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Review - Human and Animal Health

Caffeine Effect on Bone Metabolism in Rats: a Systematic Review

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HIGHLIGHTS

- Nine of 472 initially identified articles were selected for qualitative evaluation.
- Most studies show that caffeine can interfere with bone metabolism.
- Caffeine can accelerate bone loss and delay bone repair.
- In contrast, caffeine can activate osteogenesis and bone neoformation.

Abstract: Caffeine is a highly-consumed substance around the world and can be found in various food sources and certain medications. The present systematic review aimed to evaluate the effect of caffeine on bone metabolism in rats. A systematic review was conducted in the PubMed, Medline, Scopus, Cocharane, Embase, and Clinical Trials.gov databases, and the Guidelines for Preferential Reporting for Systematic Reviews and Meta-Analyzes (PRISMA) were followed. In vivo experimental studies that presented caffeine as the study object were included, and studies which did not evaluate the bone metabolism and/or evaluated the caffeine in association with other substances were excluded. The quality evaluation of the selected studies was carried out following the guidelines of the Systematic Review Center for Laboratory Animal Experimentation (SYRCLE) and the Animal Research Reporting In Vivo Experiment (ARRIVE). Nine of the 472 initially identified articles met the inclusion criteria and were selected for qualitative evaluation. There was a variation between the included studies regarding the administered caffeine doses in each experimental group, as well as their frequency and duration of ingestion. Most studies show that caffeine can interfere with bone metabolism, be it in a negative way by accelerating bone loss and delaying bone repair, or in a beneficial way by activating osteogenesis and bone neoformation. There is a need for further studies to better understand the real effect of caffeine on bone metabolism.

Keywords: caffeine; metabolism; bone; bone remodeling; rats.

INTRODUCTION

Caffeine is probably the most widely used pharmacologically active substance in the world [1]. This substance exists in some common beverages (coffee and tea), cocoa-rich products and some medications. In addition, consumption of high-caffeine energy drinks has risen sharply in recent years and has probably been the cause of problems such as increased caffeine intoxication [1,2,3,4].

Caffeine has a variety of cellular and pharmacological responses, producing biological effects such as anti-oxidation, anti-mutation, angiogenesis, antibiotic action, anti-hypercholesterolemia, anti-hypertension and anti-inflammatory action on bone metabolism [5].

The hypothesis that caffeine could exert an influence on bone metabolism has been evaluated in several experimental studies with conflicting results. Some researchers, such as Bezerra and coauthors (2008)[6] and Tsuang and coauthors (2006)[7] have suggested that caffeine predisposes the development of osteoporosis and periodontal disease, while others have found no correlation between this substance and bone loss or periodontal disease[8]. An in vitro study has shown that caffeine may increase the apoptosis rate of osteoblasts, thus exerting a potential deleterious effect on the viability of these cells. On the other hand, it has been demonstrated that caffeine can positively influence mineralization and the mechanical characteristics of bone tissues at specific doses [9].

In view of the above, the objective of this study is to investigate the real relationship between caffeine and bone metabolism in rats through a systematic review of the literature.

MATERIAL AND METHODS

The present study was conducted following the guidelines of Preferential Items of PRISMA[10].

Bibliographic research strategy and selection of studies

An electronic search using the PubMed, Medline, Scopus, Cocharane, Embase and Clinical Trials.gov databases was conducted by searching articles published in the dental literature with the following keywords: caffeine AND metabolism AND bone, without restrictions with respect to the year. As inclusion criteria, the articles should be written in the English language, present caffeine as the study object and represent experimental studies in vivo. We excluded studies which did not evaluate bone metabolism and those that evaluated caffeine in combination with other substances. The first selection stage of the work was done by analyzing the titles and abstracts by two evaluators who carried out the search in double blind format to reduce bias during the research and in selecting the works. These evaluators selected the relevant articles according to the inclusion and exclusion criteria.

Evaluation of the methodological quality of included studies

Two tools were used based on the study [11] to evaluate the quality of the studies included in the present systematic review. Thus, we used the SYRCLE, adapted to verify aspects of the bias risk of the included studies which play an important role in experiments with animals, and the ARRIVE tool[12].

