Original Article

Effect of Long-Term Captopril Administration on Bone Density and Orthodontic Tooth Movement

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Abstract

Background and Aim: Captopril is an oral angiotensin-converting-enzyme (ACE) inhibitor extensively used in the treatment of hypertension and heart failure. ACE has been suggested to function in bone cells and might therefore impact orthodontic tooth movement (OTM). Considering the controversy surrounding the effects of ACE and its inhibitors on osseous tissues, we aimed to evaluate the effect of captopril on OTM for the first time in a rat model.

Materials and Methods: Orthodontic appliances were fixed between the left first molars and incisors of 30 rats divided into three groups (n=10) receiving captopril, saline or no treatment. Following sacrifice on day 21, the amount of tooth movement was measured as the distance created between the first and second molars. Bone density was assessed by lateral cephalograms on days 1 and 21 and osteoclast number, root resorption and periodontal ligament (PDL) width were analyzed histologically. One-way ANOVA followed by post-hoc test were used for statistical analysis ($P<0.05$)

Results: OTM significantly increased in the captopril group compared to the saline and no-treatment groups ($P<0.05$). Bone density significantly decreased in the mandible of the captopril group from day 1 to 21 ($P<0.05$). No significant differences were found in the histological variables except for the significant increase in PDL width at the mesioapical aspect in the captopril group.

Conclusion: The present study showed that captopril administration could lead to increased OTM and decreased bone density in rats. Further studies are suggested to clarify its exact role at the cellular and molecular levels.

Key Words: Captopril, Tooth Movement Techniques, Rats

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Introduction

Orthodontic treatment procedures consist of continuous force applied to teeth to move them into a new more favorable position. During this process, the mechanical stimulant is transmitted to the surrounding periodontium, which results in numerous cellular-molecular changes that ultimately lead to biological adaptation to a new condition [1-3]. Many studies have shown that a number of drugs might affect this process [4].

Hypertension is a common disease and one of the major reasons for early death and disability worldwide. It is associated with cardiovascular diseases, including coronary heart disease, stroke and other vascular diseases [5,6]. Angiotensin-converting-enzyme (ACE) inhibitors including captopril are involved in the endocrine renin-angiotensin-system (RAS) and have been shown to effectively control hypertension and heart failure [7,8]. RAS modulates blood pressure and electrolyte homeostasis mainly through angiotensin II. Renin cleaves angiotensin I which in turn is converted to angiotensin II by the ACE. The specific receptors of angiotensin II are AT1 and AT2 that mediate its various functions in the body. In addition to its known systemic route of action, RAS has been suggested to regulate various "local, tissue-specific" cellular processes like proliferation/apoptosis, cell metabolism, inflammation and angiogenesis in a number of organs including the heart, kidneys, bone marrow, blood vessels and adipose tissue [9-15].

RAS plays an important role in bone metabolism, and any interference with this system and its components can affect osseous tissues. Angiotensin II can be produced by bone cells and may affect their performance through binding to its specific receptors [16-19]. The expression of RAS components has been reported in osteoblastic/ osteoclastic cells; for example, ACE was found in osteoblasts during endochondral bone formation [15] and ATI has been demonstrated in bone cells [17-26]. Similarly, studies have indicated a role for RAS in the pathogenesis and development of bone metabolic disorders [27].

There has been a widespread utilization of ACE inhibitors in the recent years. In addition to high blood pressure and heart failure, these drugs have been used for the treatment of diabetes nephropathy [28] and tissue injury resulting from RAS activity in renal disease [29]. Several studies have investigated the impact of ACE inhibitors on osseous tissues with contradicting results; both resorptive [30-32] and osteogenic [22,33] effects have been reported after the administration of this group of drugs. Hence, clarification of the role of ACE inhibitors in bone turnover and metabolism may be helpful in patient management.

There has been a recent increase in the number of adults demanding orthodontic treatments, some of which may use ACE inhibitors for various reasons. Additionally, a general understanding of the different pathways involved in orthodontic tooth movement (OTM) can be helpful in both biological and clinical aspects. Considering these facts, we sought to investigate the effect of captopril, a classic ACE inhibitor, on orthodontic tooth movement through experimental, radiographic and histological evaluations.

