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REVIEW ARTICLE

Rabbit as model for osteoporosis research

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Abstract
Osteoporosis is a major public health problem affecting more than 200 million people worldwide. The use of different animal models, for the study of its pathophysiology and treatments, is important being actually the ovariectomized rat the most widely used; although this model has several problems due its small size, lack of true closure of epiphyseal plate and bone differences with humans. This review is aimed at summarizing the most common methods published for osteoporosis induction in rabbits as model for human disease with their advantages and disadvantages. The paper shows the advantages of the use of this specie compared with the rat. All the techniques seemed to achieve the osteoporotic condition, but the one which obtained the most consistent bone mineral reduction in less time was the combination of surgery and corticoid treatment. The conclusion of the review was that rabbits are promising as a model of osteoporosis research because of their size, haversian remodelling and closure of epiphyseal plate, which solve some of the problems of the rat model. There are different techniques in the literature used to achieve the osteoporotic condition with diverse results, but there is a lack of consensus as to the best one.

Keywords Osteoporosis · Rabbit model · Bone

Introduction

Osteoporosis is a systemic skeletal disorder characterised by a decrease in bone mass and microarchitectural deterioration in bone structure, which lead to a decrease in bone strength and an increased risk of fractures. It is caused by an imbalance between the activity of osteoblasts, which form bone, and that of osteoclasts, which resorb it. This imbalance is influenced by hormones, diet, physical activity, cytokines, and clinical general status [1].

The osteoporotic disease is a major public health problem worldwide, especially in postmenopausal women and in the elderly population, leading to significant morbidity, mortality and involving high-cost health care [2]. It affects almost 75 million people in United States, Europe and Japan, more than 200 million people worldwide and it is expected to increase with the growing life span, being duplicated by 2040 [3].

Osteoporosis is generally classified as primary or secondary. Primary disease includes juvenile idiopatic, postmenopausal and senile osteoporosis and is the most prevalent in humans. Secondary osteoporosis, which is about 10% of the total number of cases, may be the result of different factors, such as treatment with glucocorticoids, endocrine disorders, gastrointestinal pathologies, lifestyle factors, and long-term immobilisation [4].

A study group of the World Health Organization (WHO) defined in 1994 the osteoporosis status as a reduction in the bone mineral density (BMD) or the bone mineral content (BMC) in at least 2.5 standard deviation (SD) of the mean reference value of a healthy 30-year-old person (same sex and race) measured in the same anatomical site and with the same technology. When BMD or BMC is between 1 and 2.5 SD below the mean, the condition is considered osteopenia [5, 6].

Although primary osteoporosis seems to be a disease restricted to humans because this condition is not naturally present in animals, studies in humans are difficult due to the slow development of the disease and the difficulty of obtaining healthy, or in the initial pathological phases, bone samples for its analysis [4]. Another challenge, when the effectiveness of therapies in humans are evaluated in clinical

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trials, is the difficulty of obtaining homogeneous groups due to the different lifestyle habits that can alter the results (alcohol consumption, smoking, diet and others). Because of these problems, animal models of the disease are really necessary in issues such as the study of pathogenesis or the evaluation of new therapies [7].

Animal models of osteoporosis can be classified in two main categories, depending on the dominant mechanism of induction: increase in bone resorption, (e.g., ovariectomy), and reduction in bone formation (e.g., glucocorticoid-induced osteoporosis) [8]. Actually, it is not an animal model that absolutely mimics all the characteristics of the human disease [9], and several animal species have been used, each of them has its pros and cons. Since 1994, the US Food and Drug Administration (FDA) have required data from at least two different animal models for the evaluation of new therapies. These models should include the ovariectomized rat model and a non-rodent large animal model with a Haversian system [10].

The ovariectomized rat is the most widely used model for osteoporosis research, due to the FDA requirement, low price, ease in handling and housing, and because the ovariectomy (OVX) in those animals has been demonstrated to induce a cancellous bone loss, mimicking the postmenopausal human osteoporosis [11, 12]. However, using rats for osteoporosis studies have several problems, such as the inability to achieve a true epiphyseal plate closure, lack of a Haversian system, and the minimal remodelling of the cortical bone [11–15]. These facts limit its suitability to test anabolic therapies. Additionally, its small size makes difficult the study of implant fixation or prosthetic devices as well as studies that need several biopsies or the collection of high blood volumes [11, 14].

Different large animals were used as osteoporosis models, including non-human primates [16–18], sheep [19, 20], dogs [21, 22] and pigs [23]. However, these animals are expensive, difficult to manage and house in large numbers [12, 24] and, in addition, their use in research is not socially accepted [25]. Rabbits as an experimental model are relatively economical, easy to handle and house, available in large genetically homogenous groups and suitable in size to evaluate conventional dental implants and some prosthetic devices [26].

In case of osteoporosis research, rabbits show several advantages compared to rats: they achieve complete closure of the epiphyseal plate at 6–8 months [27], have an active Haversian remodelling, analogous to that observed in humans, bone turnover faster than rodents and primates [28], and develop a significant bone loss. The advantages and disadvantages of using them as a model of osteoporosis are summarised in Table 1.

The aim of this review is to summarise the most commonly used methods to induce osteoporosis in rabbits found in the literature as well as to conclude the advantages and disadvantages of each of them.

Materials and methods

Eligibility criteria

1. Studies inducing osteoporosis in rabbits using one of these methods: glucocorticoid administration, OVX, glucocorticoid administration and OVX or a low-calcium diet administration and OVX.
2. Studies reporting data on bone changes and osteoporosis induction before the use of treatments or implantable devices.
3. Articles in English.

The search strategy was performed in the MEDLINE database of the US. National Library of Medicine up to and including May 2018, using the combination terms: "osteoporosis animal models", "rabbit osteoporosis", "rabbit ovariectomy", "glucocorticoid-induced osteoporosis" and "rabbit glucocorticoid osteoporosis". An extensive manual search was also performed through the references listed in the included papers.

This search resulted in 49 research studies. Thirty-three studies met the inclusion criteria and the main reason for the exclusion was the lack of osteoporotic induction data.

Table 1 Main advantages and disadvantages of using rabbits as an animal model of osteoporosis

Advantages	Disadvantages	OP induction methods
Affordable	Different microstructure with less cancellous bone	Ovariectomy (OVX)
Docile, easy to handle	Small for some prosthesis	OVX + a low-calcium diet
Easy to maintain in large groups compared with bigger animals	Osteoporosis induction only with OVX takes a long time	Glucocorticoids (GC)
Suitable size for implants	Different gait	OVX + GC
Skeletal maturity (6–8 months) (closure of epiphyseal plate)		
Haversian remodelling		

Results

There are four principal methods to induce osteoporosis in rabbits, with the goal of achieving bone loss due to different mechanisms:

- Estrogenic deprivation induced by OVX;
- combination of OVX with a reduction in the dietary calcium intake;
- administration of glucocorticoids;
- combination of OVX and glucocorticoid injection.

In the case of glucocorticoid administration, these drugs induce the depletion of bone through a reduction in the osteoblastogenesis, and the increase in osteoblast and osteocyte apoptosis [29].

Ovariectomy

Oestrogens are the major hormonal regulators of bone metabolism in humans due to bone remodelling inhibition, preferentially acting on osteocytes, and acting over the osteoclastic resorption. The direct effects of oestrogen on osteoclasts include the induction of their apoptosis and the inhibition of their formation as well as indirect effects due to RANKL and OPG [30]. The decline in oestrogen levels induced by OVX increases osteoclast recruitment, differentiation and survival; as a result, the bone resorption exceeds bone formation.