In general, the studies were considered through eight detailed aspects: selection bias (randomization and concealment of allocation), performance bias (study participant/caregiver blindness), detection bias (blindness of outcome assessors), completeness of the follow-up period, among others. Thus, they were classified as “high risk of bias” (high), “low risk of bias” (low) or “inaccurate” (?) for each of the eight sections[13].

The selected studies were considered as: (i) low risk of bias when all criteria were observed (adequate randomization and concealment of allocation; “yes” response to all questions on completeness of outcome data and blinding, and “no” response to selective reports and other sources of bias); (ii) clear risk of bias when one or more criteria were partially met; or (iii) high risk of bias when one or more criteria were not met [13].

In addition, the selected studies were submitted to another tool for evaluation of methodological quality: the ARRIVE - following the guidelines of Kilkenny and Altman, (2010)[12]., a pre-defined classification was established applied to 20 specific criteria, comprising: (1) Title (concise and precise); (2) Summary (summary of background, objectives, methods, main results and conclusions); (3) Introduction (basic objectives, relevance to human biology); (4) Introduction (primary and secondary objectives); (5) Methods (ethical

statement, national and institutional guidelines for the care and use of animals); (6) Methods (study design, measures taken to minimize bias such as concealment of allocation, blinding and randomization); (7) Methods (experimental procedure with precise details); (8) Methods (details of experimental animals, including species, gender, age, weight and source); (9) Methods (accommodation and management conditions, such as cage type, light/dark cycle, temperature, access to food and water); (10) Methods (sample size); (11) Methods (allocation of animals to experimental groups, randomization); (12) Methods (results of experiments); (13) Methods (statistical analysis); (14) Results (baseline data, animal health status); (15) Results (number of animals analyzed, reasons for exclusion); (16) Results (results and estimation, results for each analysis); (17) Outcomes (adverse events); (18) Discussion (interpretation, scientific implications, study limitations including animal model); (19) Discussion (generalization and translation, relevance to human biology); and (20) Discussion (sources of funding, role of funders, conflicts of interest).

Each criterion was classified as “0” (not reported) or “1” (reported). The combined reporting frequency for each criterion in all included studies was recorded.

RESULTS

Study selection flowchart

Initially, 472 articles were identified. In the first step, 442 publications which did not respond to the focus question or were duplicated were excluded. A total of nine studies met the inclusion criteria and were then entered into the present systematic review and processed for data evaluation. Figure 1 summarizes the literature search strategies according to the PRISMA guidelines[10].

