the studies exhibited an unclear to high risk of bias, demanding cautious interpretation of conclusions regarding bone health. The current body of literature emphasizes the continued need to investigate plausible mechanisms underlying the impact of MP on bone. This exploration should encompass an analysis of time- and dose-response relationships and the potential association with variations in body weight, taking into

account hormonal patterns, particularly considering potential differences between male and female subjects.

Keywords: Methylphenidate; Drug Evaluation, Preclinical; Physiological Effects of Drugs; Bone Remodeling; Bone Resorption; Bone Regeneration; Systematic Review.

Introduction

Attention-deficit/hyperactivity disorder (ADHD) represents a cluster of neurodevelopmental conditions marked by attention impairment, hyperactivity, and deficient impulse control [1]. This condition is primarily diagnosed in childhood and often persists into adulthood, affecting approximately 5–10% of children globally [2-4]. Stimulant pharmacotherapy has emerged as a frequently employed initial intervention for the management of ADHD. Among these pharmacological agents, methylphenidate (MP), commonly recognized as Ritalin, is classified as a Central Nervous System (CNS) stimulant within the piperidine-derived compound category and remains a primary pharmacological intervention in managing this disorder [5,6]. This drug primarily targets and modulates the monoaminergic neurotransmitter systems, including dopamine, norepinephrine, and, to a lesser extent, serotonin [7,8]. More precisely, MP's mechanism of action occurs at dopamine transporters (DAT) and norepinephrine transporters (NET), with additional activity observed at serotonergic 5-HT1A receptors. The binding of MP to these targets induces an increase in synaptic levels of these catecholamines, creating its classic stimulant effect within the CNS, mainly in the prefrontal cortex [9,10].

Previous systematic reviews of clinical investigations have underscored a possible correlation between ADHD, MP treatment, and bone-related consequences, including bone mineral density variations, susceptibility to fractures, and modifications in bone growth [11-14]. While behavioral changes associated with ADHD may contribute to these consequences, pharmacological interventions like MP are implicated in mitigating such risks by controlling behavioral symptoms [11]. However, beyond this behavioral aspect, it has been suggested that MP may directly or indirectly influence bone health through its side effects on bone cells [15].

The precise mechanism through which MP may impact bone tissue remains elusive, and various hypotheses have been proposed. It is speculated that norepinephrine exerts inhibitory effects on bone formation while concurrently facilitating bone resorption [16]. These effects may be mediated by osteoblasts, which house β-2 adrenoreceptors, whose activation releases receptor activator of nuclear factor kappa beta (RANKL), subsequently promoting osteoclast-mediated bone resorption [17-19]. Moreover, MP appears to exert an indirect skeletal effect, potentially linked to fluctuations in body weight. A clinical study revealed a decrease in serum concentrations of leptin following the initiation of MP treatment [20]. This reduction in

leptin levels correlated with a significant loss of fat and bone mass, indicative of decreased bone formation [20]. Leptin, influencing bone homeostasis through the β-2 adrenergic pathway, may be implicated in the observed appetite reduction associated with MP use [21].

Despite the available evidence, clinical investigations in this domain frequently exhibit inherent complexities in experimental design, specifically pertaining to the administration of medication to individuals contending with mental health conditions. In these instances, the underlying mental health condition itself may introduce critical confounding factors, potentially acting as a catalyst for bone-related issues [11,22]. Considering this, crucial insights have been derived from animal studies strategically designed to isolate the effects of the medication on bone tissue, thereby minimizing the incorporation of potential confounding factors. Hence, this systematic review focuses on pre-clinical investigations to explore, compare, and synthesize potential biological mechanisms that underlie the observed effects of MP, in order to improve the knowledge regarding multifaceted factors influencing bone health, as well as potential implications associated with long-term stimulant use in individuals with ADHD.

Material and methods

Protocol and registration

This systematic review adhered to the guidelines outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [23]. The study protocol was preregistered on PROSPERO (CRD42023468466) to enhance transparency and ensure a systematic and methodologically sound approach.

Eligibility criteria

The authors established the Participants, Exposure, Comparison, and Outcomes (PECO) framework to delineate the eligibility criteria for study inclusion. The structured question framed for the systematic review succinctly encapsulated the research focus. The Population (P) included animals of all species, irrespective of gender. Exposure (E) involved the administration of MP. The Comparison (C) entailed either a control group (placebo or no intervention) or a sham group. The Outcome (O) sought to evaluate alterations in bone characteristics or their metabolic pathways.

Inclusion criteria encompassed preclinical in vivo animal studies featuring experimental and control or sham groups. The administration of MP, systemically or locally, constituted an integral component of the inclusion criteria. Furthermore, eligible studies were required to provide comprehensive details regarding dosages, administration procedures, and therapeutic regimens.

Studies were included to ensure a comprehensive assessment of bone-related effects if they reported data on various parameters, including bone remodeling markers, inflammatory mediators, bone cells, microarchitecture, phenotype, morphology, and mechanical properties. Conversely, exclusion criteria were defined to exclude study designs that were not directly relevant to the relationship between MP and bone physiology, such as partial or pilot studies. Additionally, studies lacking direct drug administration to the evaluated animals, in vivo human studies, inaccessible or missing data, full-text availability, and incomplete reporting were excluded. The inclusion and exclusion criteria were designed to include various species, genders, ages, study durations, languages, and publication years to maximize the breadth of the systematic review.

Information sources and search strategy

During the development of the search strategy, guidance was sought from the Medical University of South Carolina library services to ensure a comprehensive and systematic approach. The search covered major databases, including MEDLINE, Embase, Scopus, and CINAHL, with searches conducted until December 2023. Google Scholar (https://scholar.google.com) and the System for Information on Grey Literature in Europe (SIGLE), accessed through OpenGrey (www.opengrey.eu), were additional sources consulted. The electronic search utilized a structured combination of Medical Subject Heading (MeSH) terms, text words, Emtree terms, and free terms, employing Boolean operators "OR" and "AND" to optimize precision and recall. The complete systematic search strategy across all databases is detailed in Supplementary Table 1 for reference.

In addition to electronic searches, manual searches were performed on the references of selected studies to identify potentially relevant literature not captured through the electronic search. Covidence Systematic Review Software (Veritas Health Innovation, Melbourne, Australia) was employed to streamline the review process and ensure accuracy. This software facilitated efficient data organization and aided in

identifying and removing duplicate records, contributing to the integrity of the literature search and review process.

Study Selection

The studies identified through electronic search methodologies were consolidated into a unified database utilizing Endnote for systematic tabulation and organization. Subsequently, the compiled files were transferred to Covidence, where titles and abstracts underwent independent scrutiny by two reviewers (NF and MF). Articles demonstrating features congruent with the predetermined eligibility criteria were advanced for a comprehensive full-text examination. The full-text assessments involved meticulous scrutiny of each study, ensuring alignment with the established criteria for inclusion in the systematic review. Those studies meeting the defined criteria were conclusively incorporated into the systematic review. During this stage, individual reasons for exclusion were methodically documented. In instances where discrepancies or disagreements arose at any preceding phase, discussions between the two reviewers were carried out. Should a consensus prove elusive, a third researcher (IA) intervention was provided.

Data Extraction

The pertinent information was independently extracted from the enrolled studies by collaborating between two reviewers (NF and IA). The extracted data comprehensively covered various aspects, including study characteristics, demographic details of the studied population, particulars of interventions applied, reported outcomes, and conclusive results. Specifically, the outcomes of interest encompassed the expression of chemical mediators intricately linked to bone metabolism, the quantitative and qualitative aspects of bone cell populations, microarchitectural features, morphological characteristics, and biomechanical properties. Reviewer disagreements were resolved through discussion or consultation with a third researcher (MF), ensuring methodological rigor throughout the data extraction phase.

Risk of bias assessment in individual studies

The evaluation of potential bias in the included studies was independently conducted by two reviewers (NF and IA), utilizing the Systematic Review Center for

Laboratory Animal Experimentation (SYRCLE) risk of bias tool [24]. This comprehensive tool comprises ten domains designed to identify diverse forms of bias, including selection biases such as sequence generation, baseline characteristics, allocation concealment, and performance biases related to random housing and blinding. Detection biases involving random outcome assessment and blinding, attrition biases associated with incomplete outcome data, and reporting biases linked to selective outcome reporting are also encompassed. Additionally, the tool addresses other potential biases from diverse sources in the study design.

Within each of the ten domains, the risk of bias was categorized as "low," "high," or "unclear" based on the available information. The designation "unclear" was assigned when there was insufficient information for a definitive assessment. In the context of "baseline characteristics," factors such as weight, sex, and age of the animals were deemed relevant to the assessed outcomes. Studies providing complete information were classified as having a "low" risk of bias in this domain. Conversely, studies with partial reporting were categorized as "unclear" in this specific domain.

Summary measures and synthesis of results

In conducting a systematic review, the potential consideration of a meta-analysis is contingent upon adequate similarities among studies in terms of population characteristics, intervention methodologies, and reported outcomes, enabling a coherent synthesis of findings. In this study, three of the five [25–27] articles employed similar methods. However, most results were graphically presented, with some expressed as percentages rather than raw data. Attempts were made to contact the authors for access to raw data, but there was no response. Consequently, given the acknowledged limitations, conducting a meta-analysis proved unfeasible. Qualitative descriptions were employed to systematically integrate the diverse evidence from the selected studies, providing a comprehensive narrative synthesis and elucidating the findings and patterns observed across the literature.

Results

Study Selection

A comprehensive literature search identified 758 articles related to the impact of methylphenidate on bone remodeling in animals. After removing 241 duplicates, the remaining 517 articles underwent screening based on titles and abstracts.

Subsequently, 17 articles were selected to examine their full texts thoroughly. Among these, 12 articles were excluded for not meeting the predetermined selection criteria, particularly those presenting only abstracts from conference proceedings without full texts being available. Ultimately, five articles were deemed eligible and were included in the present systematic review [25–29]. The delineation of the identification, screening, eligibility, and inclusion processes can be seen in the PRISMA flowchart (Figure 1).

