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Review

Effects of systemic administration of the retinoid Isotretinoin on bone tissue: A narrative literature review

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Abstract: Isotretinoin is an oral medication commonly utilized to treat severe acne. It belongs to the class of retinoids and is a synthetic form of vitamin A. Possible changes in bone tissue associated with the systemic administration of Isotretinoin for long periods and in high doses, such as hypercalcemia, osteopenia, diffuse hyperostosis, or even skeletal deformities, are reported in scientific studies. The objective of this literature review was to analyze the available scientific literature on the systemic administration of Isotretinoin and its effects on bone tissue. To this end, articles that demonstrate effects of Isotretinoin on bone metabolism, bone mineral density, bone formation, and resorption, in addition to possible changes generated in this tissue were considered valid for inclusion in this literature review. The search for articles that fit the premise of this work was carried out in the PubMed/MEDLINE database. In a preliminary search, 85 articles were obtained; after in-depth investigation and checking of the inclusion and exclusion criteria, 21 articles were selected, with 17

articles reporting the effect of systemic administration of Isotretinoin in humans and four articles analyzing the effect generated by this substance systemically administrated in animals, more specifically in rats. After analyzing the selected articles, it was possible to observe several reports on hyperostosis or even changes in the formation of bone structures in human patients. Concerning animals, the largest number of studies addressed the changes caused in the formation or development of bone structures by the systemic administration of Isotretinoin. From the current analyses, and given the wide use of Isotretinoin, it is necessary to know its viability and influence on tissues. Its clinical efficacy seems to overcome any of the skeletal side effects observed; however, more studies are necessary to properly evaluate and prove the effects generated on bone tissues as well as other structures.

Keywords: isotretinoin; bone repair; vitamin A; bone regeneration; retinoid; bone defects; bone

1. Introduction

Vitamin A is a micronutrient found in foods of animal origin that comes in various forms: retinol, retinyl, retinal, and retinoic acid [1]. It is a fat-soluble, unsaturated primary alcohol with a high reactive capacity; the term Vitamin A is used for all beta-ionone derivatives that have the biological activity of retinol minus the carotenoids that are present in plants such as leafy green vegetables and orange fruits. These are a naturally occurring group made up of many different substances called pro-vitamin A. Similarly, the term retinoid describes retinol or its naturally occurring derivatives and synthetic analogues [2].

Retinoic acid (RA) is a product of retinol metabolism, where the alcohol present has undergone oxidation, which regulates the growth rate of epithelial tissue and cell differentiation [3,4]. Due to possible cistrans configuration of the chain, there are several RA analogues, such as all-trans-AR (Tretinoin) and 13-cis-retinoic acid (Isotretinoin), among others [5–7]. Isotretinoin is an oral medication commonly administrated to treat severe acne, belonging to the class of retinoids and being a synthetic form of vitamin A [8]. Isotretinoin is one of the most effective medications in the treatment of acne, acute promyelocytic leukemia, and other disorders. However, it can have significant side effects [9,10], which, together with changes in the formation process of the human body's component structures, have been reported by several authors [11–17] (Figure 1).

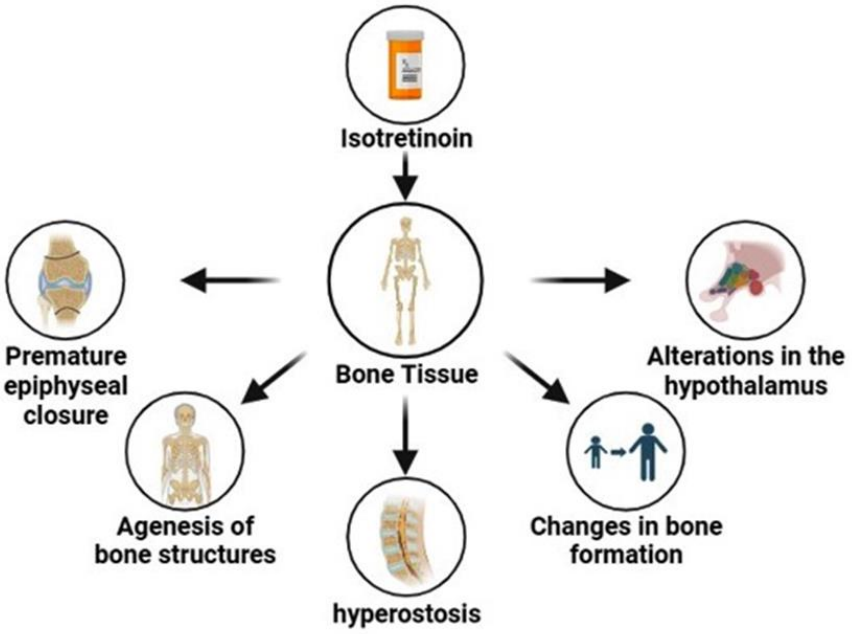

Figure 1. Evidenced effects of the systemic administration of Isotretinoin on human clinical trials.

A side effect that has been commonly associated with prolonged intake of Isotretinoin is the decrease in bone mineral density; this refers to the amount of minerals, such as calcium, phosphorus, and magnesium, present in bones [18]. Such a decrease in bone mineral density can lead to an increased risk of bone fractures, osteoporosis, and bone changes during training periods in young patients [19–23].

In addition to changes in bone tissue, it is worth noting that the systemic administration of Isotretinoin, whether in high doses or associated with long periods of treatment, may affect other structures of the human body [24,25]. Reverse congenital malformations may also be observed in human neonates born after intrauterine exposure to Isotretinoin during early pregnancy [26–30]. Depression associated with the systemic administration of Isotretinoin in the treatment of acne has received a lot of attention in scientific circles, mainly due to the way it interacts with the functioning mechanisms of the central nervous system and the effects generated, such as the pathogenesis of depression [31]. Li et al. [32] investigated the association between Isotretinoin administration and the risk of depression in patients with acne through a systematic review and meta-analysis; the findings suggested that Isotretinoin administration in acne patients is associated with significantly improved depression symptoms but, according to the author, randomized controlled trials are needed to confirm these results.

Animal studies have also demonstrated that the systemic administration of Isotretinoin can cause a decrease in the hormone AMH (anti-Müllerian hormone) and atresia in the follicles, generating the hypothesis that the toxicity of Isotretinoin may be associated with infertility and a decrease in ovarian reserve [33–36]. Prolonged administration of Isotretinoin, for more than 10–12 months, together with hormonal contraceptives shows a higher risk of developing menstrual irregularities [37]. It is also worth mentioning that, in addition to alterations being reported in human clinical trials, other authors also demonstrated changes in experiments with rats, with most results related to the formation and development of bone structures [38–41] (Figure 2).