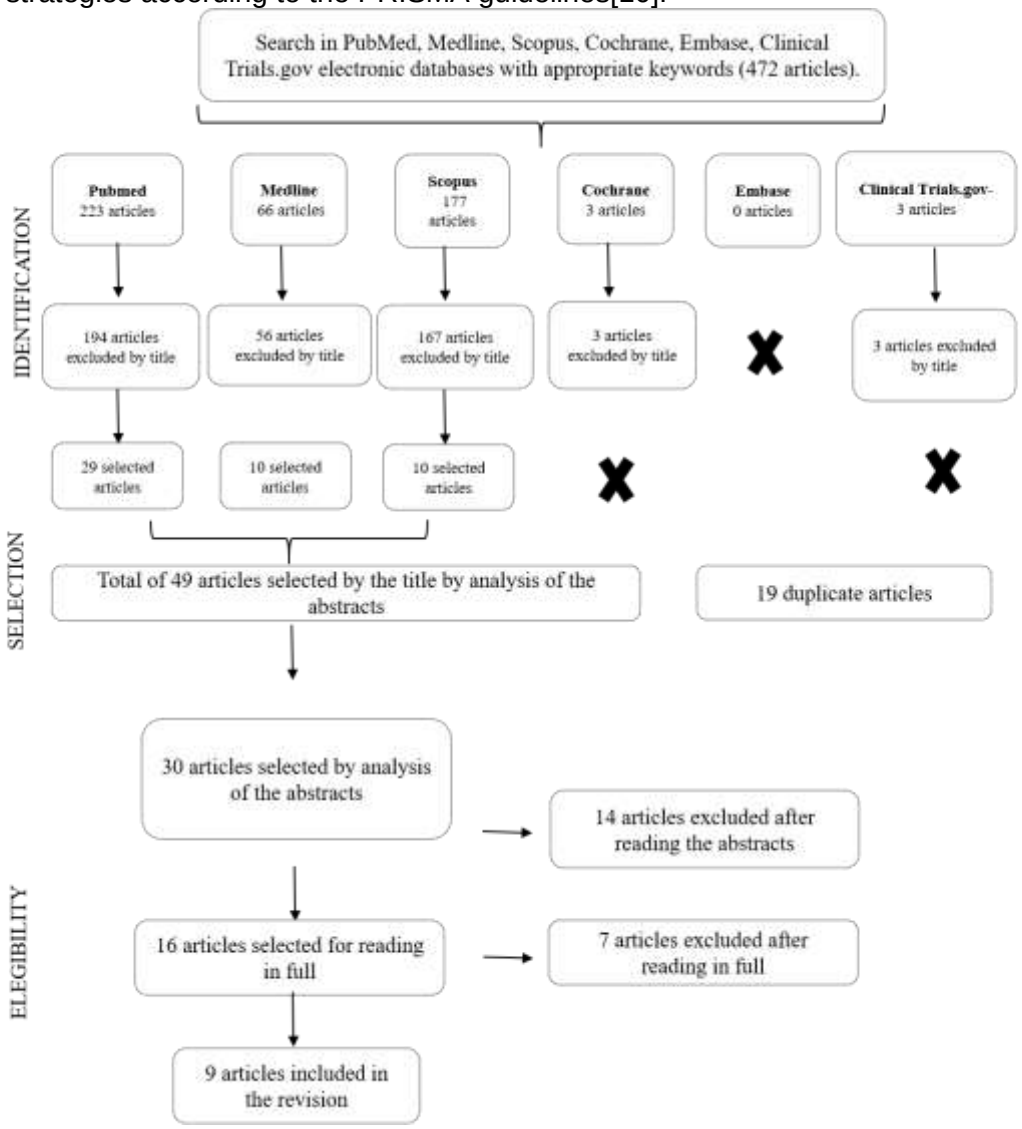

Figure 1. Study Flowchart.

General characteristics of included studies

The studies included in the present systematic review were mostly performed with male rats and only studies of Lacerda and coauthors (2010) [14] and Olchowik and coauthors (2011) [15] with females. Also, most of the rats were of the Wistar lineage, whereas only three studies used Sprague Dawley rats. The number of rats ranged from 16 to 51. In addition, the mean age of the rats ranged from 22 to 90 days. Variations regarding study groups and other details are summarized in Table 1.

Table 1. Characteristics related to the samples of the experiments.

Author/year, country	Animal/Genus	Animal age	Sample size	Study groups
Glajchen *et al*. (1988, USA)	Sprague Dawley Rats Males	77 days	27	**Control Group**, n=8; **Group 1**, n=10: 2.5 mg/100g/body weight/day of low caffeine dose; **Group 2**, n=10: 10 mg/100 g/body weight/day of high caffeine dose.
Sakamoto *et al*. (2001, Japan)	Wistar Rats Males	Not detailed	48	**Control Group**: n=16, no coffee diet; **Group 1**, n=16: supplementation of 125mg/day; **Group 2**, n=8: - 275mg/day.
Bezerra *et al*. (2008, Brazil)	Wistar Rats Males	90 days	22	**Control Group**, n = 12: no caffeine ingestion; **Group 1**, n= 10: 10 mg/100g/body weight/day of caffeine
Lacerda *et al*. (2010, Brazil)	Wistar Rats Females	Not detailed	42	**Control Group**, n=21: water; **Group 1**, n=21: 1.2mL/day 240mL/day/60kg caffeine.
Choi (2011, South Korea)	Sprague Dawley Rats Males	42 to 56 days	28	**Control Group**, n=14: water; **Group 1**, n=14: 25mg/Kg of caffeine
Olchowik *et al*. (2011, South Korea)	Wistar Rats Females	Not detailed	16	**Control Group**, n=8: water; **Group 1**: 30mg/kg of caffeine/day.
Bezerra *et al*. (2013, Brazil)	Wistar Rats Males	90 days	22	**Control Group**, n=12: no caffeine; **Group 1**, n = 10: 10 mg/100g/body weight/day/via potable water
Shin *et al*. (2015, South Korea)	Sprague Dawley Rats Males	22 days	51	**Group controle**, n=17; **Group 1,** n=34: 120 and 180 mg/kg of body weight of caffeine/day
Yi *et al*. (2016, China)	Wistar Rats Males	60 days	30	**Control Group,** n=15: orthodontic teeth movement, no caffeine ingestion); **Group 1**, n= 15: caffeine and orthodontic teeth movement.