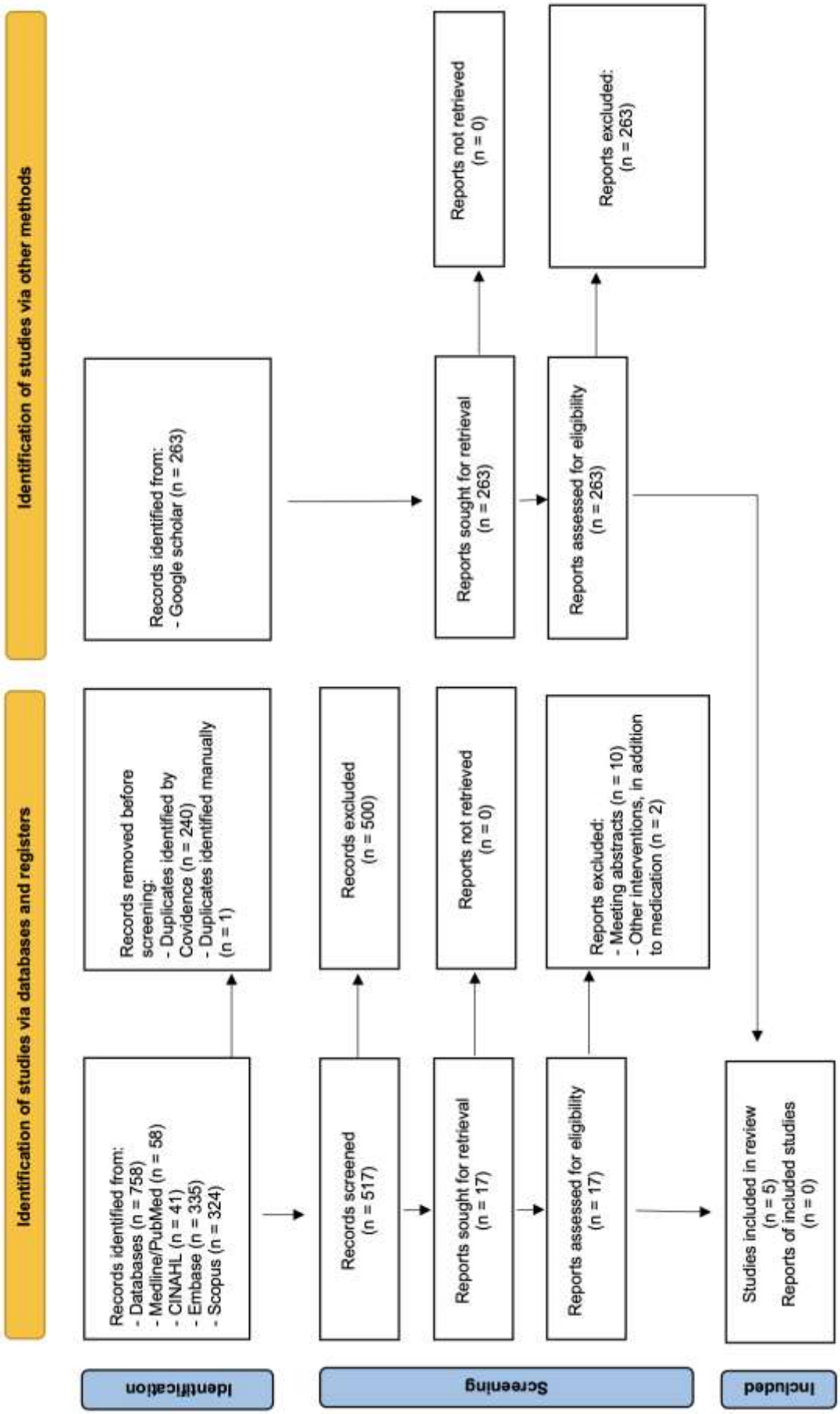

Figure 1. The flow diagram of articles selected for evaluating MP in bone tissue was adapted from the PRISMA statement.

Study Characteristics

This systematic review included five *in vivo* animal studies published between 2003 and 2023 (Table 1). The experiments exclusively involved Sprague-Dawley or Wistar rats as the chosen animal models. All studies employed male subjects, with one investigation assessing both genders [26]. MP was administered across the studies at dosages ranging from 4 to 60 mg/kg/day. Four studies specifically compared outcomes between low doses (4-10 mg/kg/day) and high doses (30-60 mg/kg/day) of MP [25–27,29]. The administration routes of the pharmacological agents included drinking water or orogastric intubation, with study durations spanning from 4 to 13 weeks. Outcome assessments were conducted through diverse methodologies, including colorimetric assays, histomorphometry, stereology, immunohistochemical analysis, micro-computed tomography (MicroCT), dual-energy X-ray absorptiometry (DXA), caliper measurements, and mechanical testing.

Table 1. Descriptive characteristics of studies evaluating the effects of MP on bone parameters.

Reference	Characteristics of animals	Groups (n/group)	Drug regimen	Method of evaluation	Evaluated parameters	Main findings
Türkbay et al. (2003)	Wistar rats Male 5 weeks old 120 ± 15 g	MP low dose (n = 10) MP high dose (n = 10) Control (water) (n = 10)	**MP low dose:** **TD:** 2 months **TOA:** 2 months **D:** 10 mg/kg/day (7 hours) **WA:** Orogastric intubation **MP high dose:** **TD:** 2 months **TOA:** 2 months **D:** 30 mg/kg/day **WA:** Orogastric intubation	DXA (femur)	BMD (g/cm^2), Length (cm)	No difference
Komatsu et al. (2012)	Sprague-Dawley rats Male 4 weeks old 100-125 g (extracted from graph)	MP low dose (n = 24) MP high dose (n = 24) Control (water) (n = 24)	**MP low dose:** **TD:** 13 weeks **TOA:** 13 weeks (n = 12) or 18 weeks (n = 12) - 13 weeks of treatment + 5 weeks without treatment **D:** 4mg/kg/day (1st hour) + 10 mg/kg/day (7 hours) **WA:** Drinking water **MP high dose:** **TD:** 13 weeks **TOA:** 13 weeks (n = 12) or 18 weeks (n = 12) - 13 weeks of treatment + 5 weeks without treatment) **D:** 30 mg/kg/day (1st hour) + 60 mg/kg/day (7 hours) **WA:** Drinking water	Caliper measurement (femur and L5vertebra)	Femur length (mm), Femur AP diameter (mm), Femur ML diameter (mm), Vertebra height (mm), Vertebra AP diameter(mm), Vertebra ML diameter (mm)	↓ AP diameter (femur, low-dose, and high-dose) *# * The difference was more significant in high doses than in low doses. # The differences were abolished after stopping the use of MP in both groups.
				DXA (femur, tibia, L5 vertebra)	BMD (g/cm^2), BMC (g), Area (cm^2)	↓ BMD (femur, tibia, high dose) # ↓ BMC (femur, tibia, high dose) # # The differences were abolished after stopping the use of MP.
				Bone mechanical testing (femur, L5 vertebra)	Ultimate force (N), Stiffness (N/mm), Energy to failure (mJ)	↓ Ultimate force (femur, high dose) # ↓ Energy to failure (femur, low-dose and high-dose) *# * The difference was more significant in high doses than in low doses. # The differences were abolished after stopping the use of MP in both groups.
				Colorimetric assay (serum)	ALP (U/L), CTX (ng/ml)	↑ ALP (high dose)! ! The differences persisted after stopping the use of MP
				Weighing	Body weight gain (g)	↓ Body weight gain (low dose) # ↓ Body weight gain (high dose) *# * The difference was more si-nificant in high doses than in low doses. # Cessation of MP led to a slight recovery of normal weight gain in both groups

Uddin et al. (2018)	Sprague-Dawley rats Male and female 4 weeks old 80-100 g (extracted from graph)	MP low dose (n = 12) MP high dose (n = 12) Low-dose pair-fed (a control group that received the same amount of food consumed by low-dose animals) (n = 12) High-dose pair-fed (a control group that received the same amount of food consumed by high-dose animals) (n = 12) Control (water) (n = 12)	**MP low dose:** **TD:** 13 weeks **TOA:** 13 weeks **D:** 4mg/kg/day (1st hour) + 10 mg/kg/day (7 hours) **WA:** Drinking water **MP high-dose:** **TD:** 13 weeks **TOA:** 13 weeks **D:** 30 mg/kg/day (1st hour) + 60 mg/kg/day (7 hours) **WA:** Drinking water	MicroCT (femur, L5 vertebra)	BV/TV (%), Trabecular BMD (g/cm^3), Tb.Th (mm), Ct.Th (mm), Ct.BMD (mgHA/cm^3), Ct. BV/TV (%)	No difference
				Bone mechanical testing (femur, L5 vertebra)	Ultimate force (N), Failure force (N), Stiffness (Nmm-1), Energy to failure (mJ)	↓ Energy to failure (femur, high-dose, male) ↓ Stiffness (femur, high-dose, male) ↓ Ultimate force (femur, high-dose, male) ↓ Failure force (femur, high-dose, male)
				Dynamic histomorphometric analyses (tibia, cancellous and cortical)	MS/BS (%), BFR/BS (µm/day)	↑ MS/BS labeling (cancellous, high-dose, male) ↑ BFR/BS labeling (cancellous, high-dose, male)
				TRAP staining (tibia)	Oc.S/BS (%), N.Oc (#/mm), N.Oc/BS (1/mm), TRAP V/TV (%)	↑ Oc.S/BS (high-dose, male** and female) ↑ N.Oc/BS (high-dose, male** and female) ↑ TRAP V/TV (high-dose, male**) ↑ N.Oc (high-dose, female) ** Compared to water and high-dose pair fed
				Caliper measurement (femur)	Length (mm), AP diameter (mm), ML diameter (mm)	No difference
				Weighing	Body weight gain (g)	↓ Body weight gain (high-dose, male and female)** ** Compared to the other 4 groups
Chirokikh et al. (2023)	Sprague-Dawley rats Male 4 weeks old ≅ 55g (extracted from graph)	MP (n = 10) Control (Water) (n = 10)	**TD:** 4 weeks **TOA:** 4 weeks **D:** 30 mg/kg/day (1st hour) + 60 mg/kg/day (7 hours) **WA:** Drinking water	Caliper measurement (femur, tibia)	Length (mm), AP diameter (mm), ML diameter (mm)	↑ Length (femur, tibia)*** *** Adjusted for body weight
				Histomorphometry (tibia)	Growth plate height (mm)	No difference
				MicroCT (tibia, femur)	TV (mm^3), BV (mm^3), BV/TV (N/A), Tb.N (1/mm), Tb.Th (mm), Tb.Sp (mm), vBMD (mgHA/cm^3), Ct.V (mm^3), Ps.V (mm^3), Ec.V (mm^3), Ct.Th (mm), TMD (mgHA/cm^3), pMOI (mm^4)	↓ BV (cancellous, tibia)**** ↓ vBMD (cancellous, tibia)**** ↑ Ct.V (cortical, femur)*** ****After adjusting for body weight, no significant difference was identified *** Adjusted for body weight
				Bone mechanical testing (femur)		↑ Yield force***

				Weighing	Yield force (N), Ultimate force (N), Stiffness (N/mm), Energy to failure (mJ) Body weight gain (g)	↑ Ultimate force*** *** Adjusted for body weight ↓ Body weight gain
Say et al. (2023)	Wistar rats Male 4 weeks old 76-106 g	MP low-dose (n = 10) MP high-dose (n = 10) Control (water) (n = 10)	**MP low dose:** **TD:** 13 weeks **TOA:** 13 weeks **D:** 4mg/kg/day (1st hour) + 10 mg/kg/day (7 hours) **WA:** Drinking water **MP high dose:** **TD:** 13 weeks **TOA:** 13 weeks **D:** 30 mg/kg/day (1st hour) + 60 mg/kg/day (7 hours) **WA:** Drinking water	Caliper measurement (femur)	Length (mm), AP diameter (mm), ML diameter (mm)	↓ Length (high dose) ↓ AP diameter (high dose) ↓ ML diameter (low-dose, high-dose)
				DXA ex vivo (femur)	BMD (g/cm^2), BMC (g)	↓ BMC (low-dose, high-dose) ↓ BMD (low-dose, high-dose)
				Bone mechanical testing (femur)	Stiffness (N/mm), Ultimate force (N)	↓ Stiffness (low-dose, high-dose) ↓ Ultimate force (low-dose, high-dose)
				Stereology (femur)	Total femoral volume (μm^3), Total cartilage volume ($x10^{-1} \mu m^3$), Total volume of growth zone (μm^3)	↓ Total femoral volume (low-dose, high-dose) ↓ Total cartilage volume (low dose)** ↓ Growth zone volume (low dose)** ** Compared to water and high-dose groups
				Immunohistochemical analysis (femur)	ALP positive cells (%), Anti-calpain positive cells (%)	No difference
				Weighing	Body weight gain (g)	↓ Body weight gain (low-dose, high dose)* * The difference was more significant in high doses than in low doses.