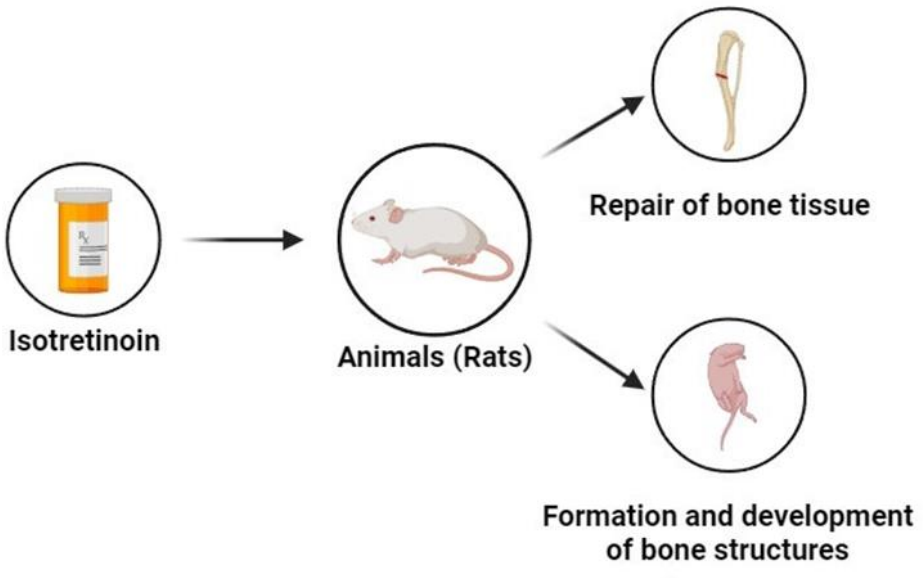

Figure 2. Changes evidenced in clinical animal trials regarding the administration of systemic Isotretinoin.

The exact mechanisms by which Isotretinoin affects bone mineral density are still unclear [42]. Some researchers suggest that Isotretinoin may inhibit the activity of osteoblasts, the cells that form bones. Others suggest that Isotretinoin may affect levels of vitamin D, an essential nutrient for bone health [43]. Regardless of the exact mechanism, it is important for doctors to regularly monitor bone mineral density in patients taking Isotretinoin to ensure that bone health is maintained during treatment [44]. It is also important to note that not only adverse effects on bone tissue are observed: retinoic acid can regulate the expression of various genes through retinoic acid receptors, being capable of generating alterations in processes such as spermatogenesis, fertilization, fetal and perinatal growth, as well as other areas [45]. Kapala et al. [46] aimed to establish the prevalence of adverse events during Isotretinoin therapy in human clinical trials through a literature review. According to the results, Isotretinoin therapy affects nearly all systems in the human body, primarily causing mild mucocutaneous conditions, with severe cases being rare. These adverse events represent individual responses to the drug and are reversible and often avoidable with specific preparations. Other authors have also evaluated sebum production and factors associated with skin hydration, obtaining positive results; in other words, the systemic administration of Isotretinoin leads to a decrease in sebum production and an increase in hydration [47–49].

Based on scientific articles collected for the preparation of this work, other possible changes in bone tissue associated with the systemic administration of Isotretinoin are hypercalcemia, osteopenia, diffuse hyperostosis, or even skeletal deformities. In relation to these changes, bone abnormalities such as premature closure of the epiphyseal disc can be observed and generate permanent sequelae for the individual, such as changes in joint angulation and the length of long bones [8,9]. Hypercalcemia refers to elevated calcium levels in the blood, which can disrupt normal bone remodeling processes. Osteopenia is characterized by reduced bone density, increasing the risk of fractures, and diffuse hyperostosis involves excessive bone growth, often leading to joint stiffness and pain [50–52]. It is important to note that researchers have observed a decrease in bone mineral density caused by the systemic administration of Isotretinoin [18,40,53–55]. Although decreased bone mineral density is a potentially serious side effect of Isotretinoin, it is usually reversible [53]. Furthermore, it

is more common in patients who take high doses or for prolonged periods [56]. Decreased bone mineral density is generally not a significant problem for patients taking Isotretinoin for a short period of time [54,55]. Its antibacterial properties were also observed in a clinical trial in which patients with periodontal changes were analyzed; the groups that used Isotretinoin saw a significant reduction in the most prevalent pathogens such as *Porphyromonas gingivalis* and *Tannerella forsythia* [57].

The aim of this study was to review the literature on the systemic administration of Isotretinoin and its effects on bone tissue. Reviewed studies involved animals (rats) as well as interventions in humans, whether adults or children, or evaluations of Isotretinoin effects during pregnancy on human fetus. Comparison of effects between other synthetic forms of vitamin A (Tretinoin) are also addressed in this review. Current recommendations for monitoring bone mineral density in patients using Isotretinoin will be discussed. The ultimate goal is to provide a comprehensive overview of the effects of Isotretinoin on bone tissue in order to help doctors make informed decisions when prescribing the drug and guide patients on how to minimize the risks and maximize the benefits of treatment with this substance.

2. Materials and methods

With the aim of reviewing literature and discussing previous research, this scientific study analyzed the systemic administration of Isotretinoin and its effects on bone tissue. Thus, articles that demonstrated the effects of Isotretinoin on bone metabolism, bone mineral density, bone formation, and resorption, as well as possible changes generated in this tissue, were considered valid for inclusion. The search for articles was carried out in the PubMed/MEDLINE database, using the following terms: "Isotretinoin and bone tissue". It is worth mentioning that the selection of articles was carried out in a single search. Articles that dealt with the effects of the systemic administration of Isotretinoin on humans and animals were selected according to the inclusion and exclusion requirements and then separated to make the table and paper.

For the inclusion criteria, articles that met the following criteria were approved and included: articles in English, complete articles published in journals, and articles that directly addressed the systemic administration of Isotretinoin and its effects on bone tissue. Articles that did not directly address the research topic, those that only evaluated the effects of Isotretinoin on the skin or other parts of the body, and opinion articles were excluded. The articles were selected by three independent researchers, who evaluated the titles and abstracts according to the established inclusion and exclusion criteria. Data extracted from selected articles include reference and year of publication, study model, intervention, results, and conclusion.

When using the terms "Isotretinoin and bone tissue", 85 articles were found. After analyzing the titles of the articles that apparently matched the objective of this work, 46 articles were obtained. Subsequently, in an in-depth investigation with a full-text reading associated with checking the inclusion and exclusion criteria, 21 articles out of the 46 were selected to prepare this literature review, with 25 articles being excluded for not meeting the inclusion criteria. Out of the 21 articles selected, 17 articles reported the effect of systemic administration of Isotretinoin in humans and 4 articles analyzed the effect generated by this substance administered systemically in animals, more specifically in rats. The article selection scheme and the associated search results can be seen in Figure 3.

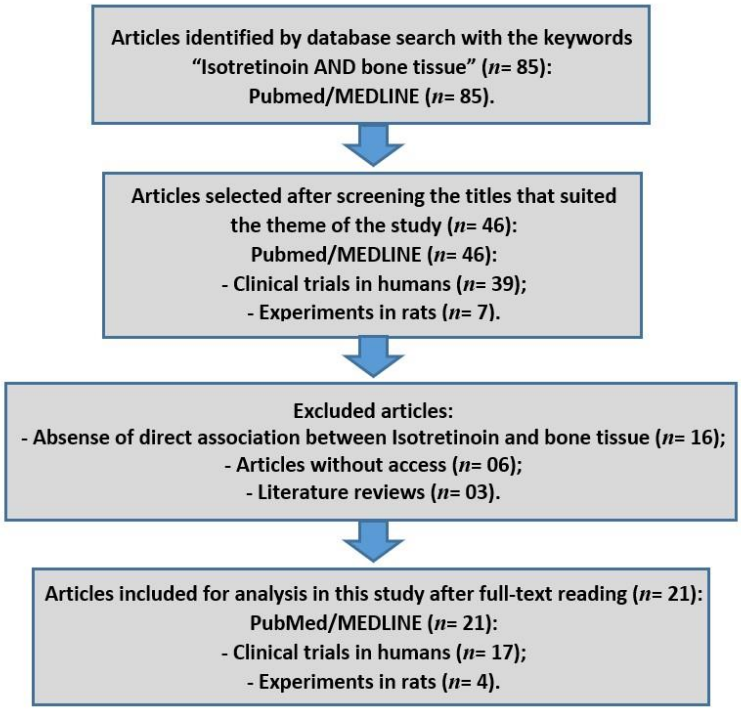

Figure 3. Diagram of the selection process for the articles reviewed and number of articles on human clinical trials and animal experiments, according to the selection phase.