Materials and Methods

Animal preparation:

The current investigation was conducted in accordance with the US National Institute of Health (publication 85-23; revised: 1985) and approved by the Ethics Committee of Tehran University of Medical Sciences (code no. 21662). Thirty male Wistar rats with an initial weight of 200-250 g were housed in transparent plastic cages and maintained on a 12-hour light-dark cycle at stable temperature of 24-25°C and 55% humidity, one week prior to appliance insertion. All animals had free access to water and food. Also, they fed with soaked standard rat chow diet to avoid excessive chewing force.

Orthodontic procedure:

After anesthesia administration with intraperitoneal injections of ketamine (Vetaset, 50 mg/kg) and xylazine (Dopaser, 14 mg/kg), orthodontic appliances consisting of 5 mm NiTi closed-coil spring (Hiek, 0.006×0.022-inch, 3M Unitek, Monrovia, CA, USA) with 0.10-inch ligature wires at each end, were tied between the incisor and first molar of the left upper jaw to exert a force of 60g at 2mm activation [34]. To secure the ligature wires on the incisors, shallow grooves were cut above the gingiva of the left tooth using a 0.8-inch diamond bur followed by application of light-cure

composite (Transbond XT, 3M Unitek, Monrovia, CA, USA) on both incisors. This served a double purpose of maintaining the wires and providing anterior anchorage to prevent distal displacement of the left incisor [35]. The lower incisors were shortened (2 mm) once a week to prevent them from dislocating the maxillary appliances. At this stage, the animals were fed with ground food pellets soaked in water. This was to prevent appliance displacement during orthodontic treatment.

The animals were randomly divided into the following groups:

1) Group A: treated with 100 mg/kg/day captopril dissolved in normal saline.

2) Group B: received 1mL/day normal saline.

3) Group C: served as the control group with no medication.

In groups A and B, injections were carried out at the same time each day, throughout the orthodontic treatment.

Tooth movement evaluation:

All rats were sacrificed by CO2 asphyxiation after three weeks of treatment (21 days) and the maxillae were separated for OTM measurements which were made between the first and second molars using a feeler gauge (Mitutoyo Co., Kawasaki-shi, Japan) calibrated to 0.01mm increments. OTM values were the mean of the two measurements carried out by two operators.

Densitometry evaluation:

Lateral cephalometric radiographs were taken with Kodak (size 2) E-speed dental X-ray films (Eastman Kodak, Rochester, NY, USA) at a constant focus–film distance (70 kV, 8 mA, exposure time: 0.3s) by using a box in which rats' necks were fixed. An automatic film processor (Velopex Extrax, Medivance, UK) was utilized to process radiograph projections obtained on days 1 and 21. Optical density was assessed by a digital densitometer (PD-504; Macbeth, London, UK) at a perimeter of 1mm around the following points (Figure 1):

Point 1: on the premaxilla between the jaw bone and lingual surfaces of the upper incisors, superior to the Bu point.

Point 2: on the hard palate between the maxillary bone and the mesial surface of the upper first molar intersection, anterior to Mu point.

Point 3 (Po point): on the skull, the most posterior point of the cranium.

Point 4 (K Point): on the mandible, the intersection between the Gonion-Menton and the line perpendicular to the Gonion-Menton through Gnathion [35].

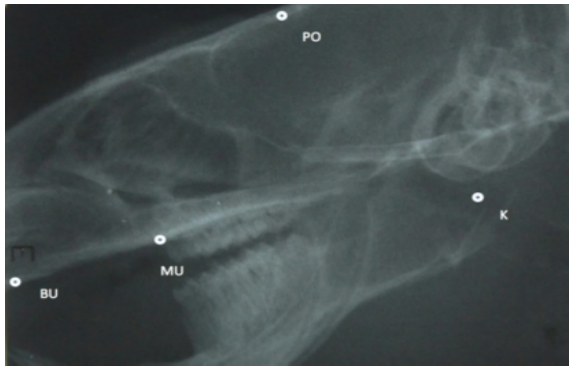

Figure 1: Four points that were assessed in densitometry

Histological evaluation:

The maxillae were placed in 10% formalin for duration of five days followed by immersion in 5% formic acid for approximately seven days. After decalcification, the left hemimaxillae were subjected to routine histopathological processing. Five micrometer sections were obtained from the paraffin blocks in a mesiodistal plane, which were stained with hematoxylin and eosin. Histological analysis was performed on the mesial roots of the first molars. The section containing the largest root area from the cementoenamel junction to the bone-containing apical area from each animal was evaluated under a light microscope (BX-51; Olympus Co., Tokyo, Japan) equipped with a digital camera (DP25; Olympus) and analysis software (DP2-BSW; Olympus). Histological variables were selected according to a previous study [34] and included width, depth and number of resorption lacunae in dentin/cementum, periodontal ligament (PDL) width and osteoclast number. The distance between crater edges and the distance between the deepest crater points to the virtual root surface, constituted lacunae width and depth, respectively.

Statistical analysis:

One-way ANOVA was used for multiple comparisons and to evaluate the differences

between groups A, B and C; when differences were significant, post hoc test was applied for pairwise comparisons between groups. SPSS version 22 was used for statistical analysis and processing of data (SPSS Inc., IL, USA). P<0.05 was considered significant

Results

The mean (± standard deviation) tooth movement in groups A, B and C was 0.62 (0.085), 0.3178 (0.079) and 0.26 (0.047), respectively. A significant increase in tooth movement was observed in the captopril group as compared to the other two groups (P=0.005).

Densitometric findings are documented in Table 1. There were no significant differences between captopril and saline groups in points 1, 2 and 3 (P>0.05), but bone density significantly decreased in captopril versus saline group in point 4. (P=0.022)

No significant differences in osteoclast counts were observed among the captopril, saline and control groups (P>0.05). Similarly, we did not find statistically significant differences in depth or length of lacunae (P>0.05). Osteoclasts and resorption lacunae are presented in Figure 2.

There was no significant difference in PDL width in any region except for the mesioapical aspect, which was significantly wider in the captopril group compared to the saline group. (P=0.044)

Table 1. Optical density (standard deviation) in the 4 main- and 4 sub-points of the three study groups

Groups	Point 1	Point 2	Point 3	Point 4	Point 1F	Point 2F	Point 3F	Point 4F
A	1.69(0.12)	1.38(0.16)	1.67(0.09)	1.66(0.09)	1.98(0.58)	1.56(0.19)	1.94(0.13)	1.98(0.15)
B	1.69(0.11)	1.38(0.22)	1.63(0.15)	1.66(0.16)	1.86(0.11)	1.37(0.14)	1.76(0.15)	1.75(0.24)
C	1.64(0.16)	1.32(0.19)	1.60(0.13)	1.59(0.13)	1.85(0.14)	1.41(0.16)	1.82(0.12)	1.83(0.15)

Point 1: Initial optical density of alveolar bone, D1F: Final optical density of alveolar bone

Point 2: Initial optical density of hard palate, D2F: Final optical density of hard palate

Point 3: Initial optical density of skull, D3F: Final optical density of skull

Point 4: Initial optical density of mandible, D4F: Final optical density of mandible

Point 1F: Final optical density of alveolar bone

Point 2F: Final optical density of hard palate

Point 3F: Final optical density of skull

Point 4F: Final optical density of mandible

Discussion

The effect of various drugs on OTM has been previously documented [4]. This could be clinically useful because some medications may impact on the rate of OTM and subsequently the duration of treatment [36]. In this study, the effect of captopril on OTM was investigated in rats.

Captopril is an ACE inhibitor that blocks the vasoconstriction function of angiotensin II through the RAS and is one of the most widely used antihypertensive drugs [33].

Bone is among the tissues that are locally impacted by RAS. This effect is mainly mediated by AT1 and AT2 receptors [25,26,37]. Additionally, considering the important role that blood flow plays in bone remodeling, this tissue can be affected through the regulatory actions of ACE inhibitors on blood flow. The bradykinin-nitric oxide pathway has a major part in this regulation [25] and has been shown to influence OTM.