TDescription of acronyms in alphabetical order: **ALP**: Alkaline Phosphatase; **AP:** Anteroposterior; **BFR/BS:** Bone formation rate over the bone surface; **BMC:** Bone mineral content; **BMD:** Bone mineral density; **BS/BV**: Bone surface/bone volume; **BV:** Bone volume; **BV/TV**: Trabecular bone fraction; **Ct. BV/TV:** Cortical bone volume fraction; **Ct.BMD:** Cortical bone mineral density; **Ct.Th:** Cortical thickness; **Ct.V:** Cortical volume; **CTX:** C-terminal collagen crosslinks; **D:** Dosage; **DXA:** Dual-energy x-ray absorptiometry; **Ec.V:** Endocortical volume: **ML:** Mediolateral; **MP:** Methylphenidate; **MS/BS:** Mineralized bone surface fraction; **N.Oc:** Number of osteoclasts; **N.Oc/BS:** Number of osteoclasts over the bone surface; **N/A:** Not applicable; **Oc.S/BS:** Osteoclast surface fraction; **pMOI:** Polar moment of inertia; **Ps.V:** Periosteal volume; **Tb.N:** Trabecular number; **Tb.Sp:** Trabecular separation; **Tb.Th:** Trabecular thickness; **TD:** Treatment duration; **TMD:** Tissue mineral density; **TOA:** Time of outcome assessment; **TRAP:** Tartrate-resistant acid phosphatase; **TRAP V/TV:** TRAP volume over tissue volume; **TV:** Total volume; **vBMD:** Volumetric bone mineral density; **WA:** Way of administration. **↑↓:** Statistically significant increase (↑) or decrease (↓) of the parameter compared to the control (water) group. The "evaluated parameters" not elucidated in the "main findings" exhibited no statistically significant difference compared to the control group

Risk of bias within studies

Table 2 displays the outcomes of the risk of bias assessment for eligible studies. Overall, the risk of bias across all included studies was categorized as "unclear." Within the ten domains assessed by the SYRCLE tool, six items consistently scored as 'unclear risk' for all studies, owing to insufficient data. These items included item 1 (sequence generation), item 3 (allocation concealment), item 4 (random housing), item 6 (random outcome assessment), item 8 (incomplete outcome data), and item 10 (other sources of bias). Notably, although some studies reported incorporating a random component in sequence generation and outcome assessment, the methodological details were not described.

Conversely, no instances of selective outcome reporting were identified, as all expected outcomes were comprehensively reported in the published articles, ensuring transparency between methods and results sections. Additionally, two articles explicitly stated that outcome assessors were blinded to the interventions administered to each animal [26,28]. Key baseline characteristics, including sex, age, and weight of the animals, were considered pivotal for this study. When the authors did not explicitly specify these characteristics, item 2 was designated as "unclear."

Table 2. Summary of risk of bias assessment of studies evaluating the effects of MP on bone parameters

STUDIES	Signaling questions									
	1	2	3	4	5	6	7	8	9	10
Türkbay et al., 2003	Unclear	Low	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Unclear
Komatsu et al., 2012	Unclear	Low	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Unclear
Uddin et al., 2018	Unclear	Low	Unclear	Unclear	Unclear	Unclear	Low	Unclear	Low	Unclear
Chirokikh et al., 2023	Unclear	Low	Unclear	Unclear	High	Unclear	Low	Unclear	Low	Unclear
Say et al., 2023	Unclear	Low	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Unclear

SYRCLE tool bias evaluation
1) Was the allocation sequence adequately generated and applied?; 2) Were the groups similar at baseline, or were they adjusted for confounders in the analysis?; 3) Was the allocation adequately concealed?; 4) Were the animals randomly housed during the experiment?; 5) Were the caregivers and investigators blinded by the knowledge of which intervention each animal received during the experiment?; 6) Were animals selected at random for outcome assessment?; 7) Was the outcome assessor-blinded?; 8) Were incomplete outcome data adequately addressed?; 9) Are reports of the study free of selective outcome reporting?; 10) Was the study free of other problems that could result in a high risk of bias?
Classification of bias: high risk, unclear risk, low risk.

Results of individual studies

1. Bone morphology

Among the included studies, three investigations that evaluated the influence of MP on the growth and development of long bones under very similar experimental conditions (administration of low-dose [4 mg/kg/day + 10 mg/kg/day] and high-dose [30 mg/kg/day + 60 mg/kg/day]) for 13 weeks via drinking water in 4-week-old male rats), reported contrasting findings. Komatsu et al. [25] demonstrated a dose-dependent reduction in the AP diameter of the femur. In contrast, Say et al. [27] indicated a decrease in this parameter in animals treated with a high dose of MP. Uddin et al. [26] found no differences in the femur AP diameter, irrespective of the dose. When assessing the femur's ML diameter and length, two studies did not report significant changes after MP use [25,26]. However, Say et al. [27] documented a reduction in femur length in the high-dose group and a decreased ML diameter at both dosage levels. No alterations were observed in the L5 vertebra under the same experimental conditions [26]. Komatsu et al. [25] further reported that the previously observed differences were no longer evident following a five-week recovery period after MP treatment.

Chirokikh et al. [28] assessed the administration of a high dose of MP for a shorter duration (4 weeks). No differences in length, AP, or ML diameters were detected in this scenario. However, when the results were statistically adjusted for body weight, an increase in the size of the femur and tibia was observed. Female rats did not show changes in femur length, AP, or ML diameters after 13 weeks of MP treatment, regardless of the dosage [26].

2. Bone mechanical properties

Femoral and vertebral biomechanical properties were assessed through a 3-point bending test following MP consumption. The three studies with similar protocols [25–27] consistently revealed an impairment of biomechanical properties for male rats treated for 13 weeks. There was a reduction in femoral ultimate force after high-dose MP [25–27], with Say et al. [27] also observing this effect in the low-dose group. In line with these findings, low-dose and high-dose treatments reported a decrease in energy to failure in the femur. A reduction in stiffness was also noted under the same experimental conditions in low-dose [27] and high-dose [26,27] treatments.

Additionally, Uddin et al. [26] reported a decrease in the femoral failure force of animals treated with a high-dose MP. The biomechanical alterations reported by Komatsu et al. [25] were reversed after discontinuing the treatment for five weeks. No vertebral changes were observed under these conditions [25,26].

Conversely, high-dose MP for a shorter period of 4 weeks did not lead to any noticeable alterations in the evaluated femoral biomechanical properties. However, an increase in ultimate force and yield force became apparent when the outcomes were normalized for the body weight [28]. No changes were identified in the femur or vertebra of female animals, regardless of the dosage [26].

3. Bone microstructure

MicroCT or DXA provided the analysis of bone microstructure parameters. Under different treatment protocols, two studies employed MicroCT to evaluate bone microarchitecture, focusing on the femur, tibia, and vertebra. In the first scenario, male and female rats treated with both low and high doses of MP for 13 weeks exhibited no discernible microarchitectural changes in the vertebra and femur [26]. Conversely, Chirokikh et al. [28], conducting a 4-week high-dose MP regimen in male rats, observed a substantial reduction of 45% in BV and 34% in vBMD in the trabecular bone of the tibia. However, no significant differences were identified after adjusting these results for body weight. Additionally, an increase in Ct.V was noted in the femur after adjusting for body weight.

DXA analyses unveiled contrasting outcomes across diverse scenarios. Türkbay et al. [29] concluded that male subjects subjected to 10 or 30 mg/kg/day of MP for two months exhibited no statistically significant differences in femur length or BMD compared to the control group. Conversely, Komatsu et al. [25] reported that high-dose treatment for 13 weeks was associated with reduced BMD and BMC in the femur and tibia. Low-dose treatment did not induce substantial alterations in the assessed parameters. The differences found with the high dose were abolished after discontinuing the use of MP. Even with varied dosages, vertebral parameters remained unaltered. In line with Komatsu et al. [25], Say et al. [27], employing the same treatment protocol, noted a reduction in both BMC and BMD in the femur, not only in high doses of MP but also in low doses. None of the included studies have assessed these parameters in female animals.

4. Histomorphometric parameters

The data suggests that the effects of MP on bone quantity are sex- and dose-dependent, mediated by the activities of both osteoblasts and osteoclasts. Uddin et al. [26] demonstrated, through dynamic histomorphometric analyses, increased trabecular bone formation and mineralization rates in male rats treated with high-dose MP for 13 weeks, with no significant differences observed in females. Furthermore, male rats treated with high-dose MP exhibited an increase in TRAP-positive cells in the tibia, with significant elevations in the number of osteoclasts over the bone surface (N.Oc/BS), osteoclast surface fraction (Oc.S/BS), and TRAP-volume fraction (TRAP V/TV). Females treated with high-dose MP displayed a similar effect on osteoclasts, albeit to a lesser extent, showing a significant increase in Oc.S/BS and N.Oc/BS but not TRAP V/TV. Low-dose MP did not induce significant changes in these parameters, regardless of sex [26].

In a similar treatment protocol for males, Say et al. [27] found a significant reduction in total femoral volume, total cartilage volume, and growth zone volume after the administration of low-dose MP and a decrease in total femoral volume was also observed with a high-dose. However, immunohistochemical analysis did not reveal differences in the femur's ALP-positive cells and anti-calpain-positive cells. Finally, in male rats treated with a high dose for four weeks, Chirokikh et al. [28] did not identify significant changes in growth plate height in the tibia.

5. Bone-related markers

Among the five included studies, only one assessed the expression of biomarkers associated with bone remodeling [25]. Administration of a high dose of MP for 13 weeks in male rats was linked to elevated serum levels of ALP. This disparity persisted even after a 5-week cessation of MP use. In contrast, administering a low dose of MP did not alter ALP expression. Additionally, serum CTX levels, a marker of bone resorption, did not exhibit significant changes, irrespective of the dosage protocol. No studies with female animals assessed bone-related markers in the context of MP treatment.

6. Body weight gain

Over time, a decrease in weight gain was associated with low [25,28] and high [25-28] doses of MP treatment, irrespective of the treatment duration. According to

Komatsu et al. [25] and Say et al. [27], the decrease in weight gain is dose-dependent. Furthermore, after a 5-week recovery protocol without the medication, this difference was no longer evident [25].

Discussion

A well-functioning skeletal status and average longitudinal growth in children and adolescents indicate good bone health, potentially predicting favorable bone health in adulthood. This condition safeguards against bone diseases, such as osteoporosis and related fractures, throughout the lifespan [10]. The influence of several chronic medications on bone metabolism, particularly CNS stimulants, has gained prominence as widely used psychopharmacologic drugs across all age groups [14,30]. Still, controversy persists regarding their impact on bone health. Multiple studies, particularly those utilizing animal models, have been conducted to elucidate the precise effects and underlying mechanisms of MP on bone. To the best of our knowledge, this systematic review represents the first comprehensive exploration of this topic, aiming to assess, compare, and synthesize the bone-related effects of MP treatment. The findings suggest that MP may induce diverse and dose-dependent effects on bone tissue, exhibiting variations across bone markers, cell activities, microarchitecture, morphology, and biomechanical properties. While the extant literature reports a few conflicting findings, a discernible inclination emerges, suggesting a prevailing trend wherein MP exhibits a propensity to exert deleterious effects on various bone parameters.

One of the primary outcomes related to the skeletal effects of MP treatment focused on bone growth, measured through caliper assessments of femur, tibia, and vertebra dimensions at the end of the experimental period. Despite quite similar protocols, distinct results were reported. While Komatsu et al. [25] documented a dose-dependent reduction in the AP diameter of the femur after 13 weeks of MP treatment, Say et al. [27] demonstrated a similar effect, but only at a high dose of the medication, and Uddin et al. [26] reported no differences under the same conditions. Furthermore, Say et al. [27] also identified a reduction in femur length and ML diameter, which was not corroborated by the other studies [25,26]. The reasons for these variations remain unclear. Moreover, these harmful effects of MP on skeletal dimensions appear to be localized to appendicular regions, as evidenced by discernible impacts on femurs.

In contrast, no observable effects on vertebrae were identified [25]. It has been hypothesized that the observed difference between axial and appendicular skeletons may be related to the preferential suppression of cortical rather than trabecular bone growth by MP [25]. Another possibility is that MP alters regular locomotor activity, and the axial sites, which are more load-responsive, are preferentially affected [25]. Further investigations are warranted to elucidate this potential correlation.