Oestrogen deprivation by OVX is the most common model for postmenopausal osteoporosis in animals. The studies conducted in rabbits using OVX for osteoporosis induction are varied in length and results since some studies obtained statistical significance in bone loss only with OVX (Wanderman et al. 2018) [28]. However, other authors found a remarkable resistance to the bone loss induced by oestrogen deprivation in rabbits [29]. In addition, the literature did not indicate the amount of bone loss induced during the post-OVX period as well as the time needed to achieve the osteopenia [31].

Our review focused on 20 articles that used the OVX as a technique for osteoporosis induction in rabbits; 8 with the aim of model validation [26, 28, 31–36], 5 with different types of implants [37–41], 5 using different treatments for osteoporosis [42–46] and 2 of periostic distraction [47, 48]. Since the aim of this review is to evaluate the effectiveness of the different techniques for osteoporosis induction in rabbits, we took into account only the studies in which authors reported the bone loss before the treatments with the validation of the model. Taking this into consideration, only 17 out of these 20 studies evaluated the bone quality before the osteoporosis treatment or before the use of implants. Thus, the others were excluded from the analysis [38, 47, 48].

The studies, number of animals, time point of osteoporosis evaluation, the techniques used for the bone loss measurement, and the principal results are reported in Table 2.

Summarising, 12 out of the 17 studies found statistical significance [28, 31–33, 39, 42–44, 46] in BMD or bone structure with respect to controls, while 5 did not found any difference [26, 34–37].

Regarding the evaluation time, huge differences were found between studies, ranging between 4 and 27 weeks with a mean of 12. The earliest BMD reduction was found at 10 weeks post-surgery [42]. Finally, the methods used to assess the osteoporosis condition were varied (from DXA to histomorphometry) dual X-ray absorptiometry (DXA) being the most used one (in 12 out of 17 studies). In conclusion, OVX alone in rabbits as a model for osteoporosis induction seems to be effective, but needs a longer time frame compared to other methods.

Ovariectomy and low-calcium diet

Nutritional deficiencies such as low-calcium diets, added to genetic and hormonal disorders, can affect the normal bone mineralisation. Calcium is the most abundant mineral in human and animal bodies, being a necessary nutrient at all ages, either for acquiring optimal bone mass or for preventing bone loss [49].

The correlation between low-calcium diets and osteoporosis in humans is controversial; even so, the current consensus is that low calcium absorption is related to an increased risk for osteoporotic bone fractures with a non-linear relationship, where only really low calcium diets are associated with osteoporotic findings [50]. In rabbits, similarly to humans, suboptimal calcium intake is known to affect bone mass [51].

The model consisting of the combination of oestrogen deprivation and low calcium intake was used in different animal models such as in rat [52], sheep [53], and goat [54] with optimal results, aimed at improving the bone loss obtained only with oestrogen depletion.

The studies that used a combination of OVX and low-calcium diets are summarised in Table 3. We have access to the full paper from 5 studies: 4 with the objective of implant evaluation [37, 55–57] and one for the validation of the model [8]. Out of these 5, 1 was excluded [55] from the final report because the authors did not provide data about the osteoporosis induction in the results section.

In summary, the evaluated studies found a BMD reduction using DXA as an evaluation method with a induction mean

Table 2 Studies which used ovariectomy for osteoporosis induction

Study	Number animals	Schedule (weeks)	Bone loss measurement	Principal results
Wanderman et al. [28]	36	17	DXA	Decrease BMD proximal tibia and distal femur*
Chen et al. [46]	24	24	DXA	Decrease BMD lumbar spine*
Qiu et al. [45]	75	12	MRS and μCT	Decrease TMD proximal femur from week 8 after surgery
Chen et al. [41]	18	17	DXA	Osteoporosis establishment (no results reported)
Dai et al. [44]	101	21	DXA	Decrease BMD spine*
Li et al. [40][a]	46 (10)	12	DXA	Decrease BMD proximal and distal femur*
He et al. [42]	21	10	DXA	Decrease BMD right femur*
Jensen et al. [43]	46	27	Histomorpho	Decrease BV/TV AND Tb.N; increase Tb.Sp; no differences in Tb.Th (spine)
Liu et al. [26]	20	6	DXA and μCT	No differences in BMD (lumbar spine and femur)
Qi et al. [39][b]	56 (16)	12	DXA	Decrease BMD right femur (ex vivo)*
Kaveh et al. [36]	15	4	X-ray	No radiological differences in radius
Baofeng et al. [35]	32	6 and 10	DXA and μCT	No differences in BMD in lumbar spine at any timepoint
Sevil and Kara [31]	24	8 and 16	DXA and CT	8 weeks no differences; 16 weeks decrease BMD* (femur)
Castañeda et al. [34]	35	6 and 16	DXA	6 and 16 weeks no differences in BMD (lumbar spine and knee)
Cao et al. [33]	12	12	Histomorpho	Decrease Tb.N; Increase Tb.Sp. No differences in BV/TV and Tb.Th
Cao et al. [32]	24	4 and 12	pQCT	4 weeks no TMD differences; 12 weeks decrease TMD
Mori et al. [37]	36	4, 12 and 24	DXA	No differences in BMD in distal tibia in any point time

DXA dual-energy X-ray absorptiometry, *MRS* magnetic resonance spectroscopy, *μCT* micro-computed tomography, *Histomorpho* histomorphometry, *pQCT* peripheral quantitative computed tomography, *BMD* bone mineral density, *TMD* total mineral density, *Tb.N* trabecular number, *Tb.Sp* trabecular separation, *BV/TV* bone volume/tissue volume, *Tb.Th* trabecular thickness

*Statistical significance

[a]The study was conducted in 46 animals but the evaluation of BMD reduction before implants placement (12 weeks) was done in 10 animals (5 ovariectomiced and 5 SHAM operated)

[b]The study was conducted in 56 rabbits but the BMD evaluation in 16

Table 3 Studies which used ovariectomy plus low calcium diet for osteoporosis induction

Study	Number animals	Schedule (weeks)	Ca (%)	Bone loss measure	Principal results
Wen et al. [57]	28	12	0.15	DXA	Decreased BMD in lumbar spine*
Martín-Monge et al. [8]	25	7	0.007	DXA	Decrease BMD in cervical spine* and calvaria but not in tibia*
Vidigal et al. [56]	20	16	0.15	DXA	Decrease BMD in femur*
Mori et al. [37]	36	4, 12 and 24	0.15	DXA	Decrease BMD in distal tibia at each time point*

DXA dual-energy X-ray absorptiometry, *BMD* bone mineral density, *Ca* daily calcium percentage on diet

*Statistical significance

time of 12.5 weeks (range 4–24 weeks) and obtaining successful results as soon as 4 weeks after the ovariectomy [37]. It was noted that two different diets were used in the literature, with different calcium concentrations (0.15% and 0.007%) the first one being the most commonly used.

Combination of ovariectomy and glucocorticoid administration

The use of glucocorticoid administration added to OVX as an osteoporosis model has two main objectives: to

accelerate the bone loss and to mimic the glucocorticoid-induced postmenopausal osteoporosis. This type of osteoporosis aggravates the postmenopausal disease, increasing the morbidity and the risk of fractures. In fact, up to 4.6% of postmenopausal women are taking oral glucocorticoids [58].