Characteristics related to caffeine

Details of the caffeine doses administered in each experimental group, as well as their frequency and time of ingestion are given in Table 2.

Table 2. Characteristics related to caffeine.

Author/year	Caffeine Doses	Ingestion frequency	Time of ingestion
Glajchen *et al*. (1988, USA)	2.5mg/100g of body weight/day of low caffeine dose and 10mg/100g of body weight/day of high caffeine dose	1 time per day	21 days
Sakamoto *et al*. (2001, Japan)	(125mg/day) and (275mg coffee/day)	1 time per day	140 days
Bezerra *et al*. (2008, Brazil)	10mg/100g of body weight	1 time per day	56 days
Lacerda *et al*. (2010, Brazil)	1.2mL/day 240mL/day/60kg	1 time per day	30 days 60 days after the rats' birth
Choi (2011, South Korea)	25mg/Kg (284mg/70kg/person)	1 time per day	42 days
Olchowik *et al*. (2011, South Korea)	30mg/day	1 time per day	21 days
Bezerra *et al*. (2013, Brazil)	10mg/100g of body weight	3 times per week	56 days
Shin *et al*. (2015, South Korea)	120 and 180 mg/kg of body weight	1 time per day	28 days
Yi *et al*. (2016, China)	25 mg/kg of body weight	1 time per day	21 days

Methodological characteristics of the experiments and the main results analyzed

The effect of caffeine on bone metabolism was evaluated by means of different experimental units from the nine selected publications, so that the evaluated parameters, the types of analyzes used and the main results were divergent. These variations are described in Tables 3 and 4.

Table 3. Methodological characteristics of the experiments.

Author/year, country	Experimental unit	Evaluated Parameters	Types of analysis
Glajchen *et al*. (1988, USA)	Blood, Tibia	1 - Trabecular bone volume, relative osteoid volume; 2 - Number of osteoclasts; 3 - Mineralisation rate in ixm per day.	1- Serum iPTH levels; 2 - Histological analysis; 3 - Histomorphometric analysis.
Sakamoto *et al*. (2001, Japan)	Blood and urine tests; Rear paws of all rats	The effect of coffee on cytokine production in vivo: 1 - Osteoclast count; 2 - Measurement amount of calcified bone matrix; 3 - Measurement of calcium, phosphorus and creatinine; 4 -TNF-alpha and IL-6 identification and measurement of osteocalcin and deoxypyridinoline.	1 - TRAP method; 2 - Azan-Malloruy staining; 3 - Colorimetry; 4 - ELISA.
Bezerra *et al*. (2008, Brazil)	First maxillary molar and maxilla	1 - Interradicular bone loss; 2 - Bone loss in the furcation of the first molars.	1 - Histological histometric analysis; 2 - Histometric analysis.
Lacerda *et al*. (2010, Brazil)	Plasma, urine and maxilla	1 - Concentration of calcium in plasma and urine; 2 - Calcium concentration in the maxilla.	1 - Calcium measurement; 2 - Bone Densitometry - Cell count.
Choi (2011, South Korea)	Femur and spine	1 - Bone mineral density of the spine and femur and bone mineral content.	1 - Measurement using PIXImus software; Little detail in the methodology.
Olchowik *et al*. (2011, South Korea)	Femur and pelvis	The assessment of the quality of bone tissue: 1 - bone density; 2 - bone volume	1 - Biomechanical tests; Hydrostatic methods; 2 - Typical load deformation, 3-point bending test.
Bezerra *et al*. (2013, Brazil)	Jaw, evident furcation area of right and left 1st molars	1 - Bone loss in furcation region.	1 - Histometric analysis.
Shin *et al*. (2015, South Korea)	Tibia, blood, serum	1 - Weight of the leg bones; 2 - Bone mineral content; 3 - Bone mineral density of the whole body; 4 - Lean mass and body fat.	1 - The final length and weight of leg bones were measured (precision scale); 2 - Histomorphometric analysis in the tibia; Dual-energy x-ray absorptiometry and 18F-NaF positron emission tomography; 3 - Histomorphometric analysis; 4 - Calculation of lean mass and body fat.
Yi *et al*. (2016, China)	First maxillary left molar, maxilla	1 - Degree of orthodontic movement; 2 - Formation of osteoclasts; 3 - Rank-L, COX2, PGE2.	1 - The degree of movement was measured using digital slide tweezers; 2 - TRAP Staining; 3 - Immunohistochemical analysis; ELISA.