Surprisingly, despite using a high dose of MP for a shorter treatment duration (4 weeks) not leading to altered bone dimensions, the length of long bones (femur and tibia) was detected after statistical adjustment for body weight [28]. This suggests that skeletal development outpaced body weight accrual under MP treatment. Overall, the duration of MP exposure may be a critical variable, and variations in weight as a side effect of MP should be considered in future research. The enhanced inclination to impair bone growth observed in pre-clinical studies aligns with the findings from human studies. A recent systematic review concluded that prolonged MP treatment could lead to a minor growth deficit, especially concerning height. However, a clinically significant impact may be discerned in a small minority of individuals [13].

As crucial as assessing characteristics of bone structure is evaluating possible correlations and their impact on functionality. A 13-week treatment with MP exhibited a clear tendency to impair the biomechanical properties of the femur, detected by a significant dose-dependent decrease in energy to failure [25,26], stiffness [26,27], ultimate force [25-27], and failure force [26]. The axial skeleton is unaffected, as the vertebra maintains its biomechanical properties unaltered [26]. Interestingly, no impairment was observed in female rats [26]. Similar to the bone morphology results, the shorter duration of high-dose treatment did not impact the biomechanical behavior of the femur [28]. However, it did demonstrate an increase in yield and ultimate forces after statistical adjustment for body weight [28]. An intriguing parallel can be drawn between biomechanical bone properties assessed in animals and the fracture risk investigated in humans. While certain clinical studies assert a reduction in fracture risk with MP use, others contend an elevated risk of stress fractures [31-34]. In children diagnosed with ADHD, characterized by symptoms such as hyperactivity and impulsiveness, the propensity for traumatic injuries is acknowledged. Treatments for ADHD, encompassing stimulant and non-stimulant modalities, are documented to mitigate these symptoms, consequently reducing the risk of traumatic fractures [32].

Conversely, stress fractures stemming from repetitive micro-trauma to the bone exhibit an increased risk associated with MP use, as evidenced by comprehensive studies in military populations [34]. This contextual background underscores the imperative of discerning the potential impacts of ADHD on bone tissue and distinguishing them from the biochemical effects attributed to drug treatments. The systematic review conducted in animal models facilitated a more nuanced evaluation of the biochemical impact of MP, mitigating the influence of environmental factors that could otherwise introduce confounding variables.

In conjunction with the reported morphological and biomechanical changes above, microstructural parameters were assessed through MicroCT and DXA and subsequently correlated. Initially, Uddin et al. [26] emphasized that, despite clear evidence of reduced mechanical strength following MP treatment, this reduction might be attributed to the degradation of the bone extracellular matrix, particularly the collagen structure, as MicroCT did not detect any microstructural variation. In contrast, in a 4-week treatment, Chirokikh et al. [28] observed a decrease in BV and vBMD from MicroCT, which was insufficient to impact biomechanical properties. Nevertheless, upon adjusting the results for body weight, the previously reported improvement in mechanical properties was supported by the increase in femoral Ct.V, while changes in BV and vBMD were no longer statistically significant. When DXA was performed, Komatsu et al. [25] and Say et al. [27] observed a reduction in BMD and BMC in long bones after both low [27] and high [25,27] MP doses for 13 weeks. This suggests that MP caused a decrease in bone mineralization, aligning with the associated biomechanical compromise. In contrast, Türkbay et al. [29] found no differences in femoral BMD assessed by the same method. This divergence may be related to the different methodological protocols employed, utilizing distinct dosages and treatment durations. Among the proposed mechanisms for elucidating the reduction in bone density and mineralization induced by psychotropic drugs is the potential role of calcium and vitamin D deficiency, attributed to the appetite-suppressing and weight-reducing effects of MP [35]. Further studies are needed to clarify this possible correlation.

Although the results of animal studies should not be directly extrapolated to the clinical context, a distinctive association can be inferred. A recent systematic review of clinical studies assessed the influence of psychostimulants on BMD and BMC at various sites, including the femur, hip, and spine. In general, 80% of the included

studies reported that psychostimulant medications, including MP, negatively impact these bone parameters in children with ADHD [14], which is in line with the findings of this study. It was noted that the duration (in years) of drug administration appears to be a contributing factor influencing BMC, BMD, and bone turnover. However, estimating the precise onset of when MP influences osseous parameters presents challenges. The authors also highlighted the difficulty in assessing the impact of gender on bone changes in this context. Additionally, it is crucial to acknowledge the potential for diverse bone-related effects in individuals with medical conditions that may compromise bone health and the underlying potential for drug interactions [14].

Histomorphometric analyses were also conducted to delve further into the cellular effects of MP. In contrast to the harmful effects reported in the assessments above, Uddin et al. [26] identified enhanced bone formation in the cortical portion of the tibia after 13 weeks of high-dose MP treatment in males. Simultaneously, TRAP staining elucidated the causative factors behind these sex-dependent effects on cortical bone. High-dose MP male rats displayed a marked increase in TRAP volume fraction, osteoclast number, and osteoclast surface in cortical bone. Conversely, analogous osteoclast number and surface trends were discerned in females without a concurrent elevation in TRAP volume fraction. Despite these observations, the results could not definitively determine whether MP directly influences osteoclasts or if these effects are mediated through secondary pathways. Ex vivo differentiation of preosteoclasts into osteoclasts following direct MP exposure demonstrated heightened osteoclastogenesis in a sex- and dose-dependent manner for both male and female cells. Furthermore, disparities in morphology and activity of osteoclasts were noted between genders, with male cultures forming progressively large multinucleated osteoclasts through cell fusion.

In contrast, female cultures exhibited delayed formation of giant multinucleated osteoclasts and a higher prevalence of small TRAP+ cell clusters, indicative of delayed cell fusion. These ex-vivo osteoclast experiments corroborate the sex- and dose-dependent effects on osteoclast activity and maturity observed in vivo [26]. More in-depth analyses of the interactions of MP with osteoclasts, DAT expression, and precise signaling pathways are required to comprehend the effects of MP on osteoclast activity fully.

Say et al. [27] conducted distinct histomorphometric analyses. They identified, through stereology, a decrease in total femoral volume, total cartilage volume, and

growth zone volume, particularly in the low-dose MP treatment. This implies that elevated doses of MP adversely affect bone formation and growth by impeding the conversion of cartilage tissue into bone tissue in the growth zone. The hypothesis has been raised that MP may block intracellular calcium accumulation in the cells of the hypertrophic zone of the growth plate, thereby inhibiting the differentiation of chondroblasts to osteocytes and suppressing bone formation and growth. Additional studies on this topic are warranted [27].

Within the scope of this systematic review, only one study investigated bone markers, focusing on ALP and CTX levels [25]. Remarkably, ALP levels were unexpectedly elevated in MP-High rats despite concurrent reductions in femoral size, disrupted mechanical properties, diminished femoral and tibial BMD, and BMC. In contrast, serum levels of CTX, a marker of bone resorption, did not differ significantly with MP treatment. The authors proposed potential explanations for this unexpected finding, suggesting that the measurement of total ALP, rather than the bone-specific isoform, might have contributed to the observed elevation. Furthermore, even if the increase in ALP was due to enhanced osteoblastic activity, a more substantial induction of osteoclastic activity could have been responsible for the other detrimental effects on bone. Further investigations are imperative to identify more pertinent and clinically applicable biomarkers.

Although not a direct bone parameter, body weight gain was a significant consideration in 4 out of the five included studies, as the anorexigenic effects of MP are well-documented in previous studies and have the potential to impact bone tissue development and homeostasis [20,36]. It was unanimous among all studies that measured this parameter that MP inhibits weight gain in a dose-dependent manner, regardless of the treatment duration and gender [25-28]. Moreover, even when the pair-feeding paradigm was applied, weight gain decreased in the high-dose MP group, suggesting that MP impairs weight gain independently of food consumption [26]. As MP treatment inhibited weight gain, it is possible that lower body mass in MP-treated rats contributed to their impaired skeletal development [25-28]. However, several reasons suggest that lower body mass cannot fully account for these findings. Some evaluated parameters showed no significant changes after body weight adjustments, indicating that MP impairs bone independently of its effects on body weight [25,28]. It should also be noted that, as body composition was not measured, it is impossible to determine if MP had differential effects on fat and lean mass, which could result in

different impacts on skeletal development [25]. Future studies should investigate the effects of weight variations on the assessed outcomes and take pair-feeding into account to achieve more accurate results.

A single study assessed the skeletal effects of MP and the extent of their reversibility after a 5-week recovery protocol without treatment [25]. Despite significantly impairing skeletal growth, mineralization, and biomechanical integrity in the appendicular sites of adolescent rats, these parameters rapidly returned to normal following the cessation of treatment. Moreover, the variation in body weight followed the same pattern. Only the serum levels of ALP detected in high-dose MP persisted after treatment discontinuation, potentially contributing to the swift skeletal normalization observed in the recovery protocol. The authors suggest that this pathway is still unclear, as the circulating level of this bone marker may be influenced by factors related to bone metabolism, hepatic interference, or intestinal changes. In this case, bone-specific ALP should be investigated.

The limitations of the current research protocol are primarily rooted in the quality of eligible studies. Systematic literature reviews face the challenge of evaluating the methodological quality of included studies through risk-of-bias evaluation tools [24]. A majority of the assessed domains received an "unclear" status. Among these limitations is the need for explicit methodology detailing the randomization process, despite some studies acknowledging a random component in sequence generation and outcome assessment. The adoption of the ARRIVE guidelines [37] by numerous journals is anticipated to enhance the reporting quality of research publications, effectively addressing this issue in the future.

Furthermore, despite some methodological homogeneity in certain studies, the lack of raw data makes the quantitative analysis of the presented data challenging. Despite efforts to obtain this task through communication with authors and data extraction from graphs, this task still needs to be completed. Due to the inherent nature of the presented data, minimal values are unreliable when obtained by approximation, potentially introducing errors in statistical analysis.

Additionally, the limited number of studies assessing specific aspects, such as bone-related markers and drug effects on female subjects, complicates drawing definitive conclusions. The intricate nature of bone remodeling processes and the multifaceted impact of MP warrant further investigation to elucidate the underlying mechanisms. The limitations in this systematic review underscore the critical role of

such reviews in animal studies, scrutinizing the validity of preclinical evidence and identifying deficiencies in research projects to encourage improvements in methodological rigor, results reporting, publication transparency, and a reduction in unnecessary duplication of animal studies [38-40].

Conclusions

This systematic literature review posits that the use of MP might predominantly negatively impact the skeletal health of rodents, with a significant association noted mainly in the appendicular bones and a direct correlation with variations in body weight. However, it is crucial to emphasize that the studies included in this review demonstrated a substantial risk of bias. Therefore, caution is warranted in interpreting conclusions regarding the effects of MP on bone health. The existing body of literature underscores the necessity for ongoing investigations to elucidate the potential mechanisms through which MP affects bone health. This includes delving into the relationships between dosage and sex responses, as well as exploring temporal effects. In a clinical context, it is advisable to consider bone health when prescribing medications of this class, particularly for individuals with pre-existing risk factors for bone-related issues.