3. Results

3.1. Selected and reviewed articles

The findings related to human clinical trials of the articles included in the present work were transcribed and organized into three tables (Table 1, Table 2, and Table 3). All selected articles were published clinical works and directly related to the topic in question: the effects on bone tissue caused by the systemic administration of Isotretinoin. Table 1 presents a summary of studies selected according to the eligibility criteria for the systemic administration of Isotretinoin in human clinical trials, focusing on studies that included a sample size of $N \geq 120$ cases. The table is organized into several columns to provide a comprehensive overview of each study: reference, model, intervention, objective, results, and conclusion. This structured format allows for a clear comparison of the various studies, highlighting the key aspects and findings of each trial.

Table 1. Studies selected according to eligibility criteria about the systemic administration of Isotretinoin that are related to human clinical trials and that presented N ≥ 120 cases.

Reference	Model	Objective	Intervention	Results	Conclusion
Carey et al. 1988 [58]	N = 120	Evaluate the musculoskeletal toxicity associated with a relatively low dose of Isotretinoin for the treatment of severe acne.	10 patients received 1 mg/kg/day, 80 received 5 mg/kg/day, and 30 received 10 mg/kg/day.	12% of the individuals selected for the study had few alterations. No clinically significant musculoskeletal alterations were observed.	Doses of 5 mg/kg/day of Isotretinoin did not produce significant musculoskeletal changes in the long term, and any possible skeletal changes related to therapy with this drug are minimal.
Digiovanna, 2001 [56]	N = 217	Evaluate the effect of Isotretinoin on the bone mineral density (BMD) of the lumbar spine and hip in adolescents with severe recalcitrant nodular acne.	Patients were treated with Isotretinoin twice a day at the total recommended dose of approximately 1 mg/kg for 16–20 weeks.	There was no significant change in BMD measured in the lumbar spine or total hip. Hyperostosis was not observed in any patient.	A course of 16–20 weeks of treatment with Isotretinoin at the recommended dose for severe acne has no clinically significant effect on the lumbar spine and total hip BMD in the adolescent population.
Duvalyan et al. 2020 [9]	N = 216	Report a rare case of bone toxicity associated with Isotretinoin exposure in a population affected by neuroblastoma.	Search the Children's Hospital Los Angeles database for patients who were diagnosed with high-risk neuroblastoma and experienced premature epiphyseal growth plate arrest to ascertain the presence, dose, agent present, or duration of retinoid exposure.	Regarding premature epiphyseal growth plate arrest after Isotretinoin exposure, 3 of the 216 patients were affected (an incidence of 1.38%). In addition, the group aged between 5 and 10 years had a higher incidence of bone abnormalities (P = 0.014).	Bone toxicity associated with exposure to Isotretinoin is a concern, requiring greater attention from professionals toward the population in a similar situation.

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Reference	Model	Objective	Intervention	Results	Conclusion
Tangrea et al. 1992 [59]	N = 269	Evaluate skeletal hyperostosis in patients receiving chronic low-dose Isotretinoin.	The lateral thoracic and cervical radiographs of subjects divided into two groups were evaluated: placebo and Isotretinoin.	Significantly more patients in the Isotretinoin group exhibited existing hyperostotic abnormalities and new hyperostotic involvement at previously unaffected vertebral levels when compared with the placebo group.	Chronic systemic administration of low doses of Isotretinoin can induce axial hyperostotic skeletal changes that resemble those reported in patients undergoing higher doses.

Table 2 presents a summary of studies selected according to the eligibility criteria for the systemic administration of Isotretinoin in human clinical trials, specifically focusing on those that included a sample size of $N \leq 36$ cases. The table is organized in the same way as Table 1: reference, model, objective, intervention, results, and conclusion. It is important to note that the smaller sample sizes in these studies may limit the generalizability and robustness of the findings. Studies with fewer participants often face challenges in achieving statistical significance and may be more susceptible to biases or random variations. Consequently, the results and conclusions drawn from these studies should be interpreted with caution. The limited number of patients can affect the power of the study, making it harder to detect true effects or differences, and may not fully represent the broader population.

Table 2. Studies selected according to eligibility criteria about the systemic administration of Isotretinoin that are related to human clinical trials and that presented N ≤ 36 cases.

Reference	Model	Objective	Intervention	Results	Conclusion
Archer et al. 1989 [60]	N = 1	Demonstrate the effects of treatment with Isotretinoin after 7 years and evaluate the systemic administration of Etretinate (another synthetic form of vitamin A) for 4 years to continue the treatment of hyperostosis.	The patient began treatment for Darier's disease with an initial dose of Isotretinoin of 40 mg/day, increasing by 40 mg/day every 2 weeks up to a maximum dose of 320 mg/day, for 7 years. The patient started the new treatment with a dose of 50 mg/day (weight 95.3 kg); after 2 months, the dose was increased to 75 mg/day and then to 100 mg of Etretinate per day.	Routine X-rays of the spine were carried out after 7 years of using Isotretinoin and showed the presence of prominent "beaklike" hyperostosis on the anterior aspect of the vertebral bodies of the cervical and thoracic spines. This finding led to the cessation of treatment with Isotretinoin and the subsequent use of Etretinate. With regards to Darier's disease, the dose of 100 mg/day of Etretinate was able to control the alteration. The use of this dosage for 4 years did not generate any symptoms in the patient.	Vertebral hyperostosis in the spine and thorax, although asymptomatic, developed/progressed during treatment with Isotretinoin over a period of 7 years. It also highlights the possibility of the disease progressing as a result of exertion and handling heavy objects related to the patient's job or recreational activities. Despite the change in dose of Isotretinoin for Etretinate over a period of 4 years, the disease progressed, although it remained asymptomatic.
Atalay et al. 2004 [61]	N = 1	Assess whether prolonged therapy with Isotretinoin can cause reversible sclerotic changes in the lumbar spine and femur.	Treatment started with Isotretinoin at a dose of 80 mg/day (1 mg/kg/day) for 17 months and 40 mg/day (0.5 mg/kg/day) for a further 5 months after clinical improvement.	The patient had ossification along the thoracic spine reminiscent of diffuse idiopathic skeletal hyperostosis, but there was no ligament calcification in the lumbar spine or pelvis. After stopping treatment, a gradual decline in bone mineral density at the lumbar and femoral sites was detected.	Although various bone abnormalities due to retinoids have been described before, reversible sclerotic changes have not been reported in the study due to the systemic administration of Isotretinoin.