Table 4. The impact of caffeine on bone metabolism. Main results found in the analyzed studies.

Author (year, country)	Results	Conclusion
Glajchen *et al*. (1988, USA)	- No difference in bone histomorphometry was observed between the three analyzed groups; - Chronic administration of caffeine in rats may slightly increase bone turnover as evidenced by increased osteocalcin	The role of caffeine as an etiological factor for osteoporosis, by itself, is still doubtful.
Sakamoto *et al*. (2001, Japan)	- The intake of caffeine at different concentrations did not affect the number of osteoclasts and the levels of urinary deoxypyridinoline and serum osteocalcin, which reflect bone resorption and bone formation respectively - Coffee consumption had no effect on the production of bone resorption cytokines, such as TNF-a and IL-6, without stimulation or lipopolysaccharide injection, despite long-term coffee consumption (140 days).	High coffee intake does not stimulate bone loss in rats.
Bezerra *et al*. (2008, Brazil)	- Caffeine intake did not have a direct effect on alveolar bone loss in teeth without periodontitis; however, a greater area of bone loss was observed in the tooth with the periodontitis induction in animals that ingested caffeine compared to those who did not.	The daily intake of high doses of caffeine may increase the progression of periodontitis induced by elastic ligation.
Lacerda *et al*. (2010, Brazil)	- Higher amount of calcium in the plasma and urine and significantly less amount in the bone; - Reduction of bone mineral density; - Lower amount of bone in animals treated with coffee sacrificed after 42 days were observed.	It can be concluded that the coffee/caffeine intake showed delay in the bone repair process.
Choi (2011, South Korea)	- Moderate (25 mg/kg) caffeine intake increased urinary calcium loss; - It did not affect the markers for the bone mineral density of the spine and femur, and the bone mineral content associated with effects on the bone quality.	Moderate consumption of coffee by young people is unlikely to be detrimental to bone metabolism.
Olchowik *et al*. (2011, South Korea)	- The deformation at the maximum load point of the femur axis in the experimental group was significantly higher than in the control group; - Changes in biomechanical parameters in the group of pregnant rats with caffeine consumption indicate their negative influence on the bone;	The results suggest that caffeine intake during pregnancy causes loss of bone tissue.
Bezerra *et al*. (2013, Brazil)	- Caffeine increased bone healing in the presence of ligation, whereas caffeine and/or estrogen deficiency negatively affected the trabecular bone area in the absence of ligation and further reduced bone healing after tooth extraction.	Postmenopausal osteopenia and caffeine consumption are potential risk indicators for impaired alveolar bone healing and reduction of trabecular bone area and bone healing.
Shin *et al*. (2015, South Korea)	- Caffeine caused a significant decrease in body mass gain, accompanied by proportional reductions in lean body mass and body fat; - Significant reductions of bone mass and osteogenic activity in vivo in caffeine-fed groups were observed; - Caffeine-fed rats showed a significantly shorter and lighter tibia and femur when compared to controls.	Caffeine alters the osteogenic activity, leading to a delay in growth and peripuberal longitudinal bone maturation; caffeine can suppress ossification by interfering in both physiological changes and in hormone secretion and osteogenic activity.
Yi *et al*. (2016, China)	- A greater area of bone loss was observed in the connected teeth of animals that ingested caffeine compared to animals that did not.	Caffeine intake increases ligature-induced periodontitis, although it was not able to induce alveolar bone loss in the absence of allele.