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Supplementary Table 1. Search Strategy According to Database

DATABASE	SEARCH STRATEGY	RESULTS
PUBMED	("Aptensio XR"[tw] OR Biphentin[tw] OR Calocain[tw] OR Centedrin*[tw] OR Concerta[tw] OR "Cotempla XR-ODT"[tw] OR Daytrana[tw] OR delmosart[tw] OR dexmethylphenidat*[tw] OR Equasym[tw] OR Focalin[tw] OR medikinet[tw] OR meridil[tw] OR Metadate[tw] OR "Methylphenidate"[Mesh] OR Methylphenidate[tw] OR "Methyl phenidat*"[tw] OR "Methyl phenidylacetat*"[tw] OR Methylin[tw] OR Methypatch[tw] OR Metilfenidato[tw] OR Penid[tw] OR Phenidylat*[tw] OR Plimasin*[tw] OR psychostimulant*[ti] OR "QuilliChew ER"[tw] OR Ritalin[tw] OR Ritaline[tw] OR Ritalin-SR[tw] OR Rubifen[tw] OR Stimdat*[tw] OR stimulant*[ti] OR Tsentedrin*[tw]) AND (bone[ti] OR "bone characteristics"[tw] OR "bone defect"[tw] OR "bone density"[tw] OR "bone development"[tw] OR "Bone Diseases, Metabolic"[Mesh] OR "bone healing"[tw] OR "bone loss"[tw] OR "bone mass"[tw] OR "bone metabolism"[tw] OR "bone mineral density"[tw] OR "bone remodeling"[tw] OR "bone resorp*"[tw] OR "bone turnover*"[tw] OR "bone regenerat*"[tw] OR "bone regeneration"[tiab:~3] OR "canalicular remodeling"[tiab:~3] OR cementoclast*[tw] OR "endochondral ossification*"[tw] OR "growth plate closure"[tw] OR "Haversian remodeling"[tw] OR odontoclast*[tw] OR ossification*[tw] OR osseo*[ti] OR osseointegrat*[tw] OR "Osteoblasts"[Mesh] OR osteoblast*[tw] OR osteoclastogenesis[tw] OR osteoclast*[tw] OR osteoconduct*[tw] OR osteoclastogeneses[tw] OR osteodensit*[tw] OR "Osteogenesis"[Mesh] OR osteogenesis[tw] OR osteolys*[ti] OR osteolyses*[tw] OR osteolysis[tw] OR osteopenia[tw] OR "Osteoporosis"[Mesh] OR osteoporosis[tw] OR osteoprogenitor*[tw] OR "perilacunar remodeling"[tiab:~3] OR "physiologic ossification"[tw] OR "skeletal development"[tw] OR "tooth depression*"[tw] OR "tooth drift*"[tw] OR "tooth intrusion*"[tw] OR "tooth migration"[tw] OR "tooth movement"[tw]) NOT ("Humans"[Mesh] NOT "Animals"[Mesh])	58
SCOPUS	#1: TITLE: "Aptensio XR" OR biphentin OR calocain OR centedrin* OR concerta OR "Cotempla XR-ODT" OR daytrana OR delmosart OR dexmethylphenidat* OR equasym OR focalin OR medikinet OR meridil OR metadate OR methylphenidate OR "Methyl phenidat*" OR "Methyl phenidylacetat*" OR methylin OR methypatch OR metilfenidato OR penid OR phenidylat* OR plimasin* OR psychostimulant* OR "QuilliChew ER" OR ritalin OR ritaline OR ritalin-sr OR rubifen OR stimdat* OR stimulant* OR tsentedrin* #2: ABSTRACT: "Aptensio XR" OR biphentin OR calocain OR centedrin* OR concerta OR "Cotempla XR-ODT" OR daytrana OR delmosart OR dexmethylphenidat* OR equasym OR focalin OR medikinet OR meridil OR metadate OR methylphenidate OR "Methyl phenidat*" OR "Methyl phenidylacetat*" OR methylin OR methypatch OR metilfenidato OR penid OR phenidylat* OR plimasin* OR psychostimulant* OR "QuilliChew ER" OR ritalin OR ritaline OR ritalin-sr OR rubifen OR stimdat* OR tsentedrin* #3: #1 OR #2 #4: TITLE: bone OR "bone characteristics" OR "bone defect" OR "bone density" OR "bone development" OR {Metabolic Bone Diseases} OR "bone healing" OR {bone loss} OR "bone mass" OR "bone metabolism" OR "bone mineral density" OR "bone remodeling" OR "bone resorp*" OR "bone turnover*" OR "bone regenerat*" OR "bone regeneration" OR "canalicular remodeling" OR cementoclast* OR "endochondral ossification*" OR "Haversian remodeling" OR odontoclast* OR ossification* OR osseo* OR osseointegrat* OR "Osteoblasts" OR osteoblast* OR osteoclastogenesis OR osteoclast* OR osteoconduct* OR osteoclastogeneses OR osteodensit* OR "Osteogenesis" OR osteogenesis OR osteolys* OR osteolyses* OR osteolysis OR osteopenia OR osteoporosis OR osteoprogenitor* OR "perilacunar remodeling" OR "physiologic ossification" OR "skeletal development" OR "tooth depression*" OR "tooth drift*" OR "tooth intrusion*" OR "tooth migration" OR "tooth movement" #5: ABSTRACT: bone OR "bone characteristics" OR "bone defect" OR "bone density" OR "bone development" OR {Metabolic Bone Diseases} OR "bone healing" OR {bone loss} OR "bone mass" OR "bone metabolism" OR "bone mineral density" OR "bone remodeling" OR "bone resorp*" OR "bone -turnover*" OR "bone regenerat*" OR "bone regeneration" OR "canalicular remodeling" OR cementoclast* OR "endochondral ossification*" OR "Haversian remodeling" OR odontoclast* OR ossification* OR osseo* OR osseointegrat* OR "Osteoblasts" OR osteoblast* OR osteoclastogenesis OR osteoclast* OR osteoconduct* OR osteoclastogeneses OR osteodensit* OR "Osteogenesis" OR osteogenesis OR osteolys* OR osteolyses* OR osteolysis OR osteopenia OR osteoporosis OR osteoprogenitor* OR "perilacunar remodeling" OR "physiologic ossification" OR "skeletal development" OR "tooth depression*" OR "tooth drift*" OR "tooth intrusion*" OR "tooth migration" OR "tooth movement" #6: #4 OR #5 #7: #3 AND #6 #8: TITLE: child* OR adolesc* OR boys OR men OR women #9: #7 AND NOT #8	324
EMBASE	('Methylphenidate'/exp OR 'Methylphenidate' [Pharmacological Action] OR 'Metadate' OR 'Equasym' OR 'Methylin' OR 'Concerta' OR 'Phenidylate' OR 'Ritalin' OR 'Ritaline' OR 'Ritalin-SR' OR 'Ritalin SR' OR 'Tsentedrin' OR 'Centedrin' OR 'Daytrana' OR 'Methylphenidate Hydrochloride' OR 'Hydrochloride, Methylphenidate') AND ('Bone Remodeling'/exp OR 'Bone-Turnover*' OR 'Bone Regeneration'/exp OR 'Bone-Regenerat*' OR 'Osteoconduct*' OR 'Bone Resorption'/exp OR 'Bone-Resorpt*' OR 'Osteoclastic-Bone-Loss' OR 'Osteoclasts'/exp OR 'Cementoclast*' OR 'Odontoclast*' OR 'Osteoclast*' OR 'Osteoblasts'/exp OR 'Osteoblast*' OR 'Osteolysis'/exp OR 'Osteolyses*' OR 'Bone Diseases, Metabolic'/exp OR 'Osteoporosis'/exp OR 'bone mass' OR 'bone defect' OR 'Osteogenesis' OR 'bone mineral density' OR 'osteodensit*' OR 'osseointegrat*' OR 'osteoprogenitor*' OR 'skeletal development' OR 'Bone Growth' OR 'Bone and Bones'/exp OR 'Bone and Bones') AND [embase]/lim NOT [medline]/lim	335
CINAHL	(("Aptensio XR" OR Biphentin OR Calocain OR Centedrin* OR Concerta OR "Cotempla XR-ODT" OR Daytrana OR delmosart OR dexmethylphenidat* OR Equasym OR Focalin OR medikinet OR	41

	meridil OR Metadate OR Methylphenidate OR (MH “Methylphenidate”) OR "Methyl phenidat*" OR "Methyl phenidylacetat*" OR Methylin OR Methypatch OR Metilfenidato OR Penid OR Phenidylat* OR Plimasin* OR psychostimulant* OR "QuilliChew ER" OR Ritalin OR Ritaline OR Ritalin-SR OR Rubifen OR Stimdat* OR stimulant* OR Tsentedrin* OR central nervous system stimulants OR (MM "Reserpine") OR (MM "Nortriptyline") OR (MM "Milnacipran Hydrochloride")) AND (TI bone OR (MH bone) OR "bone characteristics" OR "bone defect" OR "bone density" OR (MH "Bone Regeneration+") OR (MH "Bone Development+") OR (MH "Bone Diseases, Metabolic+") (MM "Bone Diseases") OR (MM "Bone Diseases, Metabolic/CO") OR "bone healing" OR "bone loss" OR "bone mass" OR "bone metabolism" OR "bone mineral density" OR "bone remodeling" OR "bone resorp*" OR "bone -turnover*" OR "bone regenerat*" OR "bone regeneration” OR "canalicular remodeling" OR cementoclast* OR "endochondral ossification*" OR "growth plate closure" OR "Haversian remodeling" OR odontoclast* OR ossification* OR osseo* OR osseointegrat* OR "Osteoblasts" OR osteoblast* OR osteoclastogenesis OR osteoclast* OR osteoconduct* OR osteoclastogeneses OR osteodensit* OR "Osteogenesis" OR osteogenesis OR osteolys* OR osteolyses* OR osteolysis OR osteopenia OR "Osteoporosis" OR osteoporosis OR osteoprogenitor* OR "perilacunar remodeling” OR "physiologic ossification" OR "skeletal development" OR "tooth depression*" OR "tooth drift*" OR "tooth intrusion*" OR "tooth migration" OR "tooth movement"))) NOT TI (child* OR adolesc* OR boys OR men OR women)	

7 CONSIDERAÇÕES FINAIS

Este estudo foi inicialmente idealizado pelos pesquisadores a partir de uma observação clínica recorrente que evidencia a crescente demanda por tratamento ortodôntico em pacientes medicados para condições de saúde mental, particularmente com antidepressivos e estimulantes do SNC. No planejamento de uma intervenção ortodôntica, é imperativo considerar os fatores que possam influenciar a biologia óssea, afetando os movimentos ortodônticos e ortopédicos e, por conseguinte, a eficácia e a eficiência do tratamento. Além disso, é relevante abordar tais aspectos não apenas no contexto da Ortodontia, mas também em outras condições clínicas potencialmente associadas à saúde óssea. Nesse sentido, o grupo de pesquisa optou por ampliar a investigação para explorar os possíveis efeitos esqueléticos gerais, visando aumentar o impacto das descobertas no campo científico. Diante das evidências científicas prévias que apontam para interações entre esses fármacos e o tecido ósseo, e considerando os resultados conflitantes, identificou-se a necessidade de uma investigação mais aprofundada sobre como essas questões estão inter-relacionadas e de levantar possíveis hipóteses sobre como o tipo de medicamento, dosagem, duração do tratamento e outros efeitos adversos podem influenciar a saúde óssea do indivíduo.

Dessa forma, as revisões sistemáticas da literatura em modelos animais foram a metodologia de escolha, pelo fato de envolver uma série de análises mais detalhadas, como testes mecânicos, variações de morfologia óssea, avaliação microestrutural, identificação de biomarcadores e seus impactos nas células ósseas, que permitem hipotetizar os mecanismos biológicos subjacentes a esse processo. Embora os resultados de estudos pré-clínicos não sejam passíveis de interpretação direta para seres humanos, os modelos animais oferecem uma vantagem significativa ao isolar muitas variáveis de confusão presentes em estudos clínicos, como os efeitos da própria doença, interações medicamentosas e influências de fatores externos, como consumo de álcool e tabagismo. Esse fator é de suma importância, considerando que tais medicamentos são prescritos não apenas para o manejo da depressão e TDAH, mas também como coadjuvantes em outras condições, como transtorno de ansiedade, bipolaridade e transtornos de humor. A crescente utilização do MF, em particular entre estudantes, como a "droga do estudo" para aumentar o foco e a concentração, suscita preocupações adicionais. Portanto, é imprescindível

aprofundar o conhecimento sobre os potenciais efeitos desses medicamentos na saúde óssea, a fim de fundamentar investigações futuras que visem correlacioná-los com essas variáveis.