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Reference	Model	Objective	Intervention	Results	Conclusion
Ellis et al. 1984 [44]	N = 22	Analyze radiographic changes in bone tissue during treatment with Isotretinoin.	Isotretinoin was administered twice a day to patients at a dosage ranging from 1.0 to 3.0 mg/kg, with dosage adjustment, when necessary, within the margin described. The average dose was 2.0 mg/kg/day.	After 1 year of treatment, 6 patients had minor skeletal hyperostosis, 5 patients had multiple hyperostosis, and only 2 patients were identified with hyperostosis after 6 months of treatment with Isotretinoin. Between 6 months and 1 year, some of the cases of hyperostosis did not change, while others progressed. Three patients had hyperostosis at 1 year, which was no longer present at the 6-month evaluation.	The authors recommend that patients taking high doses of Isotretinoin for long periods should be monitored with radiographic examinations. More studies are needed to assess the possibility of skeletal hyperostosis forming as a late sequel after treatment.
Giannoulis et al. 2005 [62]	N = 1	Observe and highlight the interactions and possible alterations of the systemic administration of Roaccutane (Isotretinoin) up to the 16th week of gestation in the developing fetus.	In addition to routine examinations, sonography, induction of labor by assessing the alterations present, measurement of the fetus, macroscopic examination, and biopsy were carried out.	Sonography revealed a single embryo with positive heart function but with malformations. The placenta was normal, and the amniotic fluid was increased. The formation of the cephalic skull up to the frontal bone region and the stomach was not observed. Measurement of the fetus showed growth retardation of 2 weeks. A biopsy revealed oesophageal atresia and a small cardiac foramen in the interventricular region.	The toxic effects of Roaccutane (Isotretinoin) on the fetus' hypothalamus are responsible for growth retardation. The authors also link damage to the hypothalamus with malformations in the spinal column and with the secretion of the alpha-melanocyte-stimulating hormone (α-MSH), which is responsible for the embryo's growth.

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Reference	Model	Objective	Intervention	Results	Conclusion
Hoemberg et al. 2017 [63]	N = 1	Observe and report on the changes present in a patient after using 13-cis-retinoic acid (Isotretinoin) for one year, considering complaints associated with pain and difficulty moving during the period of retinoic acid intake.	The patient was given oral Isotretinoin for one year, with an established dose of 160 mg/m²/day in two doses divided into 14 days, followed by 14 days of rest, which was considered to be one cycle. The patient underwent a total of 6 cycles, and after a rest period of 3 months, they underwent an additional 3 cycles.	After 6 cycles of Isotretinoin, during a rest period of 3 months, the patient reported pain and difficulty moving. At the age of 7, the patient's growth slowed down. With the presence of premature closure of the epiphyseal cartilages, the pain reported by the patient in the knees and both legs, as well as the short stature, were understood to be side effects of the Isotretinoin therapy, caused partly by a growth hormone deficiency.	Although rare, premature closure of the epiphyseal lines is a possible factor to be associated with or studied in cases of leg pain reported by patients or changes in growth in patients treated with Isotretinoin. The use of radiographic examinations before, during, and after treatment can be valuable tools for highlighting these changes and enabling treatment to be stopped or changes to be made.
Kindmark et al. 1998 [53]	N = 11	Investigate the early effects of oral Isotretinoin therapy on bone remodeling and calcium homeostasis in nodular cystic acne.	Subjects received Isotretinoin at an initial dose of 0.71 mg/kg, which was subsequently adjusted to 0.88 mg/kg/day as a maintenance dose after 1–3 months, administered once a day with food.	A significant drop in the concentration of both osteoblastic activity markers (bALP, PICP, and osteocalcin) and bone resorption markers (U-Ca/tU Crea) was observed around the 5th day of treatment. Calcium levels were significantly reduced during the first 5 days, associated with a significant increase in serum parathyroid hormone (PTH) levels. For both findings, by day 14, concentrations had returned to normal. Regarding bone mineral density, there was a significant change even after 6 months' intake of Isotretinoin.	During the administration of Isotretinoin, it was possible to observe changes in the biochemical markers associated with bone remodeling and calcium levels; after 14 days, even with continuous administration of the drug, the changes returned to normal levels, even in a subsequent six-month control.

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Reference	Model	Objective	Intervention	Results	Conclusion
Luthi et al. 2012 [64]	N = 1	Present a case of premature epiphyseal closure, as well as discuss the tests and examinations carried out, associating the systemic administration of Isotretinoin in the formation of the diagnosed alteration and the treatment carried out to resolve the case.	An assessment of the patient's medical history revealed the systemic administration of Isotretinoin for approximately 3 years at a dosage of 0.5 mg/kg. X-rays were taken of the region referred to by the symptoms and magnetic resonance imaging was also carried out on both knees.	Magnetic resonance imaging showed irregularities in the growth plate and significant metaphyseal-epiphyseal edema, most evident in the left knee region. Premature epiphyseal closure induced by the administration of Isotretinoin was diagnosed. Treatment was stopped, resulting in the resolution of the pain symptom within 2 months. After recovery, a persistent sequela lesion in the region of the growth plate was observed on a control MRI scan.	Premature epiphyseal fusion is a rare complication that can occur during the systematic administration of retinoids, for example, Isotretinoin, for the treatment of acne. An analysis of the patient's medical history, as well as imaging tests such as magnetic resonance imaging are essential. Stopping treatment usually leads to pain relief and the possibility of normal growth.
Marini et al. 1988 [65]	N = 1	Report a case of a patient who, when he was younger, took high doses of Isotretinoin to treat fibrodysplasia ossificans progressive; after 5 months of intake, the patient reported pain in the joints of his legs and a pause in growth.	At the age of 9, the patient was diagnosed with fibrodysplasia ossificans progressiva, and treatment was proposed using Isotretinoin at a dosage of 05 mg/kg/day.	A roentgenogram showed dense metaphyseal bands in the long bones of the lower limbs, associated with a pause in the patient's growth. Treatment with Isotretinoin was suspended and after 5 months it was possible to observe a reduction in the density of the bands and a continuation of the individual's growth process.	The dose of Isotretinoin affects the formation of bone elements. In the case reported, the pause in treatment with Isotretinoin was able to reduce the density of the metaphyseal bands, as well as continuing the individual's growth process.

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Reference	Model	Objective	Intervention	Results	Conclusion
Nishimura et al. 1997 [66]	N = 1	Report a case of a 6-year-old patient with generalized metaphyseal abnormalities with cone-shaped epiphyses, which developed after the administration of Isotretinoin as an adjunct in the treatment of a neuroblastoma.	After resolving the condition associated with neuroblastoma, at the age of 2 years and 7 months, as an adjunct to chemotherapy treatment, the patient began using Isotretinoin at a dosage of 40 mg/day.	Severe metaphyseal hollowing with cone-shaped epiphyses mainly affecting the rapid growth of the long bones was found. This severe epi-metaphyseal alteration, although unusual, was a remnant of premature epiphyseal closure, one of the adverse effects described by the administration of Isotretinoin. Other minor skeletal alterations were found such as posterior deformation of the vertebral bodies and increased interpedicular distances.	The precise cause of the generalized change in skeletal structure remains unknown, but it may be associated with the systemic administration of Isotretinoin.
Park et al. 2020 [67]	N = 1	Evaluate and treat the alteration "genu varum", bowed knees, in a patient who developed premature epiphyseal closure, through the systemic administration of Isotretinoin for 1 year.	After removal of the neuroblastoma and radiotherapy, the patient began using Isotretinoin at a dosage of 60 mg/day for one year to help with the process of tumor cell apoptosis.	The authors could visualize the development of a bone bridge in the region of the proximal growth plate of the tibia bilaterally, which resulted in bowed knees. Hemiepiphysiodesis was selected as the ideal treatment plan for resolving the bone bridges, and it enabled resolution on the left side, while on the right side, an arthroscopically assisted physeal bar resection was performed for complete resolution of the case.	Observation and studies to verify the alignment of the upper and lower limbs are necessary to provide an effective intervention for patients using Isotretinoin, considering the possibility of premature epiphyseal closure.