Risk of bias in the studies

Table 5 shows the overall results of the risk of bias assessment of the nine included articles. All studies reported that the experiments were randomized to some degree. Sequence allocation, similar groups at the start of the experiment and random animal lodging showed low risk of bias in 88.88 to 100% of the studies. For the analyzed studies, the blindness of caregivers or researchers, random selection to evaluate the results and incomplete data were not clear. Regarding the blindness of the outcome evaluator, there was a high risk of bias since 66.7% of the studies did not reveal this data. In general, about 50% of each study was scored as low risk of bias, since they adequately addressed only half of the evaluated criteria, and others were not well informed.

Table 5. Risk of bias verified through the SYRCLE tool, average per item. Legend: NI: Not informed.

No.	Risk of bias (SYRCLE)	Glajchen *et al*. (1988)	Sakamoto *et al*. (2001)	Bezerra *et al*. (2008)	Lacerda *et al*. (2010)	Choi (2011)	Olchowik *et al*. (2011)	Bezerra *et al*. (2013)	Shin *et al*. (2015)	Yi *et al*. (2016)	Total (%)
1	Was the allocation sequence properly generated and applied?	Yes	Yes	Yes	NI	Yes	Yes	Yes	Yes	Yes	Low bias (88.88%)
2	Were the groups similar at baseline or were they adjusted for confounders in the analysis?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Low bias (100%)
3	Was the allocation sequence was properly hidden?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Low bias (100%)
4	Were the animals were randomly housed during the experiment?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Low bias (100%)
5	Were the caregivers and/or researchers were blinded to the knowledge of the intervention that each animal received during the experiment?	NI	NI	NI	NI	NI	NI	NI	NI	NI	Inaccurate data (100%)
6	Were the animals randomly selected for outcome evaluation?	NI	NI	NI	NI	NI	NI	NI	NI	NI	Inaccurate data (100%)
7	Was the outcome evaluator blinded?	NI	NI	Yes	NI	NI	NI	Yes	Yes	NI	Inaccurate data (66.7 %)
8	Were incomplete results data adequately addressed?	NI	NI	NI	NI	NI	NI	NI	NI	NI	Inaccurate data (100%)

Quality assessment of included studies

The total score of included studies based on the ARRIVE criteria is summarized in Table 6. The average score for the nine included studies of this tool was 16.44 from a maximum of 20 points. Regarding the individual criteria, the highest reporting frequencies were recorded for: title, abstract, baseline information in the introduction, study design, animal details, experimental results, statistical analysis in the methods, number of animals analyzed, results and estimation of each analysis in the results, interpretation, scientific implications, generalization and translation in the discussion (100%).

Table 6. ARRIVE criteria: list of criteria reported by included studies.

Nº.	ARRIVE criteria	Glajchen *et al*. (1988)	Sakamoto *et al*. (2001)	Bezerra *et al*. (2008)	Lacerda *et al*. (2010)	Choi (2011)	Olchowik *et al*. (2011)	Bezerra *et al*. (2013)	Shin *et al*. (2015)	Yi *et al*. (2016)	Total (%)
1	**Title**	1	1	1	1	1	1	1	1	1	100%
2	**Abstract**	1	1	1	1	1	1	1	1	1	100%
	Introduction										
3	**General information**	1	1	1	1	1	1	1	1	1	100%
4	**Primary and secondary objectives**	1	1	1	1	1	1	1	1	1	100%
	Methodology										
5	**Ethical statement**	0	0	1	1	0	1	1	1	0	55.5%
6	**Study design, allocation concealment, blinding and randomization**	1	1	1	1	1	1	1	1	1	100%
7	**Experimental procedure with precise details**	1	1	1	1	0	1	1	1	1	88.88%
8	**Details of experimental animals, including species, sex, age, weight and source**	1	1	1	1	1	1	1	1	1	100%
9	**Housing and breeding conditions such as cage, light/dark cycle, temperature, access to food and water**	0	1	1	1	1	1	1	1	1	88.88%
10	**Sample size**	1	1	1	1	1	1	1	1	1	100%
11	**Allocation of animals to experimental groups, randomization**	0	0	1	1	1	1	1	1	1	100%
12	**Experimental results**	0	0	0	0	1	1	1	1	1	55.5%
13	**Statistical analysis**	1	1	1	1	1	1	1	1	1	100%
	Results										
14	**Baseline, animal health status**	1	1	1	1	1	1	1	1	1	100%
15	**Number of animals analyzed, reasons for exclusion**	0	0	0	0	0	0	0	0	0	0
16	**Results and estimation, results for each analysis**	1	1	1	1	1	1	1	1	1	100%
17	**Adverse events**	0	0	0	0	0	0	0	0	0	0
	Discussion										
18	**Interpretation, scientific implications, study limitations**	1	1	1	1	1	1	1	1	1	100%
19	**Generalization and translation, relevance to human biology**	1	1	1	1	1	1	1	1	1	100%
20	**Sources of financing, conflict of interest**	1	1	1	0	1	1	1	1	1	88.88%
	Total Score	14	15	17	15	16	18	18	18	17	16.44