O presente trabalho foi conduzido com o apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001. Adicionalmente, a doutoranda foi beneficiada com uma bolsa do Programa de Doutorado Sanduíche no Exterior (PDSE), durante o qual teve a oportunidade de vivenciar por oito meses (outubro/2022 a maio/2023) o ambiente acadêmico na área de Ortodontia na Medical University of South Carolina. Durante esse período, além do desenvolvimento dos estudos principais desta tese, uma pesquisa colaborativa foi realizada com o Dr. Subramanya Pandruvada (Professor do Departamento de Ciências da Saúde Bucal), com o objetivo de avaliar, por meio de cultura celular in vitro, a influência das cininas nas células ósseas. Os experimentos realizados foram complementares a um estudo anteriormente iniciado em colaboração com a Universidade Federal de Minas Gerais, visando demonstrar em um modelo animal in vivo a influência do sistema calicreína-cinina no processo de remodelação óssea induzido por força mecânica. O manuscrito encontra-se nas etapas finais de elaboração para submissão a uma revista científica.

Essa tese gerou como produtos principais os artigos intitulados “The role of selective serotonin reuptake inhibitors (SSRIs) in bone health: A systematic review of in vivo animal studies” e "Does the use of Methylphenidate affect bone health? A systematic review of preclinical studies", como requisitos parciais para obtenção do título de Doutora em Clínicas Odontológicas pela Pontifícia Universidade Católica de Minas Gerais.

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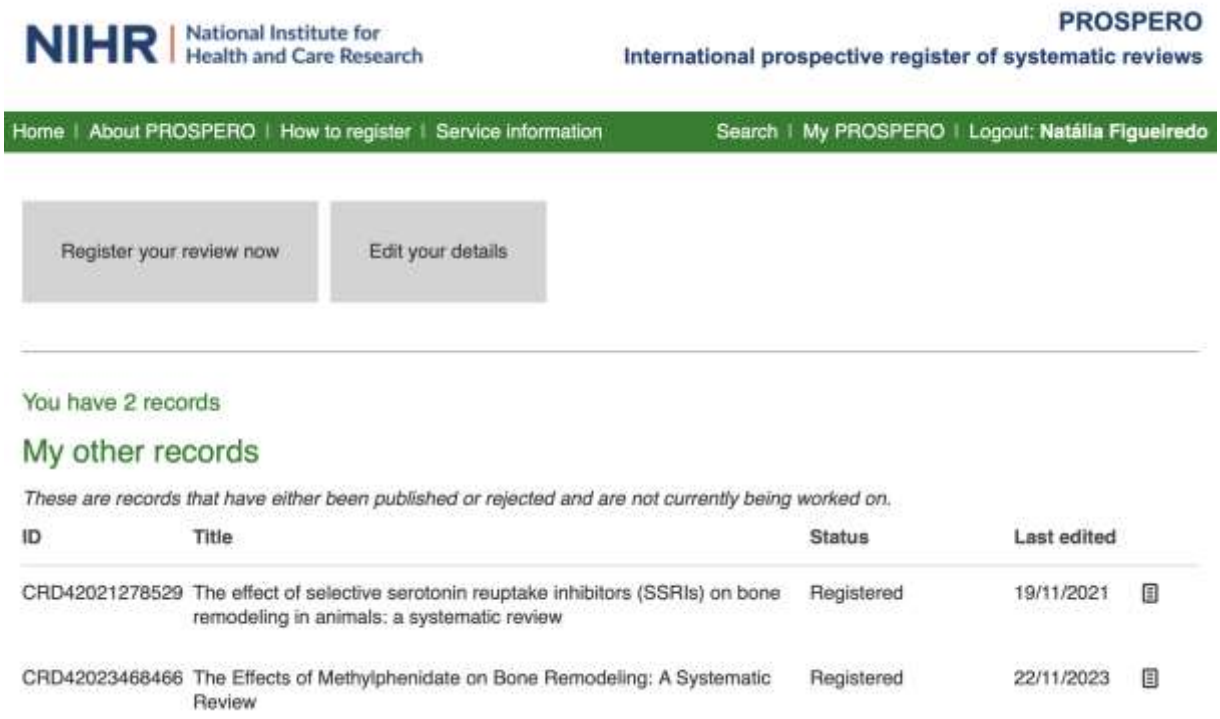
ANEXO A – Registro PROSPERO



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CRD42021278529	The effect of selective serotonin reuptake inhibitors (SSRIs) on bone remodeling in animals: a systematic review	Registered	19/11/2021
CRD42023468466	The Effects of Methylphenidate on Bone Remodeling: A Systematic Review	Registered	22/11/2023

ANEXO B - Produção intelectual do aluno durante o Curso de Doutorado

1. Artigos completos publicados em periódicos

ANDRADE JR, Ildeu; PASCHOAL, Marco Aurélio Benini; **FIGUEIREDO, Natália Couto**. Modified Arnold expander: an alternative for mandibular arch expansion. **Dental Press Journal of Orthodontics**, v. 26, 2021.

Dental Press Journal of ORTHODONTICS

Volume 26 - Number 5 - Online

SPECIAL ARTICLE

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Modified Arnold expander: an alternative for mandibular arch expansion

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Eto, V. M., **Figueiredo, N. C.**, Eto, L. F., Azevedo, G. M., Silva, A. I. V., & Andrade Jr, I. (2023). Bone thickness and height of the buccal shelf area and the mandibular canal position for miniscrew insertion in patients with different vertical facial patterns, age, and sex. *The Angle Orthodontist*, *93*(2), 185-194.

Original Article

Bone thickness and height of the buccal shelf area and the mandibular canal position for miniscrew insertion in patients with different vertical facial patterns, age, and sex

Vitor Mascarenhas Eto[a]; Natália Couto Figueiredo[b]; Luiz Fernando Eto[c]; Gabriel Maia Azevedo[a]; Amaro Ilídio Vespasiano Silva[d]; Ildeu Andrade Jr.[e]

ABSTRACT

Objectives: The objectives of this article were the following: (1) to analyze bone thickness and height (BTH) of the buccal shelf area (BS) quantitatively in four different potentially eligible sites for miniscrew insertion; (2) to compare and contrast BTH and the changes in spatial position of the inferior alveolar nerve canal (IANC); and (3) to assess differences with age among vertical facial patterns (hypodivergent, normodivergent, and hyperdivergent) and sex.

Materials and Methods: Cone-beam computed tomography scans of 205 individuals (110 women and 95 men) were divided into groups according to age, vertical facial pattern, and sex. The BTH of the BS and the BTH to the IANC were measured in the mesial and distal roots of the first and second molars.

Results: BTH progressively increased in a posterior direction ($P < .001$), while BTH to the IANC increased and decreased ($P < .001$) for thickness and height, respectively, in the same direction in all age groups, for the three different vertical facial patterns, and in both sexes. Women showed significantly less BTH to the IANC ($P < .002$). Hypodivergent patients had greater BTH ($P < .024$) and a smaller bone height to the IANC ($P < .018$) only in the first molar region. Patients over 40 years of age had lower bone height in the second molar area ($P < .003$).

Conclusions: The ideal place for BS miniscrew insertion is the region of the distal root of the second molars, regardless of facial pattern, sex, and age. The BS in women has less BTH and less BTH to the IANC. (*Angle Orthod.* 2023;93:185–194.)

KEY WORDS: Mini dental implant; Trabecular bone; Cortical bone; Mandibular canal

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INTRODUCTION

Miniscrews have been widely used to obtain temporary skeletal anchorage and to facilitate orthodontic biomechanics.[1] The overall failure rate of miniscrews was shown[2] to be 13.5%, and a failure rate of 7% has been reported[3] for extra-alveolar miniscrews. The stability of these devices is directly linked to bone density, bone thickness and height (BTH), insertion site, and proximity to roots, nerves, and vessels.[4–7]

An optimal place for installing mandibular miniscrews is the buccal shelf area (BS) because of the quantity and quality of bone available,[8–12] the high rate of stability of the devices, and the distance from the dental roots.[3] However, the best insertion site in the BS and how vertical facial pattern, age, and sex influence site suitability remain unclear given anatomic variability.[8–12]

2. Capítulo de livro publicado

FIGUEIREDO, Natália Couto. Capítulo 3 - Movimentação dentária ortodôntica e reações teciduais. *In:* LIMA, Yasmin Caroline Furtado de, *et al.* (org.). **Livro eletrônico: Reações teciduais frente à movimentação dentária ortodôntica e suas implicações clínicas.** Belo Horizonte: Editora PUC Minas, 2014. ISBN: 978-65-88547-59-5. Disponível em: https://www.editora.pucminas.br/obra/reacoes-teciduais-frente-a-movimentacao-dentaria-ortodontica-e-suas-implicacoes-clinicas

3. Resumos publicados em Anais de Eventos

FIGUEIREDO, N. C.; REIS, A. C. A. ; GUEDES, A. S. G. ; FERES, M. F. N. ; ARAUJO, V. E. ; ANDRADE JUNIOR, I Maxillary protraction in children with cleft lip and palate: a systematic review and meta-analysis. In: 37a Reunião Anual da Sociedade Brasileira de Pesquisa Odontológica, 2020. Proceedings of the 37th SBPqO Virtual Annual Meeting, 2020. v. 34. p. 436-436.

RS058 **Maxillary protraction in children with cleft lip and palate: a systematic review and meta-analysis**

Figueiredo NC, Reis ACA, Guedes ASG, Feres MFN, Araújo VE, Andrade-Júnior I*
Odontologia - PONTIFÍCIA UNIVERSIDADE CATÓLICA DE MINAS GERAIS.

Não há conflito de interesse

Maxillary protraction has been widely implemented in patients with cleft lip and palate (CLP) and skeletal Class III malocclusion, although there still remains a controversy about its skeletal effects and stability. The aim of this systematic review and meta-analysis was to evaluate the short and long-term effects of maxillary protraction in patients with CLP and skeletal Class III malocclusion. The search was performed without restrictions on MEDLINE, Embase, PubMed, LILACS and all EBM Reviews electronic databases. Randomized or prospective controlled clinical trials enrolling children with CLP (up to 12 years) treated with maxillary protraction, which had included untreated patients with cleft as a control group were selected. The risk of bias was assessed by ROBINS-I tool and four CCT´s were included. Statistically significant increases were observed in ANB (mean average difference (MAD) of 4.10°), SNA (MAD of 2.41°), overjet (MAD of 5.08 mm) and incisor inclination (MAD of 1.53°) in the treatment group, which also showed a statistically significant decrease in SNB (MAD of -1.70°).

In conclusion, moderate level of evidence indicates that maxillary protraction in patients with CLP can be a short-term effective therapy and might improve sagittal skeletal and dental relationships in the medium term. The skeletal modifications are forward displacement of maxilla, clockwise rotation of the mandible, and counterclockwise rotation of the maxillary plane. The dental changes are flaring of maxillary incisors and greater overjet. PROSPERO number: 42018117952

Natália Couto Figueiredo*, Vitor Mascarenhas Eto, Luiz Fernando Eto, Amaro Ilídio Vespasiano Silva, Ildeu Andrade Jr. Variabilidade da região buccal shelf e do canal do nervo alveolar inferior em pacientes com diferentes padrões verticais, idade e sexo. Em: Anais do 13º Congresso Internacional da Associação Brasileira de Ortodontia e Ortopedia Facial, 2022. p. 129.