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Reference	Model	Objective	Intervention	Results	Conclusion
Tekin et al. 2008 [68]	N = 36	Investigate the effect of standard, single-course Isotretinoin therapy on bone mineral density (BMD) and bone remodeling markers in patients with nodular cystic acne.	The patients were treated with Isotretinoin for 4–6 months until they reached a cumulative dose of 120 mg/kg.	No significant changes were found in lumbar spine and femoral BMD between groups either at the start of treatment or in a start vs. end comparison. The bone remodeling markers chosen also showed no significant change between groups.	Single-course therapy with Isotretinoin did not generate significant clinical effects on bone metabolism.
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Torok et al. 1989 [69]	N = 15	Evaluate whether the systemic administration of retinoids administered in moderate doses for a few months can be considered safe regarding the skeletal system.	Patients in the acne group received Isotretinoin at a dose of 1 mg/kg/day for 4 months and the psoriasis group received Etretinate at a dose of 0.7–1 mg/kg/day also for 4 months.	In the group treated with Isotretinoin, there was pathological uptake of the radiomarker in three cases, while in the Etretinate group no significant bone alterations attributable to the treatment were found.	An early increase in metabolic activity in the bone is to be expected after administration of Isotretinoin. In treatment with Etretinate, bone changes were not evidenced.
Yang et al. 2022 [70]	N = 1	Describe a case of embryopathy with rare agenesis of the parietal bones and athelia due to exposure to Isotretinoin during gestation.	Prenatal ultrasound was carried out at 21 weeks and 2 days of gestation. It was decided to terminate the pregnancy and consent to a full autopsy.	Histological examination revealed the presence of fibrous tissue in the parietal region without intramembranous ossification. The fetus also had multiple craniofacial dysmorphisms, agenesis of the thymus, and alterations associated with the circulatory and neurological systems.	Agenesis of the parietal bones and athelia are considered rare anomalies, with no previous reports associated with embryopathies caused by retinoic acids. Based on these findings, the authors propose an expansion in the phenotype of retinoic acid–associated embryopathy.

Among the articles selected for inclusion on this study, some of them described experiments in rats. Therefore, to better organize the information gathered, Table 3 presents a summary of studies selected according to the eligibility criteria for the systemic administration of Isotretinoin in experiments conducted on rats.

Table 3. Studies selected according to eligibility criteria about the systemic administration of Isotretinoin related to experiments in rats.

Reference	Model	Objective	Intervention	Results	Conclusion
Bergoli et al. 2011 [38]	N = 32	Evaluate the effects of Isotretinoin on alveolar bone repair after maxillary incisor extraction and analyze serum calcium levels in rats.	Experimental group received a daily dose of Isotretinoin (7.5 mg/kg) for 30 days before the surgical procedure and until the day of euthanasia (7, 21, 28, and 90 days).	There was an acceleration in the alveolar repair process at all data collection times. Serum calcium levels showed a significant drop between the first and second data collection (21, 28, and 90).	Daily administration of Isotretinoin in doses corresponding to those used to treat cystic acne caused an acceleration in the alveolar repair process.
Cashin et al. 1984 [39]	N = 15	Evaluate tibial bone breaking stress in rats after using Etretinate and Isotretinoin.	The doses obtained using the drug Roacutan (Roche) were administered to groups of five rats every day for 15 days. The volume administered was 5 mg/kg of body weight.	The experiment showed that Etretinate induced significant bone changes after measuring tibial tensile strength. Isotretinoin also showed a slight influence, but in a milder way compared with Etretinate.	This method provides an effective means of assessing bone changes resulting from hypervitaminosis A in rats.
Hotchkiss et al. 2006 [40]	N = 60 for Experiment 1 and N = 72 for Experiment 2	Compare the effects of Isotretinoin and all-trans-retinoic acid (Tretinoin) in rats.	Experiment 1: The rats received 1 mL/kg of soybean oil by gavage to acclimatize them to the dosing process. At 82 days, treatment with Isotretinoin and Tretinoin began and lasted for 15 weeks. Experiment 2: Followed the same acclimatization process and at 59 days, the rats began treatment with Isotretinoin for 20 weeks.	Spontaneous fractures in long bones were observed in some rats treated daily with 15 mg/kg Tretinoin. Bone mineral density, bone mineral content, bone diameter, and cortical thickness of the femur were reduced in rats treated daily with 10 or 15 mg/kg Tretinoin or 30 mg/kg Isotretinoin. The lumbar spine showed no changes.	Although the effects of Isotretinoin are not as dramatic as those of Tretinoin, further studies are recommended to evaluate the effects of Isotretinoin on long bones.

Continued on next page

Reference	Model	Objective	Intervention	Results	Conclusion
De Oliveira et al. 2013 [41]	N = 33	Analyze the bone repair process in rats given a dose of 7.5 mg/kg/day of oral Isotretinoin.	Isotretinoin was administered in combination with sunflower oil by gavage at a concentration of 7.5 mg/kg daily for 30 days.	The highest percentages of bone formation were observed in the experimental group, with 25.37% formation on the 21st day, 41.78% on the 28th day, and 57.51% on the 90th day, compared to the control group, which showed 17.10% on the 21st day, 34.42% on the 28th day, and 48.49% on the 90th day.	The results showed an acceleration in the process of bone formation in the calvaria of the rats but without statistical significance.

3.2. Literature evaluation results

The data obtained that was transcribed into the tables and information associated with the number of articles and the change evidenced was collected and used to create two graphics, to better observe the prevalence found in the collection of theoretical material. Figure 4 shows the alterations highlighted by the authors of the papers selected for this study as well as the number of articles that referenced them in human clinical trials. A higher prevalence of cases associated with hyperostosis, followed by changes in bone formation, can be observed following systemic administration of Isotretinoin in the clinical human trials selected for this study.

It is also worth pointing out that some studies found no changes following the systemic administration of Isotretinoin in human patients.