DISCUSSION

The present systematic review aimed to find the best scientific evidence regarding the influence of caffeine on bone metabolism. In the initial research, a significant number of studies were found on the subject; however, after a careful analysis it was observed that most did not present the necessary inclusion criteria. Therefore, only 9 experimental studies performed on rats were included in this study[6,8,14,15,16,17,18,19,20].

Among the studies included in the present systematic review (although four studies had information which was not detailed by the authors), all showed low risk of bias and frequency of 16.44 in methodological quality, thus demonstrating good qualification. From a total of 9 studies, 6 showed that caffeine modified some aspect of bone metabolism[6,14,15,16,19,20], while three showed that there was no relationship between caffeine consumption and bone metabolism[8,17,18].

The studies by Bezerra and coauthors (2008) [6]; Bezerra and coauthors (2013) [16]; Lacerda and coauthors (2010) [14]; Olchowik and coauthors (2011)[15]; Shin and coauthors (2015) [19]; Yi and coathors (2016) [20] which found a relationship between caffeine and bone metabolism, suggest that caffeine is associated with a significantly increased risk of periodontal disease, delayed bone repair, loss of bone tissue in pregnant women, reduction of bone neoformation in extracted teeth, decrease in mass gain and the activation of orthodontic movement, with the potential impact of this substance on the bone tissue usually attributed to its capacity to increase calcium excretion [21]. In addition, studies by Lacerda and coauthors (2010)[14], Shin and coauthors (2015)[19] and Yi and coauthors (2016)[20] agree that caffeine acts to increase calcium levels in plasma and urine, which decreases bone mineral density, thus interfering with the bone repair process.

In addition, the studies[6,8,14,,16,17,18,19,20] which similarly evaluated the effect of caffeine on bone metabolism in male or female rats aged 21-90 days, another study [15] was also carried out which expanded this investigation to evaluate this effect in rats during the gestational period. Findings from this last experiment revealed that caffeine in fact exerted a negative influence on the bone tissue of pregnant rats. For these authors, the impact of caffeine on bone tissue is also related to calcium metabolism, since this substance slightly impairs its absorption by the intestine, diverging from the previously reported findings of Lacerda and coauthors (2010) [14], Shin and coauthors (2015) [19] and Yi and coauthors (2016) [20]; however, such a substance has no effect on urinary calcium excretion. Thus, the authors suggest that caffeine intake causes a decrease in bone mass and an increased risk of bone fracture.

In agreement with the previous study [15] other reports [22,23] in the literature have shown that pregnancy exerts a negative influence on the bone tissue. Therefore, the gestational status of the rats investigated in the study by Olchowik and coauthors (2011) [15] may have increased the predisposition to loss of bone tissue, which may represent a bias in the aforementioned study. Corroborating this, Namgumg and Tsuang (2003) [23] found that gestational markers of bone resorption increase in the first trimester of pregnancy, whereas bone formation markers only increase in the last trimester. Such events may be justified by the hormonal component involved in the gestational period to maintain maternal calcium homeostasis and meet the requirements of fetal development.