13º CONGRESSO INTERNACIONAL ABOR

VARIABILIDADE DA REGIÃO BUCCAL SHELF E DO CANAL DO NERVO ALVEOLAR INFERIOR EM PACIENTES COM DIFERENTES PADRÕES VERTICAIS, IDADE E SEXO

Autor(es)

Natália Couto Figueiredo*, Vitor Mascarenhas Eto, Luiz Fernando Eto, Amaro Ilídio Vespasiano Silva, Ildeu Andrade Jr

Orientador(es)

Ildeu Andrade Jr

Instituição

Pontifícia Universidade Católica de Minas Gerais

Resumo

Os objetivos deste estudo retrospectivo transversal foram analisar a espessura e a altura da área do Buccal Shelf (BS), bem como a posição espacial do canal do nervo alveolar inferior (CNAI) no corpo mandibular, em 4 diferentes sítios potencialmente elegíveis para a colocação de mini-implantes. A amostra foi composta por tomografias computadorizadas de feixe cônico de 205 indivíduos, divididos por faixa etária, padrão esquelético vertical e sexo. As raízes dos primeiros e segundos molares mandibulares foram usadas como referência para medir os parâmetros estipulados. A altura e a espessura óssea aumentam progressivamente em direção posterior ($p < 0,001$), enquanto a distância vertical e a espessura óssea para o CNAI diminui e aumenta ($p < 0,001$), respectivamente, na mesma direção, em todos os grupos avaliados. A distância da junção cemento-esmalte (JCE) para o CNAI ($p<0,002$) e a espessura óssea para o CNAI ($p<0,001$) é significativamente menor em mulheres. Os pacientes hipodivergentes apresentaram maior espessura ($p<0,01$) e altura ($p<0,024$) ósseas, e menor distância JCE-CNAI ($p<0,018$) na região primeiro molar. Pacientes acima de 40 anos apresentaram menor espessura óssea à 6 mm da JCE no primeiro molar ($p< 0,001$), e menor altura óssea à 5 mm da região raiz distovestibular do segundo molar ($p<0,003$). O local ideal para a colocação do mini-implante BS é na região vestibular da raiz distal dos segundos molares, independente do padrão facial, sexo e idade. As mulheres têm uma menor altura e espessura ósseas, uma menor distância JCE-CNAI e uma menor espessura óssea para o CNAI.

4. Premiações

13º Congresso Internacional ABOR 2022 - 3º lugar do Fórum Científico - Categoria Doutorado. Variabilidade da região do buccal shelf e do canal do nervo alveolar inferior em pacientes com diferentes padrões verticais, idade e sexo. **Natália Couto Figueiredo***, Vitor Mascarenhas Eto, Luiz Fernando Eto, Amaro Ilídio Vespasiano Silva, Ildeu Andrade Jr.

13º CONGRESSO INTERNACIONAL ABOR

15 A 18DE JUNHO DE 2022
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FORTALEZA • CEARÁ • BRASIL

REALIZAÇÃO ABOR

CERTIFICADO

Certificamos que,

o Trabalho Científico intitulado **VARIABILIDADE DA REGIÃO BUCCAL SHELF E DO CANAL DO NERVO ALVEOLAR INFERIOR EM PACIENTES COM DIFERENTES PADRÕES VERTICAIS, IDADE E SEXO**

da autora, NATÁLIA DO COUTO FIGUEIREDO, foi premiado como terceiro melhor trabalho (3º lugar) das apresentações do Fórum Científico – Categoria Doutorado no **13° Congresso Internacional de Ortodontia e Ortopedia Facial ABOR**, realizado no Centro de Eventos do Ceará, em Fortaleza (CE), de 15 a 18 de junho de 2022.

Fortaleza, 18 de junho de 2022

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NADJA GURGEL
PRESIDENTE DO CONGRESSO

ADRIANA BEZERRA
DIRETORA CIENTÍFICA DO CONGRESSO

REALIZAÇÃO ABOR CE · APOIO · PATROCÍNIO DIAMANTE clearcorrect iD-Logical MORELLI · ORGANIZAÇÃO ARX

54º Encontro do Grupo Brasileiro de Professores de Ortodontia e Odontopediatria – 3º lugar do Fórum Científico de Ortodontia. Avaliação da espessura óssea palatina em indivíduos com diferentes faixas etárias, padrões sagitais e verticais: um estudo transversal. Azevedo GM*, Silva AIV, Guimarães FAM, **Figueiredo NC**, Souki BQ, Andrade I Jr.

Fórum Científico de Ortodontia

3° Lugar

AVALIAÇÃO DA ESPESSURA ÓSSEA PALATINA EM INDIVÍDUOS COM DIFERENTES FAIXAS ETÁRIAS, PADRÕES SAGITAIS E VERTICAIS: UM ESTUDO TRANSVERSAL

Autores: Azevedo GM*, Silva AIV, Guimarães FAM, Figueiredo NC, Souki BQ, Andrade I Jr

Apresentador: Gabriel Maia Azevedo

Instituição: Pontifícia Universidade Católica de Minas Gerais, Belo Horizonte, Brasil

54º Encontro do Grupo Brasileiro de Professores de Ortodontia e Odontopediatria

Certificado

UNIFASE

Certificamos que o trabalho intitulado **FCO-03 - AVALIAÇÃO DA ESPESSURA ÓSSEA PALATINA EM INDIVÍDUOS COM DIFERENTES FAIXAS ETÁRIAS, PADRÕES SAGITAIS E VERTICAIS: UM ESTUDO TRANSVERSAL**, dos autores Azevedo GM*, Silva AIV, Guimarães FAM, Figueiredo NC, Souki BQ, Andrade I Jr , foi apresentado na sessão de FÓRUM CIENTÍFICO DE ORTODONTIA, durante o 54° Encontro do Grupo Brasileiro de Professores de Ortodontia e Odontopediatria, realizado no período de 10 a 12 de agosto de 2023.

Petrópolis, 12 de agosto de 2023.

Profa. Dra. VERA SOVIERO
Coordenadora do 54º Encontro

Prof. Dr. LEANDRO S. MARQUES
Presidente do GRUPO

PATROCÍNIO MASTER

PATROCÍNIO

APOIO

5. Apresentação de trabalhos em congressos

Nome
A influência da sinalização dos receptores B2 do sistema calicreína-cinina na movimentação dentária ortodôntica

Instituição
Pontifícia Universidade Católica de Minas Gerais

Categoria
Fórum Científico (Tese de Doutorado)

Evento
14º CONGRESSO INTERNACIONAL DA ABOR - [2023]

Data de Inscrição
06/08/2023

Situação
Aceito

Autor(es)
Natália Couto Figueiredo*, Carina Cristina Montalvany Antonucci, Tarcília Aparecida da Silva, Soraia Macari, Ildeu Andrade Júnior

Resumo
O sistema calicreína-cinina é um complexo multienzimático que regula diversos processos inflamatórios através de dois receptores, B1 e B2, presentes em diferentes células, incluindo osteoblastos. No entanto, ainda não está claro como esse sistema afeta a remodelação óssea induzida mecanicamente. O objetivo deste estudo foi investigar o papel dos receptores B1 e B2 na remodelação óssea induzida por força mecânica. Uma força ortodôntica foi aplicada aos primeiros molares superiores em camundongos deficientes para os receptores B1 (B1-/-) e B2 (B2-/-), assim como em animais selvagens (WT). A análise microtomográfica foi usada para avaliar a microarquitetura óssea da maxila, a quantidade de perda óssea alveolar e o movimento dentário ortodôntico (MDO). O número de osteoblastos e osteoclastos foi determinado por análise histopatológica. A expressão de RANK, RANKL, OPG, B1, IL-1 β, IL-6 e TNF-α foi avaliada por qPCR. Os resultados demonstraram que B2-/- e B1-/- exibiram redução da espessura trabecular e aumento do número de células TRAP-positivas, em comparação com WT. No entanto, B2-/- apresentou maior perda óssea alveolar horizontal e MDO, quando comparado a B1-/- e WT. Esses efeitos foram correlacionados com o aumento da expressão de receptores B1, IL-1 β, RANK e razão RANKL/OPG nas maxilas de animais B2-/-. Portanto, os achados sugerem que a falta de receptores B2 pode contribuir para a modulação da resposta inflamatória, que afeta a perda óssea em um modelo de remodelação óssea induzida por força mecânica, e também regula a expressão de IL-1β na via RANK/RANKL/OPG, aumentando a osteoclastogênese. Assim, o eixo cinina-B2 pode ser considerado como um alvo para modular o MDO.

Nome
Variabilidade da região buccal shelf e do canal do nervo alveolar inferior em pacientes com diferentes padrões verticais, idade e sexo

Instituição
Pontifícia Universidade Católica de Minas Gerais

Categoria
Fórum Científico

Evento
13º CONGRESSO INTERNACIONAL DA ABOR (2022)

Data de Inscrição
30/03/2022

Situação
Aceito

Autor(es)
Natália Couto Figueiredo*, Vitor Mascarenhas Eto, Luiz Fernando Eto, Amaro Ilídio Vespasiano Silva, Ildeu Andrade Jr

Resumo
Os objetivos deste estudo retrospectivo transversal foram analisar a espessura e a altura da área do buccal shelf (BS), bem como a posição espacial do canal do nervo alveolar inferior (CNAI) no corpo mandibular, em 4 diferentes sítios potencialmente elegíveis para a colocação de mini-implantes. A amostra foi composta por tomografias computadorizadas de feixe cônico de 205 indivíduos, divididos por faixa etária, padrão esquelético vertical e sexo. As raízes dos primeiros e segundos molares mandibulares foram usadas como referência para medir os parâmetros estipulados. A altura e a espessura óssea aumentam progressivamente em direção posterior ($p < 0,001$), enquanto a distância vertical e a espessura óssea para o CNAI diminui e aumenta ($p < 0,001$), respectivamente, na mesma direção, em todos os grupos avaliados. A distância da junção cemento-esmalte (JCE) para o CNAI ($p<0,002$) e a espessura óssea para o CNAI ($p<0,001$) é significativamente menor em mulheres. Os pacientes hipodivergentes apresentaram maior espessura ($p<0,01$) e altura ($p<0,024$) ósseas, e menor distância JCE-CNAI ($p<0,018$) na região primeiro molar. Pacientes acima de 40 anos apresentaram menor espessura óssea à 6 mm da JCE no primeiro molar ($p< 0,001$), e menor altura óssea à 5 mm da região raiz distovestibular do segundo molar ($p<0,003$). O local ideal para a colocação do mini-implante BS é na região vestibular da raiz distal dos segundos molares, independente do padrão facial, sexo e idade. As mulheres têm uma menor altura e espessura ósseas, uma menor distância JCE-CNAI e uma menor espessura óssea para o CNAI.

RS058 Maxillary protraction in children with cleft lip and palate: a systematic review and meta-analysis

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Não há conflito de interesse

Maxillary protraction has been widely implemented in patients with cleft lip and palate (CLP) and skeletal Class III malocclusion, although there still remains a controversy about its skeletal effects and stability. The aim of this systematic review and meta-analysis was to evaluate the short and long-term effects of maxillary protraction in patients with CLP and skeletal Class III malocclusion. The search was performed without restrictions on MEDLINE, Embase, PubMed, LILACS and all EBM Reviews electronic databases. Randomized or prospective controlled clinical trials enrolling children with CLP (up to 12 years) treated with maxillary protraction, which had included untreated patients with cleft as a control group were selected. The risk of bias was assessed by ROBINS-I tool and four CCT´s were included. Statistically significant increases were observed in ANB (mean average difference (MAD) of 4.10°), SNA (MAD of 2.41°), overjet (MAD of 5.08 mm) and incisor inclination (MAD of 1.53°) in the treatment group, which also showed a statistically significant decrease in SNB (MAD of -1.70°).

In conclusion, moderate level of evidence indicates that maxillary protraction in patients with CLP can be a short-term effective therapy and might improve sagittal skeletal and dental relationships in the medium term. The skeletal modifications are forward displacement of maxilla, clockwise rotation of the mandible, and counterclockwise rotation of the maxillary plane. The dental changes are flaring of maxillary incisors and greater overjet. PROSPERO number: 42018117952

6. Produção técnica científica

Foram elaborados e publicados 22 vídeos didáticos, em português e Inglês, sobre temas diversos relacionados à Ortodontia. Esse material encontra-se disponível na plataforma do YouTube, no canal “[gotas de conhecimento em odontologia] PUC Minas”, disponível em:
https://www.youtube.com/@gotasdeconhecimentoemodont9680

1. ETO, V. M. ; **FIGUEIREDO, N. C.** ; LANZA, G. L. ; ANDRADE JUNIOR, I. . Gota #62 EXPANSÃO MAXILAR: EFEITOS DENTOESQUELÉTICOS. 2020. (Desenvolvimento de material didático ou instrucional - Vídeo didático).