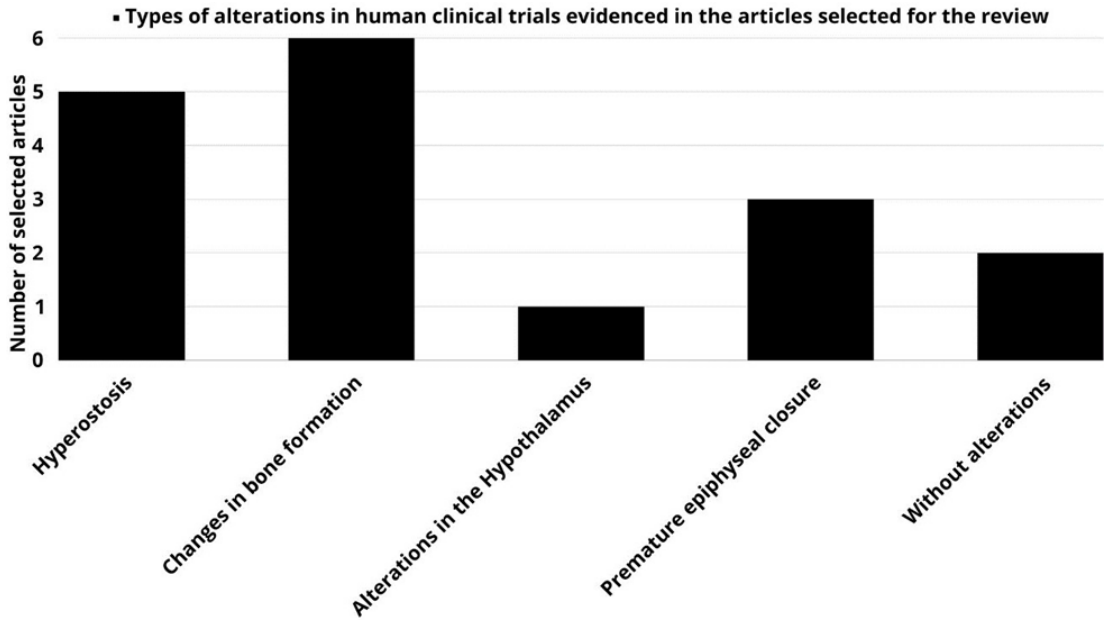

Figure 4. Number of articles presenting results on the administration of systemic Isotretinoin in human clinical trials as well as the types of alterations found in those articles.

Figure 5 shows the organization of data obtained from the articles that showed alterations in experiments on rats. They have been organized in a way that allows visualizing the alterations as well as the number of articles that reported them. It is worth noting that a high number of articles associated with the formation of bone structures can be observed, and only one article reported a positive effect on the process of repairing bone structures.

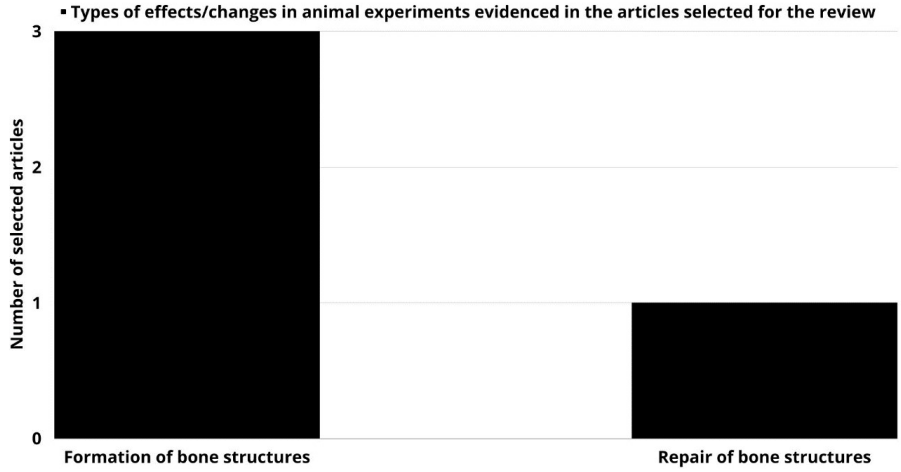

Figure 5. Number of articles that presented results on the administration of systemic Isotretinoin in experiments with rats as well as the types of alterations found by those articles.

4. Discussion

Isotretinoin is a synthetic form of vitamin A that has the function of regulating, directly or indirectly, biological processes in different cell types [46]. This substance has been widely used for the treatment of lamellar ichthyosis, severe cystic acne, severe recalcitrant nodular acne, xeroderma pigmentosum, organ or bone marrow–transplant recipients, and diseases with abnormal keratinization [23,71–74]. Draghici et al. [75] reported that Isotretinoin is the first-choice treatment for acne vulgaris, causing a reduction in sebum production and the formation of new acne lesions, with its main function being the apoptosis of human sebocytes.

The need to warn the patient that they will be using Isotretinoin is fundamental, and the professional responsible should also be aware of the patient's condition and possible factors that could lead to interactions or side effects. Alshaalan [76], in a study of 768 female patients with acne vulgaris who were using Isotretinoin, was able to observe that although the patients had a certain level of knowledge about the side effects and types of alterations resulting from the systemic administration of Isotretinoin, more preventive health actions were needed to alert these individuals to its teratogenic effects. Layton et al. [23] summarized the potential adverse effects through a literature review and suggested that both patients and prescribers must be fully aware of its teratogenicity. Patients should acknowledge the risks by signing a consent form and undergo detailed counseling by the clinician before and during treatment.

Ellis et al. [44] managed to obtain interesting results regarding the systemic administration of Isotretinoin. During an experiment with 8 patients affected by keratinization disorders and treated with Isotretinoin for 9 months (1 patient) or 1 year (7 patients), with an average dose of 2 mg/kg/day, it was possible to demonstrate through radiographic examinations that after 1 year of therapy, 6 of the 8 patients presented a small skeletal hyperostosis. In an analysis of previous results obtained at the 6-month mark, they reported that some of the patients did not present skeletal hyperostosis, while others already showed signs that advanced in the subsequent collection of results. This study is similar to that of Archer et al. [60] who concluded that vertebral hyperostosis in the cervical and thoracic spinal region, despite being asymptomatic, developed during treatment with Isotretinoin for a period of 7 years.

Tangrea et al. [59] observed that chronic systemic administration of low doses of Isotretinoin could induce axial hyperostotic skeletal changes: excessive growth or thickening of bone tissue that resembles those reported in patients subjected to higher doses. A randomized, double-blind, placebo-controlled study was carried out, with a sample of 269 white women and men between 40 and 75 years old who had two or more biopsy-proven basal cell carcinomas in the last 5 years before randomization. Significantly more patients in the Isotretinoin group exhibited existing hyperostotic abnormalities and new hyperostotic involvement at previously unaffected vertebral levels than in the placebo group. Zhao and Goodson [77] were also able to observe a case of hyperostosis in a 35-year-old patient who complained of back pain and stiffness, associated with long-term administration of Isotretinoin.

Giannoulis et al. [62], prepared a case report on a 16-year-old female patient, primigravida at 27 weeks, who until the 16th week of gestation used 60 mg daily of Roaccutane, a drug commonly used for severe acne therapy because of its active principle Isotretinoin and known for causing serious malformations to the embryo if utilized during pregnancy. The objective was to observe possible changes in the developing fetus and how they would present. Fetal measurements, macroscopic examination, and biopsy were performed. The cephalic skull was not formed up to the region of the frontal bone, there was an absence of the stomach, and the presence of a central lagostroma. During measurement, it was possible to observe a delay in the growth process of approximately 2 weeks. The biopsy showed esophageal atresia and a small cardiac foramen in the interventricular region. Brzezinski et al. [78] carried out a study to compare the adverse effects on the formation of newborns with and without exposure to Isotretinoin in 1459 female patients, divided into two groups according to exposure to the substance. There was no significant association between exposure to Isotretinoin and teratogenic effects. Still on alterations associated with heart disease, Pepe et al. [25], through a case report, demonstrated heart failure associated with dilated cardiomyopathy and concomitant renal infarction linked to the systemic administration of Isotretinoin for 5 months.

Still on the subject of possible alterations during an individual's development as a result of contact with retinoic acids, Yang et al. [70] described a case of retinoic acid embryopathy secondary to maternal Isotretinoin administration until the ninth week of gestation, expanding the phenotype to include the rare features of parietal bone agenesis and athelia. Histology of the parietal region showed fibrous tissue with no intramembranous ossification. The fetus also had multiple craniofacial dysmorphisms, thymic agenesis, and transposition of the great arteries with double-outlet right ventricle and subaortic perimembranous ventricular septal defect. Neuropathology revealed enlarged ventricles with agenesis of the cerebellar vermis, focal duplication of the central canal and scattered parenchymal ependymal rests, and possible cerebral heterotopias with associated abnormal neuronal lamination. A chromosomal microarray was made, and the results were normal. Parietal bone agenesis and athelia are rare congenital anomalies not previously reported in retinoic acid embryopathy, but retinoic acid or its degrading enzyme has been shown to exert effects in both developmental pathways, offering biological plausibility.