The reason to use glucocorticoids as an adjuvant of OVX is because the achievement of statistical significant bone loss only with OVX in rabbits is variable; while some studies reached significance only with OVX [28, 46], others did not see differences with SHAM animals [34, 35] and also is time consuming (up to 6 months) [46]. Because of that, the combination of OVX and glucocorticoid administration was presented as a fast and reproducible method to induce osteoporosis in rabbits [34, 35]. The administration of glucocorticoids in ovariectomised animals seems to accelerate the bone loss during the osteoporosis induction [34] and the recovery of BMD after the discontinuation of corticoid treatment seems to be avoided [34, 59].

According to our database search, there are 15 studies using this combination method; 5 for implant evaluation [29, 60–63], 6 for model validation [34–36, 59, 64, 65], 3 for the evaluation of the treatment effects [66–68] and one for other purposes [69]. Out of the initial 15 studies, 5 were removed from this review due to the lack of data on the reduction of bone mineral density [62, 63, 66, 68, 70].

Table 4 shows the main findings of the evaluated studies using the combination of OVX and glucocorticoid administration. In brief, the studies ranged between 2 and 16 weeks (mean 6.47 weeks), using DXA as a preferential BMD evaluation method (in 7 articles) and authors reported the osteoporotic state in all the studies as soon as 4 weeks of treatment. With regard to the type and dose of corticoid, the most used was 1 mg/kg of methylprednisolone administered daily by intramuscular injection for 4 weeks, starting 2 weeks after surgery [29, 34, 61, 65].

The results of the models using the combination of OVX and glucocorticoid administration showed that compared to

Table 4 Studies which used ovariectomy plus glucocorticoid administration for osteoporosis induction

Study	Number animals	Schedule (weeks)	Corticoid (type/dose (mg/kg)/via/start point/Trt period)[a]	Bone loss measure	Principal results
Oue et al. [61]	12	6	MP/1/IM/2w/4w	I. torque	Decrease max. mechanical strength*
Wen et al. [57]	40	4, 6 and 8	MP/1/IM/2w/4,6,8w[b]	DXA and µCT	Decrease BMD lumbar from week 4 and femur from week 6*
Zhang et al. [67]	60	8	MP/1/?/?/4w[c]	DXA	Decrease BMD lumbar, decreased vertebral BMC and BMD (p = unknown)
Almagro et al. [29]	38	6	MP/1/IM/2w/4w	DXA	Decrease BMD lumbar spine and knee subchondral bone*
Liu et al. [26]	20	6	MP/1/IM/2w/4w	DXA and µCT	Decrease BMC in femur*
Li et al. [64]	40	2,4,8 and 10	MP/1.5/0w/4w[d]	MRS and µCT	Decrease vBMD from 8 weeks*;BV/TV decrease from week 4*
Baofeng et al. [35]	32	6 and 10	MP/1/IM/2w/8w	DXA and µCT	Decrease BMD lumbar spine at 6 weeks and 10 weeks*
Kaveh et al. [36]	15	4	MP/1/IM/0w/4w[b]	X ray	Radiological osteoporotic state (p = unknown)
Castañeda et al. [34]	35	6 and 16	MP/0.5-1-2/IM/2w/4w[e]	DXA	Decrease BMD at 6 and 16 weeks (group 1 mg/kg treatment) at lumbar spine, knee and subchondral bone*
Castañeda et al. [59]	29	6	MP/1/IM/2w/4w	DXA	Decrease BMD spine, knee and subchondral bone*

DXA dual-energy X-ray absorptiometry, *MRS* magnetic resonance spectroscopy, *µCT* micro-computed tomography, *X ray* radiography, *BMD* bone mineral density, *vBMD* volumetric bone mineral density, *BMC* bone mineral content, *Tb.Sp* trabecular separation, *BV/TV* bone volume/tissue volume, *MP* methylprednisolone, *IM* intramuscular, *w* weeks

*Statistical significance

[a]Type of glucocorticoid used/ dose (mg/kg)/ administration via/ time between surgery and start of the glucocorticoid administration/ treatment period

[b]4,6,8w: study with 3 groups of animals with different time of glucocorticoids administration: 4 weeks, 6 weeks and 8 weeks

[c]0w: started treatment the same day of the ovariectomy

[d]Authors did not include in the text the administration via and not specify the time they started the methylprednisolone treatment (they said "after wound healing")

[e]Authors used three different doses of methylprednisolone (0.5 mg/kg; 1 mg/kg; 2 mg/kg)

the OVX alone, the combination induces more significant bone loss (almost in vertebral cancellous bone) in less time [57]. Moreover, the longer the treatment continued, the more severe were the osteoporotic changes in bone [35, 69].

Glucocorticoid administration

The use of glucocorticoid administration alone for the osteoporosis induction was more focused on the study of secondary osteoporosis (Glucocorticoid-induced osteoporosis or GIOP), due to chronic corticoid administration in humans, which is the most frequent secondary osteoporosis and the most prevalent in young people [71]. Glucocorticoid therapy is an important treatment for inflammatory diseases [72] and it is estimated that 0.5% of the world population was treated with these drugs as well as more than 1.75% of the women over 55 years of age (which is the population with the greatest incidence of primary osteoporosis) [71].

The mechanisms underlying the glucocorticoid-induced osteoporosis are different from those of the oestrogen deprivation and consist mainly of the reduction of the number and function of the osteoblasts [73] with the impairment of bone formation [74] and the increase of osteoblast and osteocyte apoptosis [29] (Almagro et al. 2013). The amount of bone loss depends on the cumulative glucocorticoid dose and the rate of this bone depletion is higher in the first 3–6 months of therapy [75]. One relevant fact is that bone architecture can be recovered after discontinuing the treatment [76].

The depletion in the number of osteoblasts is mainly due to three mechanisms: an impairment in the differentiation of mesenchymal cells towards the osteoblastic lineage [77], the reduction of the terminal differentiation of mature osteoblasts [78] and the enhanced apoptosis of mature osteoblasts [79]. These mechanisms are potentiated due to the apoptosis of osteocytes mediated by the activation of caspase3 [65]. In relation to the function of osteoblasts, glucocorticoid administration alters the synthesis of collagen type I by transcriptional and post-transcriptional mechanisms [80], leading to a decrease in the amount of bone matrix available for mineralisation. BMD loss is the immediate consequence of the glucocorticoid administration and affects trabecular bone more than the cortical one in a cumulative way [81].

In the models using only glucocorticoids to induce the bone loss, several points have to be addressed: first of all, it should be known whether the osteopenia derived from glucocorticoid administration can be recovered after treatment discontinuation [76]; second, the doses of glucocorticoid have to be adjusted because if they are too low there will be no bone loss, whereas too high doses can produce osteonecrosis [82] or systemic problems leading to the animal's death [34].

10 full articles were found in the literature using glucocorticoids for osteoporosis induction in rabbits and they could be divided into: 2 for model characterisation [34, 35], 2 for osteoporosis treatment [83, 84], 1 for the evaluation of biomaterials [85], 4 for the evaluation of bone changes in GIOP [86–89] and 1 for the investigation of a possible disease mechanism [90].