In the present study, statistical analysis indicated that the rate of tooth movement was significantly higher in the captopril-treated group compared to the control and saline groups. As expected, saline and control animals showed no significant

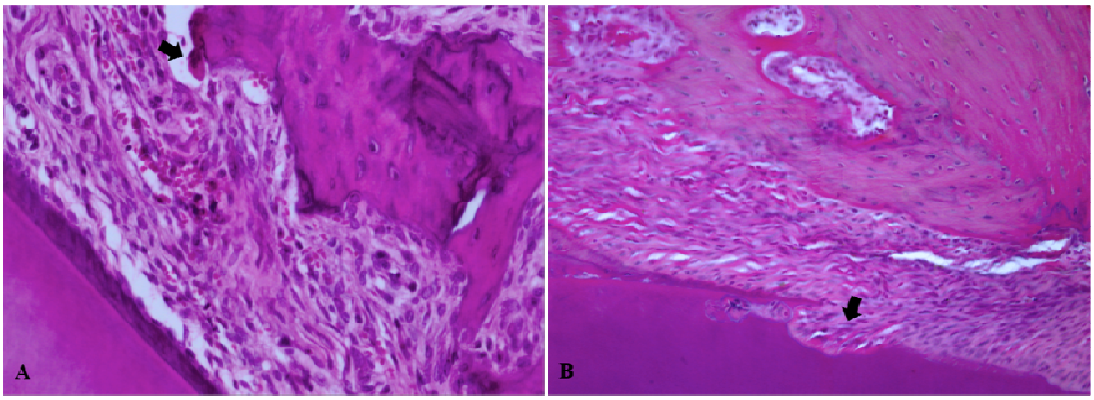

Figure 2. Representative images of hematoxylin/eosin-stained tissue samples depicting an osteoclast in micrograph A (original magnification×400) and root resorption demonstrated by a resorption lacuna in micrograph B (original magnification ×200)

difference in the rate of tooth movement. The densitometric findings of the current investigation showed a significant decrease in mandibular bone density at point 4 from day one to 21, which confirms our OTM results. The fact that this specific point achieved significance could be explained by the fact that the lower jaw has been suggested to be more susceptible to drug-induced bone loss [35].

We were not able to find a previous study in the English literature that evaluated the effect of captopril on OTM, which limits a thorough comparison of our findings with former research.

The reports of ACE inhibitors on osseous tissues indicate both resorptive and promoting effects on bone density. In contrast to our findings, some studies have shown a relation between ACE inhibitor treatment and reduced risk of bone fractures [38,39] along with increased bone mineral density [38-40]. It has been suggested that individuals who have less ACE activity genetically show greater bone mineral density compared to those with higher ACE activity [41,42]. ACE inhibition by perindopril was demonstrated to promote the healing of bone fracture [15]. Also, inhibition of AT1 receptor by losartan resulted in increased bone strength and trabeculae in the femur of ovariectomized rats [43]. Shimizu et al. [25] reported inhibition of osteoporosis following deletion and blocking of AT1 receptors.

On the other hand, a number of studies similar to the current investigation, have shown negative effects of ACE inhibitors on bone. Application of ACE inhibitors in a previous study caused underdeveloped calvarium in human fetuses [44]. It was shown that perindopril significantly decreased the BMD of rat's femur [15]. Also, ACE inhibitors have been suggested to exert an increasing effect on bone loss [30].

Contrary to our assumption, we did not find a significant difference in osteoclast count between the captopril and control groups. It is noteworthy that the results obtained in the present study only reflect the number of osteoclasts, while the number and function of these cells may not be necessarily related [45], i.e. captopril may merely have increased the function of osteoclasts without impacting on their number. Liu et al. [33] demonstrated positive influence of captopril on osteoblasts; while they did not find a significant difference in "osteoclast surface" following captopril administration and proposed that the mechanism of action of this drug may be unrelated to resorption. Various cellular-molecular interactions, especially osteoblastic-osteoclastic coupling may be involved in the results obtained in the present study. Further investigation is suggested in order to help clarify the cellular events that take place following captopril application.

At the end, it should be emphasized that duration of force application, dosage of drug, bone structure and force level in rats are different from humans; thus, the results of animal studies should be generalized to human subjects with caution [34]

Conclusion

The present study showed that captopril administration increased tooth movement in rats, which was corroborated by our densitometric findings demonstrating subtractive effects of this drug on the mandibular bone. Further studies are suggested to elucidate the molecular events behind the observed clinical effects of captopril and its possible impact on human subjects.