It is believed that an early increase in metabolic activity in bone is expected after administration of Isotretinoin [46], according to the study by Torok et al. [69] where 15 male patients with chronic psoriasis received treatment with Etretinate and 18 men with acne severe received treatment with Isotretinoin, both for 4 months. In the group treated with Isotretinoin, there was pathological uptake of the radiotracer in three cases. It is worth mentioning that the changes associated with the systemic administration of Isotretinoin will not always be related only to cystic nodular acne and may be linked to the treatment of other changes or diseases. Nishimura et al. [66], through a case report of a 6-year-old patient, were able to observe the systemic administration of Isotretinoin at a dose of 40 mg/day. After the start of use, changes could be observed. Initial radiographic examinations suggested the formation

of osteocytes in the cervical region of the spine, which is a very common manifestation of retinoic acid toxicity. Severe metaphyseal hollowing with cone-shaped epiphysis mainly affecting the rapid growth of the long bones was noted, which represented impairment in endochondral ossification. Other minor skeletal alterations were found, such as posterior deformation of the vertebral bodies and increased interpedicular distances.

The systemic administration of Isotretinoin to combat neuroblastoma is well-known and promising, particularly due to its ability to induce differentiation in neuroblastoma cells [79,80]. Despite this, Isotretinoin is only administrated to treat residual neuroblastoma as a maintenance therapy, and studies are being carried out to find retinoic acid derivatives to increase this effect or even the possibility of synergism with other existing drugs [81,82]. Duvalyan et al. [9] carried out a survey of patients at Children's Hospital Los Angeles (CHLA) diagnosed with high-risk neuroblastoma and who experienced premature arrest of the epiphyseal growth plate and compared with patients with high-risk neuroblastoma without premature arrest of the epiphyseal growth plate. Of the 216 patients selected for the research, 3 developed premature arrest of the epiphyseal growth plate after exposure to Isotretinoin, generating an incidence of 1.38%. Authors also showed a higher incidence of bone abnormalities in patients belonging to the group diagnosed between 5 and 10 years of age compared to the other two groups studied ($P = 0.014$). This study is similar to the study carried out by Steineck et al. [83], where 2 patients who used Isotretinoin in addition to exposure to Fenretinide developed premature closure of the epiphysis of the long bones, resulting in limb length discrepancy. In addition to changes in bone length due to the systemic administration of Isotretinoin, the age of the bones can also be affected, directly affecting the growth of the individual [84].

Hoemberg and Reinhard [63] also carried out a case report of a patient diagnosed with neuroblastoma. In this case, a female patient, after remission of neuroblastoma associated with chemotherapy, used Isotretinoin for 1 year at an established dose of 160 mg/m²/day in two doses for 14 days followed by 14 days of rest; this routine was considered as 1 cycle. After 6 cycles, the patient had a rest period of 3 months and subsequently underwent 3 more cycles. During the 3-month rest period, the patient reported pain and difficulty moving. At first, no explanations or justifications were found, and the procedure continued. At the age of 7 years, the patient's growth had slowed down, and it was possible to observe the premature closure of the epiphyseal cartilages, generating an important point of attention and understanding of the possible reason associated with the symptoms reported by the patient.

Park et al. [67] evaluated a 9-year-old male patient diagnosed with neuroblastoma who showed premature epiphyseal closure after 1 year of treatment with Isotretinoin, correlated with the presence and formation of bowed knees "genu varum"). Through the proposed imaging tests, it was possible to visualize the development of a bone bridge in the region of the proximal tibial growth plate bilaterally, which resulted in the bowed knees. On the right side, an arthroscopically assisted physeal bar resection was performed for complete resolution of the case; on the left side, hemiepiphysiodesis was selected as the ideal treatment plan for resolving the bony bridges and was able to resolve the case. Hemiepiphysiodesis consists of the induction of an asymmetric growth arrest in the physeal plate, thus allowing gradual correction of the deformity with limb growth over time [85]. Matsuoka et al. [86] also observed premature closure of the epiphysis in a 10-year-old female patient, related to the systemic administration of Isotretinoin to treat a neuroblastoma. Because of the closure, a deformity was found in the knee region, requiring surgical intervention to resolve the case. Cardoso-Demartini et al. [17] were able to observe, through a literature review, that premature epiphyseal closure has been reported in patients with neuroblastoma treated with high doses of Isotretinoin and in acne patients receiving lower doses. While many adverse effects of Isotretinoin in pediatric patients are transient, premature

epiphyseal closure and bone abnormalities can cause both temporary and permanent deformities, negatively impacting longitudinal growth and final height.

The effect of Isotretinoin on epiphyseal closure is related to the total dose, average daily dose, or duration of treatment [87,88]. Retinoids bind to one or more receptors, leading to premature closure of the epiphyses by proliferation and differentiation of epithelial cells [67,89]. Luthi et al. [64] were able to observe, through a case report, the changes associated with premature closure of the epiphyses. A 16-year-old male patient sought medical attention due to progressive pain in the anterior region of the knees lasting approximately 2 months. The patient was referred for physiotherapeutic treatment, relieving the pain on the right side, but with persistence on the left side. After evaluating the patient's medical history, the administration of Isotretinoin for the treatment of acne for 3 years at a dosage of 0.5 mg/kg was observed. Radiological examinations were carried out in the region referenced by the symptoms, as well as an imaging examination using magnetic resonance imaging. The exams revealed the presence of metaphyseal-epiphyseal edema and irregularities in the growth plate. With the findings and information obtained, it was possible to diagnose premature epiphyseal closure induced by the administration of retinoids.

Marini et al. [65], through a case report, obtained similar results to those mentioned above. A 13-year-old male patient was selected for the study. He had a history of using Isotretinoin when he was 9 years old to treat fibrodysplasia ossificans progressive, commonly caused by pathogenic variants in the activin A receptor type 1 (ACVR1) gene and characterized by heterotopic ossification of soft tissues [90]. The patient received a dosage of 5 mg/kg/day for 5 months. After complaining of pain in the joints of his legs and a pause in growth, tests were carried out and showed the presence of dense metaphyseal bands. After stopping treatment, the patient returned for another consultation and the authors were able to see a decrease in the density of the metaphyseal bands as well as a return to the growth process.

DiGiovanna [56] also concluded that a 16–20 week course of treatment with Isotretinoin at the recommended dose for severe acne has no clinically significant effect on lumbar spine and total hip BMD in the adolescent population (12–17 years). The study sample consisted of 217 adolescents, out of which 81 were girls, with severe recalcitrant nodular acne and treated with Isotretinoin twice a day at the recommended total dose of approximately 1 mg/kg for 16–20 weeks. The expected effectiveness of Isotretinoin in treating acne was observed.