Table 5 exposes the principal features of the evaluated studies (two were removed because they did not provide data on the model [86, 90]. In summary, the schedule ranges between 4 and 18 weeks, with a mean of 8.71 weeks; in most of them (6) the evaluation method used was DXA and in all the studies, independently of the evaluation method and the time point used, authors reported osteoporotic conditions in the animals. With regard to the used glucocorticoid, two main drugs (methylprednisolone and dexamethasone) were found at different doses and routes of administration (which are described in Table 5).

Discussion

For the study of postmenopausal osteoporosis, the ovariectomised rat is considered as the "gold standard" and the US-FDA recommend its use for the study of new osteoporosis therapies in addition to a larger animal model [10]. Nevertheless, this model has several disadvantages due to its size, inability to achieve complete epiphyseal plate closure and lack of Haversian remodelling. Its small size makes this animal unable for the study of some prosthetic devices. Haversian cortical remodelling is of extreme importance in maintaining bone strength (a critical point in the osteoporosis pathogenesis), thus, the use of animals with this feature, such as rabbits or sheep [14] is vital for the study of anabolic therapies. Finally, the skeletal maturity of the animal model is important to study the effects of osteoporotic therapies (because they are destined to human adults) and rabbits, unlike rats, do reach this stage. Moreover, compared to bigger models, they do it within a shorter time frame (between 6 and 8 months old) [27]; for these reasons, the characterisation of a rabbit model seems to be relevant. Rabbits also have the advantage over other models of being easy to house and handle, and big groups with similar genetic background may be available for study.

There is inconsistency in the literature regarding the efficacy of oestrogen deprivation alone in rabbits for inducing osteoporosis. Several studies lack in achieving significant bone loss only by OVX [26, 34–37], while others demonstrated osteopenia without the use of concomitant treatments [28, 31–33, 39–46]. In the present review, we found 4 studies that compared OVX animals with animals who were submitted to a combination of OVX and glucocorticoid treatment [26, 34–36] using periods of time between surgery and determination of osteoporosis between 4 [36] and 16 weeks [34]. All of them said that, in the time they evaluated bone

Table 5 Studies which used glucocorticoids for osteoporosis induction

Study	Number animals	Schedule (weeks)	Corticoid (type/ dose (mg/kg)/via/Trt period)[a]	Bone loss measure	Principal results
Li et al. [40]	20	8 and 12	MP/1.5/SC/4w[b]	MRI and DXA	Decrease BMD in femur from 8 weeks*
Zhang et al. [84]	60	6 and 12	DEX/3/?/6,12w[c]	Histology	Decrease Tb.N*, BV/TV, Tb.Th; Increase Tb.Sp*
Lozano et al. [85]	8	4	MP/1.5/IM/4w	DXA	BMD similar values than Castañeda et al., 2008
Baofeng et al. [35]	32	6 and 10	MP/1/IM/8w	DXA and µCT	Decrease BMD at 10 weeks*
Carvas et al. [83]	18	8 and 18	MP/0.35/SC/18w	DXA	Decrease BMD in tibia at 8 weeks and 18 weeks*
Castañeda et al. [34]	35	6 and 16	MP/1.5/IM/4w	DXA	Decrease BMD at 6 at lumbar spine, knee and subchondral bone. No data of the 16 weeks group*
Takahashi et al. [87]	20	4 and 8	DEX/0.2–0.4/SC/8.5w[d]	DXA and µMRI	Decrease BMD both doses at 4 and 8 weeks*
Eberhardt et al. [88]	12	4	MP/0.07; 0.17/IM/4w[e]	X ray and histology	Decrease Tb.Th, Tb.V and BV/TV using both doses*

DXA dual-energy X-ray absorptiometry, *MRI* magnetic resonance imaging, *µCT* micro-computed tomography, *µMRI* micro-magnetic resonance imaging, *BMD* bone mineral density, *Tb.N* trabecular number, *Tb.Sp* trabecular separation, *BV/TV* bone volume/tissue volume, *Tb.Th* trabecular thickness, *Tb.V* trabecular volume, *MP* methylprednisolone, *DEX* dexamethasone, *IM* intramuscular, *SC* subcutaneous, *w* weeks

*Statistical significance

[a]Type of glucocorticoid used/ dose (mg/kg)/ administration via/ time between surgery and start of the glucocorticoid administration/ treatment period

[b]In this study after the administration of 1.5 mg/kg of MP daily for 4 weeks authors used a maintenance dose of 0.35 mg/kg three times a weeks for 8 weeks

[c]Authors administered dexamethasone twice a week during 6 or 12 weeks. The administration route was not described in the text

[d]The methodology of this article was the use of subcutaneous pellets of dexamethasone what is equivalent to 0.2 mg/kg/day with duration of 60 days (8.5 weeks). Authors implant one or two pellets per animal (equivalent to 0.2 or 0.4 mg/Kg/day)

[e]Two groups of treatment with different methylprednisolone dose: 0.07 mg/kg and 0.17 mg/kg

quality, using DXA [26, 34, 35] or radiography [36] the group treated with the combination of OVX + GC reached an osteoporosis status while the OVX alone not.

Authors that did not observe osteoporosis in their studies, using only hormonal deprivation, explained this in two ways: as the effect of reflex ovulations or the possibility of an ectopic production of sexual hormones such as estrone, estriol or adrenal estrogens [12, 34]. Anyway, the studies which reached statistical significance only with OVX achieved these results over a longer period of time compared to the use of the combination of glucocorticoids (e.g., 4–6 months [28, 46] vs. 6 weeks [34]. Thus, the problem may be that hormonal deprivation alone in rabbits needs much longer time to reach differences and when glucocorticoids were added, this time is deeply diminished. According to this premise, Castañeda [34] reported that, although their results in the ovariectomised group were not significant, there was a tendency to decrease the bone mineral density measured by DXA in 6 weeks post-surgery.

When low-calcium diets were added to the effects of ovariectomy, the results of the evaluated studies found a significant reduction in BMD as soon as 1 month after the surgery [37], although most of them evaluated the effects of the combination at 2 [8], 3 [37, 57] or 4 months [56]. Furthermore, in the study conducted by Mori and colleagues [37], the authors compared the BMD variation in ovariectomised rabbits with and without a low-calcium diet and concluded that the animals who were fed with reduced mineral content food presented a reduction of the tibial bone mineral density measured by DXA on three occasions (at 1, 3 and 6 months), while the animals on normal diets did not show any bone mineral density variations. The amount of calcium used in the rabbits' diet for the low doses differed from author to author, ranging from 0.07 [8, 55] to 0.15% [37, 56, 57], taking into account that a normal maintenance diet for rabbits has approximately 0.85% of calcium. These results are in accordance with the findings in other animal models, such as rat [52], sheep [53], and goat [54].

The type of glucocorticoid used for osteoporosis induction, alone or in combination with ovariectomy, as well as the dose administered are not clearly established; with regard to the type of drug, in the literature we found reports of the use of methylprednisolone acetate [61], methylprednisolone hemisuccinate [24, 29, 35] and dexametasone [68]; all of

them seem to induce bone loss in a similar way. Eberhard et al. [88] demonstrated that the doses of glucocorticoids ranging between 0.5 and 1 mg/kg/day had effect only on bone turnover without inflammation, necrosis or changes in the subarticular bone, while doses under 0.5 mg/kg/day did not produce significant changes in bone. In the study of Castañeda et al. [34], the doses of 1.5 mg/kg/day of methylprednisolone over 4 weeks induced significant bone loss in lumbar spine and knee, whereas 2 mg/kg/day was lethal in all the cases. For this reason, and to minimise certain deleterious effects, they recommended 1 mg/kg/day of methylprednisolone hemisuccinate for osteoporosis induction when combined with ovariectomy [34]. This drug and dose are the most commonly used in the evaluated studies [29, 34, 35]. The drug was administered by intramuscular injection every day over 4 weeks and beginning 2 weeks after the surgery. With this treatment, a consistent and significant bone loss was achieved in different anatomical sites, such as the lumbar vertebras, the global knee and the subchondral bone [34], and they demonstrated that it was possible to reduce BMD in bones with different cortical and cancellous composition.