2. ETO, V. M. ; **FIGUEIREDO, N. C.** ; LANZA, G. L. ; GUIMARAES, F. A. M. ; AZEVEDO, G. M. ; ANDRADE JUNIOR, I. Drop #62 MAXILLARY EXPANSION: DENTOSKELETAL EFFECTS. 2020. (Desenvolvimento de material didático ou instrucional - Vídeo didático).

3. ETO, V. M. ; **FIGUEIREDO, N. C.** ; LANZA, G. L. ; ANDRADE JR, I. Gota #63 EXPANSÃO MAXILAR: QUAL APARELHO DEVO UTILIZAR?. 2020. (Desenvolvimento de material didático ou instrucional - Vídeo didático).

4. ETO, V. M. ; **FIGUEIREDO, N. C.** ; LANZA, G. L. ; GUIMARAES, F. A. M. ; AZEVEDO, G. M. ; ANDRADE JR, I. Drop #63 MAXILLARY EXPANSION: WHAT APPLIANCE SHOULD I USE?. 2020. (Desenvolvimento de material didático ou instrucional - Vídeo didático).

5. ETO, V. M. ; **FIGUEIREDO, N. C.** ; LANZA, G. L. ; GUIMARAES, F. A. M. ; AZEVEDO, G. M. ; ANDRADE JR, I. Gota #70 EXPANSÃO MAXILAR: SARPE X MARPE. 2020. (Desenvolvimento de material didático ou instrucional - Vídeo didático).

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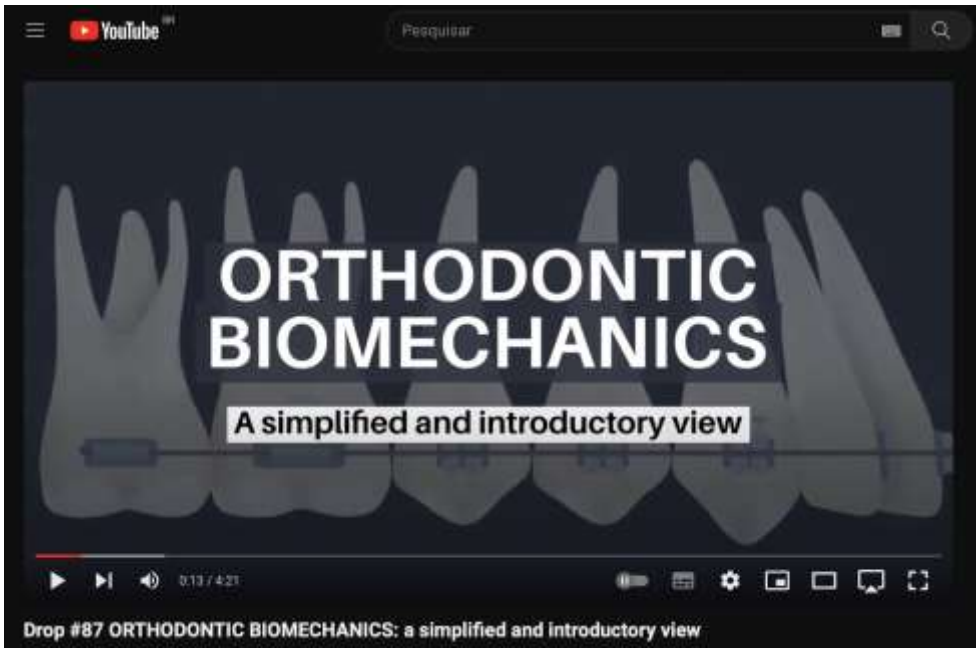
7. GUIMARAES, F. A. M. ; **FIGUEIREDO, N. C.** ; LANZA, G. L. ; ETO, V. M. ; AZEVEDO, G. M. ; ANDRADE JR, I. Gota #87 BIOMECÂNICA ORTODÔNTICA: uma visão simplificada e introdutória. 2020. (Desenvolvimento de material didático ou instrucional - Vídeo didático).

8. GUIMARAES, F. A. M. ; **FIGUEIREDO, N. C.** ; LANZA, G. L. ; ETO, V. M. ; AZEVEDO, G. M. ; ANDRADE JR, I. Drop #87 ORTHODONTIC BIOMECHANICS: a simplified and introductory view. 2020. (Desenvolvimento de material didático ou instrucional - Vídeo didático).



9. AZEVEDO, G. M. ; **FIGUEIREDO, N. C.** ; LANZA, G. L. ; GUIMARAES, F. A. M. ; ETO, V. M. ; ANDRADE JR, I. Gota #92 ALINHADORES ORTODÔNTICOS: conceitos básicos. 2020. (Desenvolvimento de material didático ou instrucional - Vídeo didático).

10. AZEVEDO, G. M. ; **FIGUEIREDO, N. C.**; LANZA, G. L. ; GUIMARAES, F. A. M. ; ETO, V. M. ; ANDRADE JR, I. Drop #92 ORTHODONTIC ALIGNERS: basic concepts. 2020. (Desenvolvimento de material didático ou instrucional - Vídeo didático).

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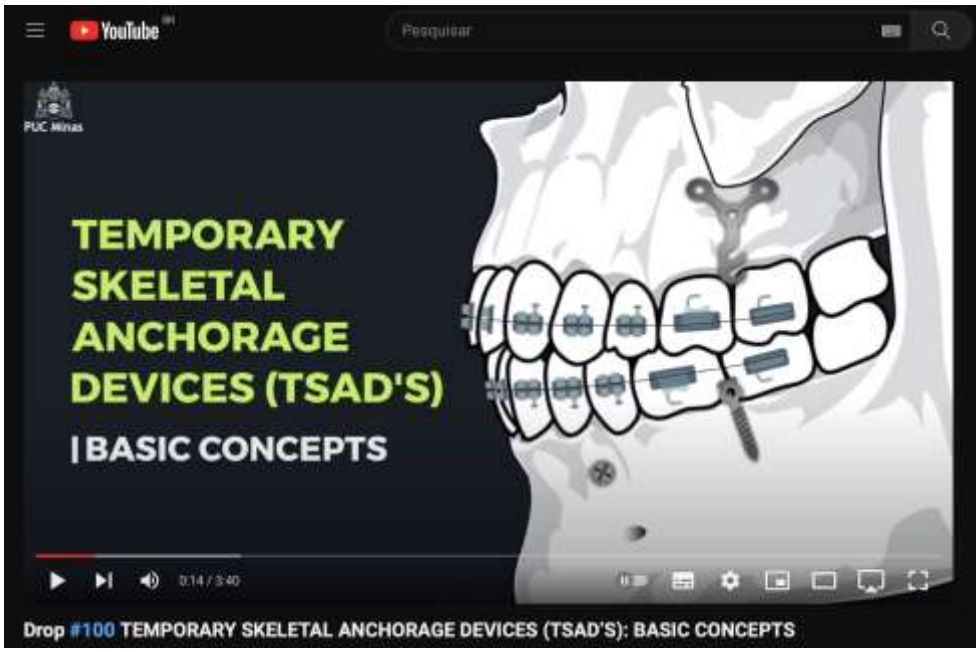

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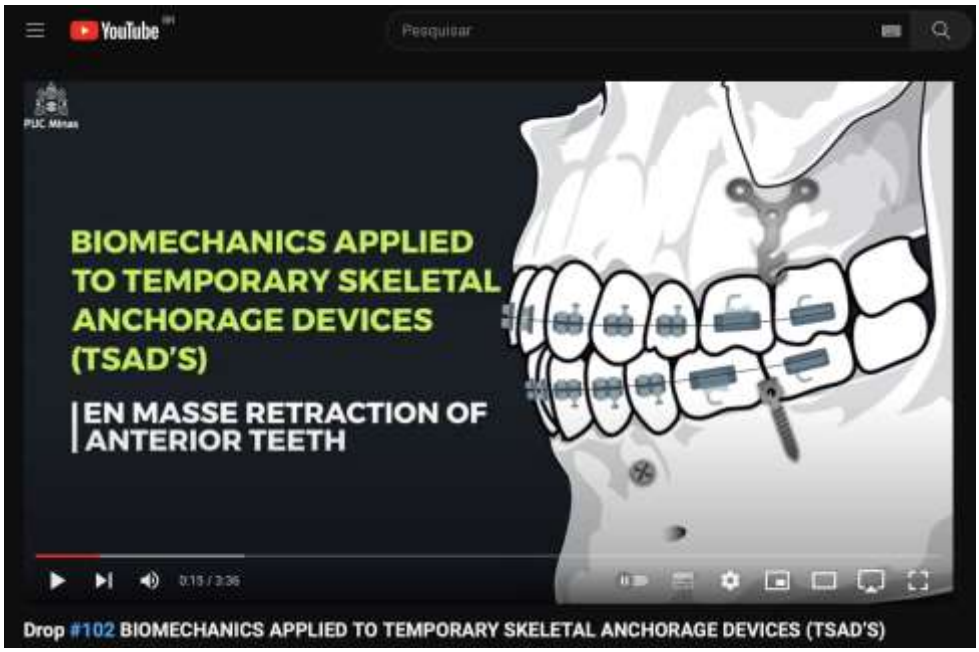
15. **FIGUEIREDO, N. C.**; ETO, V. M. ; GUIMARAES, F. A. M. ; LANZA, G. L. ; AZEVEDO, G. M. ; ANDRADE JR, I. Gota #102 BIOMECÂNICA APLICADA A DISPOSITIVOS DE ANCORAGEM ESQUELÉTICA TEMPORÁRIA (DAET'S). 2020. (Desenvolvimento de material didático ou instrucional - Vídeo didático).

16. **FIGUEIREDO, N. C.**; ETO, V. M. ; GUIMARAES, F. A. M. ; LANZA, G. L. ; AZEVEDO, G. M. ; ANDRADE JR, I. Drop #102 BIOMECHANICS APPLIED TO TEMPORARY SKELETAL ANCHORAGE DEVICES (TSAD'S). 2020. (Desenvolvimento de material didático ou instrucional - Vídeo didático).



17. **FIGUEIREDO, N. C.**; AZEVEDO, G. M. ; ETO, V. M. ; GUIMARAES, F. A. M. ; LANZA, G. L. ; ANDRADE JR, I. Gota #108 FORÇAS ORTODÔNTICAS E REAÇÕES TECIDUAIS. 2020. (Desenvolvimento de material didático ou instrucional - Vídeo didático).

18. **FIGUEIREDO, N. C.**; AZEVEDO, G. M. ; ETO, V. M. ; GUIMARAES, F. A. M. ; LANZA, G. L. ; ANDRADE JR, I. Drop #108 ORTHODONTIC FORCES AND TISSUE REACTIONS. 2020. (Desenvolvimento de material didático ou instrucional - Vídeo didático).

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20. **FIGUEIREDO, N. C**.; AZEVEDO, G. M. ; MASCARENHAS ETO, V. ; GUIMARAES, F. A. M. ; LANZA, G. L. ; ANDRADE JR, I. Drop #119 ORTHODONTIC FORCES AND TISSUE REACTIONS: types of tooth movement. 2021. (Desenvolvimento de material didático ou instrucional - Video didático).

21. AZEVEDO, G. M. ; **FIGUEIREDO, N. C.** ; ETO, V. M. ; GUIMARAES, F. A. M. LANZA, G. L. ; ANDRADE JR, I. Gota #120 ELÁSTICOS ORTODÔNTICOS: conceitos básicos. 2021. (Desenvolvimento de material didático ou instrucional - Vídeo didático).

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