Even with the evidence of both positive and negative results associated with the systemic administration of Isotretinoin for bone tissue, some authors have demonstrated in experiments that there is no significant change in the administration of retinoids. Tekin et al. [68] used 36 patients (15 men and 21 women) with nodular cystic acne. Patients received treatment with Isotretinoin for 4–6 months until reaching a cumulative dose of 120 mg/kg. Through radiographic examinations, bone mineral density of the lumbar spine and femur was obtained, in addition to analysis of serum calcium, phosphate, parathyroid hormone, total alkaline phosphatase, osteocalcin, free deoxypyridinoline, and urinary calcium. When comparing results obtained before and after intervention, no significant changes were found in the analysis of bone remodeling markers or in bone mineral density.

In a study conducted by Atalay et al. [61], a 38-year-old white man was evaluated, presenting with painful nodules and abscesses on the scalp and having suffered for more than 10 years from severe cystic acne. The patient underwent treatment with Isotretinoin at a dose of 80 mg/day (1 mg/kg/day) for 17 months and 40 mg/day (0.5 mg/kg/day) for another 5 months after clinical improvement. The patient presented with ossification along the thoracic spine reminiscent of diffuse idiopathic skeletal hyperostosis, but there was no ligamentous calcification in the lumbar spine or pelvis. After stopping treatment, a gradual decline in bone mineral density at the lumbar and femoral sites was detected with measurements by a dual energy x-ray absorptiometrie (DEXA) scan. It was concluded that, although

several bone abnormalities due to retinoids have been described before, reversible sclerotic changes have not been reported.

Carey et al. [58] also concluded, after a study carried out with 120 patients (66 men and 54 women, aged between 18 and 52 years) with severe acne who were treated with Isotretinoin for 4 months and 120 patients in the control group, that doses of 5 mg/kg/day of Isotretinoin do not produce significant long-term musculoskeletal changes, and any possible skeletal changes related to therapy with this medication are minimal. They also highlighted that its clinical efficacy seems to overcome any skeletal side effects observed.

Kindmark et al. [53] obtained different results regarding the analysis of bone remodeling and calcium homeostasis in 11 patients with nodular cystic acne. The remodeling markers evaluated were serum osteocalcin, the procollagen type I carboxy-terminal propeptide, bone-specific alkaline phosphatase, carboxy-terminal telopeptide of type I collagen, and urinary levels of calcium and hydroxyproline, which had a significant drop within 5 days of treatment with Isotretinoin. In addition to a significant drop in serum calcium, with the lowest value recorded on the 5th day of treatment, there was an increase in serum parathyroid hormone. However, as reported by the authors, these values returned to normal (values obtained prior to the intervention) even with continued treatment within 14 days. The authors also emphasized that no radiological changes or changes in bone mineral density were observed.

DiGiovanna et al. [74] carried out an assessment of bone mineral density in 217 adolescents (81 females and 136 males) aged between 12 and 17 years, who took Isotretinoin for 16–20 weeks at a dosage of 1 mg/kg twice a day for the treatment of severe recalcitrant nodular acne. Data was collected at the end of use using dual-energy X-ray absorptiometry in the lumbar spine and hip regions. Despite the visualization of the effects on acne treatment, there was no clinical significance in bone mineral density, nor was the formation of hyperostosis observed.

An experiment carried out by Cashin and Lewis [39] evaluated the bone-breaking tension of the tibia in Allen and Hanbury hooded rats (AHH/R) rats after using a second-generation analogue retinoic acid, a synthetic form of vitamin A called Etretinate, and Isotretinoin in 15 rats divided into 3 groups (control, Etretinate, and Isotretinoin). Etretinate induced significant bone changes after measurement of tibial breaking stress; Isotretinoin also showed a slight influence, although in a milder way when compared to Etretinate. In the study carried out by Bergoli et al. [38], where they evaluated the effects of Isotretinoin on alveolar bone repair after tooth extraction in Wistar rats, administration in doses corresponding to those used for the treatment of cystic acne caused an acceleration in the alveolar repair process.

Still regarding the possibility of changes in rats due to the systemic administration of Isotretinoin, Hotchkiss et al. [40] carried out 2 experiments to evaluate whether different dosages are capable of leading to different changes or even different levels of impairment of the bone structure. In the first experiment, 60 Sprague-Dawley rats (30 males and 30 females) aged 82 days were used and separated into 5 groups in a randomized manner, with 6 animals of each sex: control group; group 7.5 mg/kg Isotretinoin; group 22.5 mg/kg Isotretinoin; group 10 mg/kg all-trans-retinoic acid (Tretinoin), and group 15 mg/kg Tretinoin. In the second experiment, a total of 72 rats were used (36 males and 36 females), randomly divided into 3 groups: control group; group 7.5 mg/kg Isotretinoin, and group 30 mg/kg Isotretinoin. After 15 weeks of starting to use the dosages proposed for the groups, the animals were euthanized, and the results were collected and compared. It was possible to observe spontaneous fractures in the long bones in some rats treated daily with 15 mg/kg Tretinoin. Bone mineral density, bone mineral content, bone diameter, and femoral cortical thickness were reduced in rats treated daily with 10 or 15 mg/kg Tretinoin or 30 mg/kg Isotretinoin. According to the authors, the results were able

to demonstrate that although the effects of Isotretinoin are not as dramatic as those of Tretinoin, further studies are recommended to evaluate the effects of the former on long bones.

Regarding the process of bone repair and new formation, even as it may be affected by Isotretinoin, De Oliveira et al. [41] carried out an experiment that generated favorable results, despite not showing statistical significance; namely, the experimental group achieved a higher rate of new bone formation. Thirty-three albino Wistar rats, divided into two groups (experimental and control) in a randomized manner, underwent a surgical procedure to create a 2 mm bone defect in the calvaria region and were subsequently euthanized at the 21, 28, and 90-day marks post-intervention. The established oral dose was 7.5 mg/kg/day, with Isotretinoin powder diluted in sunflower oil for oral administration. After histological analysis, the results demonstrated a higher percentage of formation in the experimental group that had contact with Isotretinoin. Regarding the percentage of bone formation, the results obtained were 25.37% (± 9.14) on day 21 for the experimental group and 17.10% (± 9.23) for the control group; on day 28, it was possible to observe 41.78% (± 7.00) in the experimental group and 34.42% (± 7.70) in the control group; on day 90, the experimental group presented 57.51% (± 11.62) and the control group 48.49% (± 16.40). In addition to the findings in rats, Standeven et al. [91], in a study with guinea pigs, were able to observe a disturbance in the epiphyseal plates. There was an induction of plate closure in a group that was subjected to intraperitoneal injection of Isotretinoin for 10 days.

5. Conclusions

Based on the researched literature, given the widespread administration of Isotretinoin, it is necessary to know the viability and influence of the material on several tissues, particularly bone tissue. Its clinical efficacy seems to surpass any of the skeletal side effects observed; however, more studies are necessary to evaluate and prove the effects generated in bone tissues and other structures, in addition to providing greater security through the establishment of protocols, treatment, and patient care and attention.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

Conflicts of interest

Rogerio Leone Buchaim is an editorial board member for AIMS Bioengineering and was not involved in the editorial review or the decision to publish this article. The authors declare no conflict of interest.

Author contributions

Conceptualization, M.J.B.M.P. and R.L.B.; methodology, B.T.P., C.H.B.R., B.F.d.M.T. and M.B.M.M.; formal analysis, M.B.M.M.; investigation, B.T.P.; writing—original draft preparation, M.J.B.M.P.; writing—review and editing, R.L.B. and D.V.B.; visualization, A.F; supervision, R.L.B.; All authors have read and agreed to the published version of the manuscript.

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