With regard to the methods used to evaluate the bone mass, most of the studies consulted for this review used DXA [8, 28, 29, 34, 35, 57], while others used different imaging methods as radiography [86], microCT [44, 57, 64], peripheral quantitative computed tomography (pQCT) [32] or magnetic resonance spectroscopy (MRS) [45, 64]. Moreover, several studies used histology or histomorphometry [62, 63, 84] as well as biomechanical tests [57, 84].

The "gold standard" method for the evaluation of bone quality is bone biopsy and histomorphometry but this method is not practical for screening a large number of patients, is invasive and time consuming as well as the study of the same bone in different time points is complicated (and impossible in most research animals). To solve some of the histomorphometry problems the use of image methods seems to be reasonable. DXA is the most used technique in both human and animals [59], and it is considered to have the necessary precision to be used on small laboratory animals as the rabbit [91, 92].

In the present review, one study compared the results of the in vivo DXA with bone histology post mortem [44] and the results of the densitometry (BMD reduction) were supported by the histologic observations (disrupted trabeculae, increased trabecular separation and decreased bone tissue). Other two studies used DXA (in vivo) and microtomography (ex vivo) to characterize bone changes in osteoporotic models [26, 69]; in both cases the microtomographic results supported the BMD reduction observed by DXA.

Using DXA, Castañeda et al. [59] found that the minimum number of rabbits needed to achieve a significant BMD reduction in 6 weeks (in a combined ovariectomy and glucocorticoid administration in rabbits) was 6 animals in global knee and 8 animals in lumbar spine [59]. With these results, they demonstrated that DXA is valid for the study of a BMD reduction in osteoporotic rabbits. Furthermore, DXA has the advantage of being an in vivo method that can be used several times in the same animal to study the progression of the disease or the effect of the treatments.

Finally, with regard to the anatomical sites used for assessing the bone loss or the effects of diverse treatments on bone, most of the studies used lumbar spine as the main site for densitometric evaluation [34, 44, 46, 59]. These studies are in accordance with those in humans, where DXA of lumbar spine has the highest sensibility for bone mineral density measurements [57]. Regardless, in the studies considered for this review, there were different opinions about the preferential anatomical site for the osteoporosis evaluation. While Wen [57] found greater and earlier changes in vertebral bodies (using DXA and microCT) than in femoral condyles, Castañeda [59] stated that the global knee could be an excellent site for bone mineral density evaluation, due to the low number of animals needed to achieve statistical significance.

Conclusions

The combination of ovariectomy and glucocorticoid administration is able to induce osteoporotic bone changes in only 6 weeks, and it is an easy, consistent and reproducible method. It produces a bone loss different from the postmenopausal osteoporosis [34] and the effects of the glucocorticoids persist over time after the treatment discontinuation.

Models using low-calcium diets as an adjuvant of the oestrogen depletion had to be taken into account, but only a few studies used this model in rabbits, with inconsistency in the time needed to achieve the osteoporotic status. The use of glucocorticoids alone should be reserved for the study of secondary osteoporosis. Finally, the use of ovariectomy alone, although with promising results, provided huge differences in terms of the time needed to achieve enough BMD reduction.

In conclusion, all the studied methods seemed to achieve osteoporotic condition in rabbits with a huge difference in the time. There is no best method, because ovariectomy and corticoids, which seem to be the most reproducible one, is not perfect for the study of postmenopausal osteoporosis (the most prevalent in humans), whereas the ovariectomy alone, which would be the best for primary osteoporosis, lacks this consistency and needs a long time to induce it. Thus, the choice of the best osteoporotic method has to be made by the researchers according to their specific study characteristics.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights This article does not contain any studies with human or animal subjects performed by any of the authors.