The experiment by Yi and coauthors (2016)[20] demonstrated that caffeine, at least in a specific concentration, can increase the osteoclastogenesis induced by cells of the periodontal ligament under static compression, which may stimulate bone resorption and accelerate orthodontic movement. Therefore, coffee consumption is not counter-indicated during orthodontic treatment. In contrast, the study by Shin and coauthors (2015)[19] found that caffeine compromises osteogenic activity in vivo and may interfere with the physiological alterations of hormone secretion and metabolic activity relevant to the osteogenesis necessary for the bone repair process. Still, further studies will be needed to investigate the molecular and cellular mechanisms by which caffeine affects osteogenesis.

The way that caffeine interferes in the bone repair process is not yet fully elucidated in the literature [14,16]. It is known that such a substance can modulate various aspects of the innate and adaptive immune and inflammatory response [24]. In relation to this, Lacerda and coauthors (2010)[14] suggested a change in the role of macrophages by their reduced activity and/or apoptosis, as well as a decrease in the production of interferon-gamma (IFN-γ) by T lymphocytes, with consequent decrease in the stimulatory effects of this mediator on the phagocytic and secretory functions. In view of the reduced viability of the macrophages at the repair site, there is also a reduction in the levels of cytokines and growth factors secreted by these cells, especially those that stimulate fibroblasts. As several of these mediators secreted by macrophages act on chemotaxis, proliferation, collagen production and granulation tissue formation, it is possible that their

reduction causes a deficiency in granulation tissue formation and maintenance of blood clotting. Moreover, interferences in the differentiation, proliferation, bone matrix production and mineralization processes by osteoblasts and osteocytes could also act as a synergic factor, increasing the intensity of the alterations in the dynamic process of alveolar bone repair [7,25].

Although most of the studies in this review showed that there was an association of caffeine with alterations in some aspect of bone metabolism, there was no significant association between these factors in three studies. In the study [18], chronic administration of high caffeine doses resulted in a slight increase in osteocalcin serum levels in rats. However, caffeine at different doses did not cause changes in serum parathyroid levels and no change in bone morphometric parameters was observed, therefore there is no corroborating evidence that caffeine alters bone metabolism or is a risk factor for osteoporosis.

The study by Sakamoto and coauthors (2001) [8] also did not find data in its findings which justified the influence of caffeine on bone metabolism. There were no significant differences in body weight changes, serum or urinary biochemical markers of bone metabolism and bone histomorphometry between the diet and coffee groups. Only the urinary excretion of phosphorus after 140 days of both diets was significantly increased ($p < 0.05$) compared to the control. In addition, coffee diets did not show any association with the cytokines TNF-α and IL-6, which together with IL-1β have been implicated in the pathogenesis of bone loss. The study concluded that caffeine does not stimulate bone loss in rats. Similarly, the study by Choi (2011)[14] showed that moderate (25 mg/kg) caffeine intake by rats increases urinary calcium loss, but does not affect any of the markers used to assess bone density or the bone mineral content of the spine and femur associated with detrimental effects on bone quality, thus concluding that coffee is unlikely to stimulate bone loss in rats.

The controversial results of this systematic review may be associated with several confounding factors, including caffeine dosage, age variation of animals, experimental units and different evaluation methods used. For example, caffeine dosages varied in the studies from 2.5 mg/kg of body weight to 180 mg/kg. With regard to age, the variation occurred from 22 to 90 days. Also, the maxilla molars, tibia, femur, pelvis, blood, urine and mandible were investigated regarding the experimental units. Finally, there was a wide variation in relation to the analysis type, including histochemical analysis for the quantification of osteoclasts by analysis of tartrate-resistant acid phosphatase (TRAP), histological and histomorphometric analyses, biomechanical tests, Elisa method and the three-point bending test. Altogether this means that such a lack of standardization in the methodologies used in the experiments constitutes a limitation of the present study, since it is difficult to compare the results from experiments with diverging methodologies [6,8,14,15,16,17,18,19,20].

CONCLUSION

Despite the existence of still quite controversial results in the literature and the lack of methodological standardization in the conducted studies, most of the studies in this systematic review have reported that caffeine can interfere in bone metabolism, whether in a negative form by accelerating bone loss and retarding bone repair, or in a beneficial way by activating osteogenesis and consequently new bone formation. Therefore, it is necessary to conduct additional well-delineated studies in order to better understand the real effect of caffeine on bone metabolism.

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