References

1. Komori T (2015) Animal models for osteoporosis. Eur J Pharmacol 759:287–294. https://doi.org/10.1016/j.ejphar.2015.03.028
2. Compston J (2010) Osteoporosis: social and economic impact. Radiol Clin N Am 48:477–482. https://doi.org/10.1016/j.rcl.2010.02.01
3. Reginster JY, Burlet N (2006) Osteoporosis: a still increasing prevalence. Bone 38:S4–S9. https://doi.org/10.1016/j.bone.2005.11.024
4. Bonucci E, Ballanti P (2014) Osteoporosis—bone remodeling and animal models. Toxicol Pathol 42:957–969. https://doi.org/10.1177/0192623313512428
5. Kanis JA, Melton LJ, Christiansen C, Johnston CC, Khaltaev N (1994) The diagnosis of osteoporosis. J Bone Miner Res 9:1137–1141. https://doi.org/10.1002/jbmr.5650090802
6. Kanis JA, McCloskey EV, Johansson H, Oden A, Melton LJ, Khaltaev N (2008) A reference standard for the description of osteoporosis. Bone 42:467–475. https://doi.org/10.1016/j.bone.2007.11.001
7. Lelovas PP, Xanthos TT, Thoma SE, Lyritis GP, Dontas IA (2008) The laboratory rat as an animal model for osteoporosis research. Comp Med 58:424–430
8. Martin-Monge E, Tresguerres IF, Blanco L, Khraisat A, Rodríguez-Torres R, Tresguerres JA (2011) Validation of an osteoporotic animal model for dental implant analyses: an in vivo densitometric study in rabbits. Int J Oral Maxillofac Implants 26:725–730
9. Oheim R, Schinke T, Amling M, Pogoda P (2016) Can we induce osteoporosis in animals comparable to the human situation? Injury 47(Suppl 1):S3–S9. https://doi.org/10.1016/S0020-1383(16)30002-X
10. Thompson DD, Simmons HA, Pirie CM, Ke HZ (1995) FDA guidelines and animal models for osteoporosis. Bone 17:125S–133S. https://doi.org/10.1016/8756-3282(95)00285-L
11. Kalu DN (1991) The ovariectomized rat model of postmenopausal bone loss. Bone Miner 15:175–191
12. Turner AS (2001) Animal models of osteoporosis—necessity and limitations. Eur Cell Mater 1:66–81. https://doi.org/10.22203/eCM
13. Mosekilde L (1995) Assessing bone quality—animal models in preclinical osteoporosis research. Bone 17:343S–352S. https://doi.org/10.1016/8756-3282(95)00312-2
14. Turner RT, Maran A, Lotinun S, Hefferan T, Evans GL, Zhang M, Sibonga JD (2001) Animal models for osteoporosis. Rev Endocr Metab Disord 2:117–127
15. Rodgers JB, Monier-Faugere MC, Malluche H (1993) Animal models for the study of bone loss after cessation of ovarian function. Bone 14:369–377. https://doi.org/10.1016/8756-3282(93)90166-8
16. Appt SE, Clarkson TB, Hoyer PB, Kock ND, Goode AK, May MC, Persyn JT, Vail NK, Ethun KF, Chen H, Sen N, Kaplan JR (2010) Experimental induction of reduced ovarian reserve in a nonhuman primate model (*Macaca fascicularis*). Comp Med 60:380–388
17. Smith SY, Jolette J, Chouinard L, Komm BS (2015) The effects of bazedoxifene in the ovariectomized aged cynomolgus monkey. J Bone Miner Metab 33:161–172. https://doi.org/10.1007/s00774-014-0580-z
18. Saito M, Kida Y, Nishizawa T, Arakawa S, Okabe H, Seki A, Marumo K (2015) Effects of 18-month treatment with bazedoxifene on enzymatic immature and mature cross-links and non-enzymatic advanced glycation end products, mineralization, and trabecular microarchitecture of vertebra in ovariectomized monkeys. Bone 81:573–580. https://doi.org/10.1016/j.bone.2015.09.006
19. Oheim R, Simon MJK, Steiner M, Vettorazzi E, Barvencik F, Ignatius A, Amling M, Clarke IJ, Pogoda P, Beil FT (2017) Sheep model for osteoporosis: the effects of peripheral hormone therapy on centrally induced systemic bone loss in an osteoporotic sheep model. Injury 48:841–848. https://doi.org/10.1016/j.injury.2017.02.009
20. Dias IR, Camassa JA, Bordelo JA, Babo PS, Viegas CA, Dourado N, Reis RL, Gomes ME (2018) Preclinical and translational studies in small ruminants (sheep and goat) as models for osteoporosis research. Curr Osteoporos Rep 16:182–197. https://doi.org/10.1007/s11914-018-0431-2
21. Martin RB, Butcher RL, Sherwood LL, Buckendahl P, Boyd RD, Farris D, Sharkey N, Dannucci G (1987) Effects of ovariectomy in beagle dogs. Bone 8:23–31. https://doi.org/10.1016/8756-3282(87)90128-1
22. Dannucci GA, Martin RB, Patterson-Buckendahl P (1987) Ovariectomy and trabecular bone remodeling in the dog. Calcif Tissue Int 40:194–199
23. Kim SW, Kim KS, Solis CD, Lee MS, Hyun BH (2013) Development of osteoporosis animal model using micropigs. Lab Anim Res 29:174–177. https://doi.org/10.5625/lar.2013.29.3.174
24. Reinwald S, Burr D (2008) Review of nonprimate, large animal models for osteoporosis research. J Bone Miner Res 23:1353–1368. https://doi.org/10.1359/jbmr.080516
25. Quigley M (2007) Non-human primates: the appropriate subjects of biomedical research? J Med Ethics 33:655–658. https://doi.org/10.1136/jme.2007.020784
26. Liu X, Lei W, Wu Z, Cui Y, Han B, Fu S, Jiang C (2012) Effects of glucocorticoid on BMD, micro-architecture and biomechanics of cancellous and cortical bone mass in OVX rabbits. Med Eng Phys 34:2–8. https://doi.org/10.1016/j.medengphy.2011.06.010
27. Gilsanz V, Roe TF, Gibbens DT, Schulz EE, Carlson ME, Gonzalez O, Boechat MI (1988) Effect of sex steroids on peak bone density of growing rabbits. Am J Physiol 255:E416–E421. https://doi.org/10.1152/ajpendo.1988.255.4.E416
28. Wanderman NR, Mallet C, Giambini H, Bao N, Zhao C, An KN, Freedman BA, Nassr A (2018) An ovariectomy-induced rabbit osteoporotic model: a new perspective. Asian Spine J 12:12–17. https://doi.org/10.4184/asj.2018.12.1.12
29. Almagro MI, Roman-Blas JA, Bellido M, Castañeda S, Cortez R, Herrero-Beaumont G (2013) PTH [1–34] enhances bone response around titanium implants in a rabbit model of osteoporosis. Clin Oral Implants Res 24:1027–1034. https://doi.org/10.1111/j.1600-0501.2012.02495.x
30. Faienza MF, Ventura A, Marzano F, Cavallo L (2013) Postmenopausal osteoporosis: the role of immune system cells. Clin Dev Immunol 2013:575936. https://doi.org/10.1155/2013/575936
31. Sevil F, Kara ME (2010) The effects of ovariectomy on bone mineral density, geometrical, and biomechanical characteristics in the rabbit femur. Vet Comp Orthop Traumatol 23:31–36. https://doi.org/10.3415/VCOT-08-09-0085

32. Cao T, Shirota T, Yamazaki M, Ohno K, Michi KI (2001) Bone mineral density in mandibles of ovariectomized rabbits. Clin Oral Implants Res 12:604–608. https://doi.org/10.1034/j.1600-0501.2001.120608.x
33. Cao T, Shirota T, Ohno K, Michi KI (2004) Mineralized bone loss in partially edentulous trabeculae of ovariectomized rabbit mandibles. J Periodontal Res 39:37–41. https://doi.org/10.1111/j.1600-0765.2004.00703.x
34. Castañeda S, Calvo E, Largo R, González-González R, de la Piedra C, Díaz-Curiel M, Herrero-Beaumont G (2008) Characterization of a new experimental model of osteoporosis in rabbits. J Bone Miner Metab 26:53–59. https://doi.org/10.1007/s00774-007-0797-1
35. Baofeng L, Zhi Y, Bei C, Guolin M, Qingshui Y, Jian L (2010) Characterization of a rabbit osteoporosis model induced by ovariectomy and glucocorticoid. Acta Orthop 81:396–401. https://doi.org/10.3109/17453674.2010.483986
36. Kaveh K, Ibrahim R, AbuBakar MZ, Ibrahim TA (2010) Osteoporosis induction in animal model. Am J Anim Vet Sci 5:139–145
37. Mori H, Manabe M, Kurachi Y, Nagumo M (1997) Osseointegration of dental implants in rabbit bone with low mineral density. J Oral Maxillofac Surg 55:351–361. https://doi.org/10.1016/S0278-2391(97)90124-5
38. Oberg S, Johansson C, Rosenquist JB (2003) Bone formation after implantation of autolysed antigen extracted allogeneic bone in ovariectomized rabbits. Int J Oral Maxillofac Surg 32:628–632. https://doi.org/10.1054/ijom.2003.0428
39. Qi M, Hu J, Li J, Dong W, Feng X, Yu J (2012) Effect of zoledronate acid treatment on osseointegration and fixation of implants in autologous iliac bone grafts in ovariectomized rabbits. Bone 50:119–127. https://doi.org/10.1016/j.bone.2011.10.011
40. Li JP, Li P, Hu J, Dong W, Liao NN, Qi MC, Li JY (2014) Early healing of hydroxyapatite-coated implants in grafted bone of zoledronic acid-treated osteoporotic rabbits. J Periodontol 85:308–316. https://doi.org/10.1902/jop.2013.130046
41. Chen X, Giambini H, Ben-Abraham E, An KN, Nassr A, Zhao C (2015) Effect of bone mineral density on rotator cuff tear: an osteoporotic rabbit model. PLoS One 14:10. https://doi.org/10.1371/journal.pone.0139384
42. He J, Yang L, Qing Y, He C (2014) Effects of electroacupuncture on bone mineral density, oestradiol level and osteoprotegerin ligand expression in ovariectomised rabbits. Acupunct Med 32:37–42. https://doi.org/10.1136/acupmed-2012-010271
43. Jensen PR, Andersen TL, Pennypacker BL, Duong LT, Delaissé JM (2014) The bone resorption inhibitors odanacatib and alendronate affect post-osteoclastic events differently in ovariectomized rabbits. Calcif Tissue Int 94:212–222. https://doi.org/10.1007/s00223-013-9800-0
44. Dai L, Wu H, Yu S, Zhao H, Xue L, Xu M, Shen Z, Hu M (2015) Effects of OsteoKing on osteoporotic rabbits. Mol Med Rep 12:1066–1074. https://doi.org/10.3892/mmr.2015.3551
45. Qiu Y, Yao J, Wu X, Zhou B, Shao H, Hua T, Xiong Z, Tang G (2015) Longitudinal assessment of oxytocin efficacy on bone and bone marrow fat masses in a rabbit osteoporosis model through 3.0-T magnetic resonance spectroscopy and micro-CT. Osteoporos Int 26:1081–1092. https://doi.org/10.1007/s00198-014-2933-9
46. Chen J, Liu W, Zhao J, Sun C, Hu K, Zhang L, Ding Y (2017) Gelatin microspheres containing calcitonin gene-related peptide or substance P repair bone defects in osteoporotic rabbits. Biotechnol Lett 39:465–472. https://doi.org/10.1007/s10529-016-2263-4
47. Arslan H, Ketani A, Gezici A, Kapukaya A, Necmioğlu S, Kesemenli C, Subasi M (2003) The effects of osteoporosis on distraction osteogenesis: an experimental study in an ovariectomised rabbit model. Acta Orthop Belg 69:67–73
48. Bayar GR, Gunaydin Y, Ortakoglu K, Gunhan O, Aydintug YS, Sencimen M (2012) Histomorphometric analysis of new bone obtained by osteogenic periosteal distraction in ovariectomized rabbits. Oral Surg Oral Med Oral Pathol Oral Radiol 113:472–479. https://doi.org/10.1016/j.tripleo.2011.04.012
49. Lani A, Kourkoumelis N, Baliouskas G, Tzaphlidou M (2014) The effect of calcium and vitamin D supplementation on osteoporotic rabbit bones studied by vibrational spectroscopy. J Biol Phys 40:401–412. https://doi.org/10.1007/s10867-014-9358-8
50. Warensjö E, Byberg L, Melhus H, Gedeborg R, Mallmin H, Wolk A, Michaëlsson K (2011) Dietary calcium intake and risk of fracture and osteoporosis: prospective longitudinal cohort study. BMJ 342:d1473. https://doi.org/10.1136/bmj.d1473
51. Duque G, Watanabe K (2011) Osteoporosis research–animal models. Springer, London
52. Gao X, Ma W, Dong H, Yong Z, Su R (2014) Establishing a rapid animal model of osteoporosis with ovariectomy plus low calcium diet in rats. Int J Clin Exp Pathol 7:5123–5128
53. Zarrinkalam MR, Beard H, Schultz CG, Moore RJ (2009) Validation of the sheep as a large animal model for the study of vertebral osteoporosis. Eur Spine J 18:244–253. https://doi.org/10.1007/s00586-008-0813-8
54. Alt V, Cheung WH, Chow SK, Thormann U, Cheung EN, Lips KS, Schnettler R, Leung KS (2016) Bone formation and degradation behavior of nanocrystalline hydroxyapatite with or without collagen-type 1 in osteoporotic bone defects—an experimental study in osteoporotic goats. Injury 47(Suppl 2):S58–S65. https://doi.org/10.1016/S0020-1383(16)47010-5
55. Tresguerres IF, Clemente C, Donado M, Gómez-Pellico L, Blanco L, Alobera MA, Tresgerres JA (2002) Local administration of growth hormone enhances periimplant bone reaction in an osteoporotic rabbit model. Clin Oral Implants Res 13:631–636. https://doi.org/10.1034/j.1600-0501.2002.130609.x
56. Vidigal GM, Groisman M, Gregório LH, GdeA Soares (2009) Osseointegration of titanium alloy and HA-coated implants in healthy and ovariectomized animals: a histomorphometric study. Clin Oral Implants Res 20:1272–1277. https://doi.org/10.1111/j.1600-0501.2009.01739.x
57. Wen B, Zhu F, Li Z, Zhang P, Lin X, Dard M (2014) The osseointegration behavior of titanium–zirconium implants in ovariectomized rabbits. Clin Oral Implants Res 25:819–825. https://doi.org/10.1111/clr.12141
58. Zhang Z, Ren H, Shen G, Qiu T, Liang Yang Z, Yao Z, Tang J, Jiang X, Wei Q (2016) Animal models for glucocorticoid-induced postmenopausal osteoporosis: an updated review. Biomed Pharmacother 84:438–446. https://doi.org/10.1016/j.biopha.2016.09.045
59. Castañeda S, Largo R, Calvo E, Rodríguez-Salvanés F, Marcos ME, Díaz-Curiel M, Herrero-Beaumont G (2006) Bone mineral measurements of subchondral and trabecular bone in healthy and osteoporotic rabbits. Skelet Radiol 35:34–41. https://doi.org/10.1007/s00256-005-0022-z
60. Luo X, Barbieri D, Duan R, Yuan H, Bruijn JD (2015) Strontium-containing apatite/polylactide composites enhance bone formation in osteopenic rabbits. Acta Biomater 26:331–337. https://doi.org/10.1016/j.actbio.2015.07.044
61. Oue H, Doi K, Oki Y, Makihara Y, Kubo T, Perrotti V, Piattelli A, Akagama Y, Tsuga K (2015) Influence of implant surface topography on primary stability in a standardized osteoporosis rabbit model study. J Funct Biomater 6:143–152. https://doi.org/10.3390/jfb6010143
62. Oki Y, Doi K, Makihara Y, Kubo T, Oue H, Tsuga K (2016) Intermittent administration of parathyroid hormone enhances primary stability of dental implants in a bone-reduced rabbit model. J Oral Sci 58:241–246. https://doi.org/10.2334/josnusd.15-0717
63. Oki Y, Doi K, Makihara Y, Kobatake R, Kubo T, Tsuga K (2017) Effects of continual intermittent administration of

parathyroid hormone on implant stability in the presence of osteoporosis: an in vivo study using resonance frequency analysis in a rabbit model. J Appl Oral Sci 25:498–505. https://doi.org/10.1590/1678-7757-2016-0561
64. Li GW, Tang GY, Liu Y, Tang RB, Peng YF, Li W (2012) MR spectroscopy and micro-CT in evaluation of osteoporosis model in rabbits: comparison with histopathology. Eur Radiol 22:923–929. https://doi.org/10.1007/s00330-011-2325-x
65. Liu Y, Porta A, Peng X, Gengaro K, Cunningham EB, Li H, Dominguez LA, Bellido T, Christakos S (2004) Prevention of glucocorticoid-induced apoptosis in osteocytes and osteoblasts by calbindin-D28k. J Bone Miner Res 19:479–490. https://doi.org/10.1359/JBMR.0301242
66. Bellido M, Lugo L, Castañeda S, Roman-Blas JA, Rufián-Henares JA, Navarro-Alarcón M, Largo R, Herrero-Beaumont G (2014) PTH increases jaw mineral density in a rabbit model of osteoporosis. J Dent Res 89:360–365. https://doi.org/10.1177/0022034510363082
67. Zhang J, Chen SG, Habaerxi K, Alimujiang S, Chen Y, Peng MZ, Yue R, Wu YL, Wang DQ, Chen YM (2014) Reinforcing effect of calcium sulfate cement bovine bone morphogenetic protein on vertebral in the rabbit model of osteoporosis. Asian Pac J Trop Med 7:382–385. https://doi.org/10.1016/S1995-7645(14)60060-2
68. Jing D, Hao X, Xu F, Liu J, Luo E, Meng G (2016) Effects of local delivery of BMP2, zoledronate and their combination on bone microarchitecture, biomechanics and bone turnover in osteoporotic rabbits. Sci Rep 6:28537. https://doi.org/10.1038/srep28537
69. Wen XX, Xu C, Wang FQ, Feng YF, Zhao X, Yan YB, Lei W (2015) Temporal changes of microarchitectural and mechanical parameters of cancellous bone in the osteoporotic rabbit. Biomed Res Int 2015:263434. https://doi.org/10.1155/2015/263434
70. Luo X, Barbieri D, Duan R, Yuan H, Bruijn JD (2015) Strontium-containing apatite/polylactide composites enhance bone formation in osteopenic rabbits. Acta Biomater 26:331–337. https://doi.org/10.1016/j.actbio.2015.07.044
71. Walsh LJ, Wong CA, Pringle M, Tattersfield AE (1996) Use of oral corticosteroids in the community and the prevention of secondary osteoporosis: a cross sectional study. BMJ 313:344–346. https://doi.org/10.1136/bmj.313.7053.344
72. Morand EF (2007) Effects of glucocorticoids on inflammation and arthritis. Curr Opin Rheumatol 19:302–307. https://doi.org/10.1097/BOR.0b013e32805e87d0
73. Sambrook PN (2007) Anabolic therapy in glucocorticoid-induced osteoporosis. N Engl J Med 357:2084–2086. https://doi.org/10.1056/NEJMe0706770
74. Canalis E, Mazziotti G, Giustina A, Bilezikian JP (2007) Glucocorticoid-induced osteoporosis: pathophysiology and therapy. Osteoporos Int 18:1319–1328. https://doi.org/10.1007/s00198-007-0394-0
75. Steinbuch M, Youket TE, Cohen S (2004) Oral glucocorticoid use is associated with an increased risk of fracture. Osteoporos Int 15:323–328. https://doi.org/10.1007/s00198-003-1548-3
76. Ding M, Cheng L, Bollen P, Schwarz P, Overgaard S (2010) Glucocorticoid induced osteopenia in cancellous bone of sheep: validation of large animal model for spine fusion and biomaterial research. Spine (Phila Pa 1976) 35:363–370. https://doi.org/10.1097/BRS.0b013e3181b8e0ff
77. Pereira RC, Delany AM, Canalis E (2002) Effects of cortisol and bone morphogenetic protein-2 on stromal cell differentiation: correlation with CCAAT-enhancer binding protein expression. Bone 30:685–691. https://doi.org/10.1016/S8756-3282(02)00687-7
78. Pereira RM, Delany AM, Canalis E (2001) Cortisol inhibits the differentiation and apoptosis of osteoblasts in culture. Bone 28:484–490. https://doi.org/10.1016/S8756-3282(01)00422-7
79. Weinstein RS, Jilka RL, Parfitt AM, Manolagas SC (1998) Inhibition of osteoblastogenesis and promotion of apoptosis of osteoblasts and osteocytes by glucocorticoids. Potential mechanisms of their deleterious effects on bone. J Clin Invest 102:274–282. https://doi.org/10.1172/JCI2799
80. Delany AM, Gabbitas BY, Canalis E (1995) Cortisol downregulates osteoblast alpha 1 (I) procollagen mRNA by transcriptional and posttranscriptional mechanisms. J Cell Biochem 57:488–494. https://doi.org/10.1002/jcb.240570314
81. Briot K, Roux C (2015) Glucocorticoid-induced osteoporosis. RMD Open 1:e000014
82. Grardel B, Sutter B, Flautre B, Viguier E, Lavaste F, Hardouin P (1994) Effects of glucocorticoids on skeletal growth in rabbits evaluated by dual-photon absorptiometry, microscopic connectivity and vertebral compressive strength. Osteoporos Int 4:204–210
83. Carvas JS, Pereira RM, Caparbo VF, Fuller P, Silveira CA, Lima LA, Bonfa E, Mello SB (2010) A single dose of zoledronic acid reverses the deleterious effects of glucocorticoids on titanium implant osseointegration. Osteoporos Int 21:1723–1729. https://doi.org/10.1007/s00198-009-1125-5
84. Zhang KJ, Zhang J, Kang ZK, Xue XM, Kang JF, Li YW, Dong HN, Liu DG (2012) Ibandronate for prevention and treatment of glucocorticoid-induced osteoporosis in rabbits. Rheumatol Int 32:3405–3411. https://doi.org/10.1007/s00296-011-2074-9
85. Lozano D, Trejo CG, Gómez-Barrena E, Manzano M, Doadrio JC, Salinas AJ, Vallet-Regí M, García-Honduvilla N, Esbrit P, Buján J (2012) Osteostatin-loaded onto mesoporous ceramics improves the early phase of bone regeneration in a rabbit osteopenia model. Acta Biomater 8:2317–2323. https://doi.org/10.1016/j.actbio.2012.03.014
86. Southard TE, Southard KA, Krizan KE, Hillis SL, Haller JW, Keller J, Vannier MW (2000) Mandibular bone density and fractal dimension in rabbits with induced osteoporosis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 89:244–249. https://doi.org/10.1067/moe.2000.102223
87. Takahashi M, Wehrli FW, Hilaire L, Zemel BS, Hwang SN (2002) In vivo NMR microscopy allows short-term serial assessment of multiple skeletal implications of corticosteroid exposure. Proc Natl Acad Sci USA 99:4574–4579. https://doi.org/10.1073/pnas.062037499
88. Eberhardt AW, Yeager-Jones A, Blair HC (2001) Regional trabecular bone matrix degeneration and osteocyte death in femora of glucocorticoid- treated rabbits. Endocrinology 142:1333–1340. https://doi.org/10.1210/endo.142.3.8048
89. Li GW, Xu Z, Chen QW, Chang SX, Tian YN, Fan JZ (2013) The temporal characterization of marrow lipids and adipocytes in a rabbit model of glucocorticoid-induced osteoporosis. Skelet Radiol 42:1235–1244. https://doi.org/10.1007/s00256-013-1659-7
90. Yongtao Z, Kunzheng W, Jingjing Z, Hu S, Jiangiang K, Ruiyu L, Chunsheng W (2014) Glucocorticoids actiate the local renin-angiotensin system in bone: possible mechanism for glucocorticoid-induced osteoporosis. Endocrine 47:598–608. https://doi.org/10.1007/s12020-014-0196-z
91. Griffin MG, Kimble R, Hopfer W, Pacifici R (1993) Dual-energy X-ray absorptiometry of the rat: accuracy, precision, and measurement of bone loss. J Bone Miner Res 8:795–800. https://doi.org/10.1002/jbmr.5650080704
92. Casez JP, Muehlbauer RC, Lippuner K, Kelly T, Fleisch H, Jaeger P (1994) Dual-energy X-ray absorptiometry for measuring total bone mineral content in the rat: study of accuracy and precision. Bone Miner 26:61–68