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References

1. Krishnan V, Davidovitch Z. Cellular, molecular and tissue-level reactions to orthodontic force. Am J Orthod Dentofacial Orthop. 2006 Apr; 129(4): 469. e1-32.
2. Meikle MC. The tissue, cellular, and molecular regulation of orthodontic tooth movement: 100 years after Carl Sandstedt. Eur J Orthod. 2006 Jun; 28(3):221-40.
3. Sandy JR, Farndale RW, Meikle MC. Recent advances in understanding mechanically induced bone remodeling and their relevance to orthodontic theory and practice. Am J Orthod Dentofacial Orthop. 1993 Mar;103(3):212-22.
4. Krishnan V, Davidovitch Z. The effect of drugs on orthodontic tooth movement. Orthod Craniofac Res. 2006 Nov;9(4):163-71.
5. Lawes CM, Vander Hoorn S, Rodgres A. Global burden of blood-pressure related disease, 2001. Lancet. 2008 May;371(9623):1513-18.
6. Singh GM, Danaei G, Farzadfar F, Stevens GA, Woodward M, Wormser D, et al. The age –specific quantitative effect of metabolic risk factors on cardiovascular diseases and diabetes: a pooled analysis. PLOS One. 2013 Jul;8(7):e65174.
7. SOLVD Investigators, Yusuf S, Pitt B, Davis CE, Hood WB, Cohn JN. Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure. N Engl J Med. 1991 Aug;325(5):293-302.
8. Solomon SD, Skali H, Anavekar NS, Bourgoun M, Barvik S, Ghali JK, et al. Changes in ventricular size and function in patients treated with valsartan, captopril, or both after myocardial infarction. Circulation. 2005 Jun;111(25):3411-9.
9. Paul M, PoyanMehr A, Kreutz R. Physiology of local renin-angiotensin systems. Physiol Rev. 2006 Jul;86(3):747-803.
10. Phillips MI. Functions of angiotensin in the central nervous system. Annu Rev Physiol. 1987; 49:413-35.
11. Steckelings UM, Bottari SP, Unger T. Angiotensin receptor subtypes in the brain. Trends Pharmacol Sci. 1992 Sep;13(9):365-8.
12. Aceto JF, Baker KM. Angiotensin II receptor-mediated stimulation of protein synthesis in chick heart cells. Am J Physiol. 1990 Mar;258(3 Pt 2):806-13.
13. Millan MA, Jacobowitz DM, Aguilera G, Catt KJ. Differential distribution of AT1 and AT2 angiotensin II receptor subtypes in the rat brain during development. Proc Natl Acad Sci USA. 1991 Dec;88(24):11440-4.
14. Guide to Receptors and Channels (GRAC). 4th edition. Br J Pharmacol. 2009 Nov;158 Suppl 1: S1-254.
15. Garcia P, Schwenzer S, Slotta JE, Scheuer C, Tami AE, Holstein JH, et al. Inhibition of angiotensin-converting enzyme stimulates fracture healing and periosteal callus formation-role of a local renin-angiotensin system. Br J Pharmacol. 2010 Apr;159(8):1672-80.
16. Schurman SJ, Bergstrom WH, Shoemaker LR, Welch TR. Angiotensin II reduces calcium uptake into bone. Pediatr Nephrol. 2004 Jan;19(1):33-5.
17. Hagiwara H, Hiruma Y, Inoue A, Yamaguchi A, Hirose S. Deceleration by angiotensin II of the differentiation and bone formation of rat calvarial osteoblastic cells. J Endocrinol. 1998 Mar; 156(3): 543-50.
18. Hiruma Y, Inoue A, Hirose S, Hagiwara H. angiotensin II stimulates the proliferation of osteoblast–rich populations of cells from rat calvariae. Biochem Biophys Res Commun. 1997 Jan; 230(1):176-8
19. Hatton R, Stimpel M, Chambers TJ. Angiotensin II is generated from angiotensin I by

bone cells and stimulates osteoclastic bone resorption in vitro. J Endocrinol. 1997 Jan; 152(1): 5-10.
20. Sacchetti B, Funari A, Michienzi S, Cesare S, Piersanti S, Saggio I, et al. Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. Cell. 2007 Oct;131(2):324-36
21. Sanada M, Taguchic A, Higashi Y, Tsuda M, Kodama I, Yoshizumi M, et al. Forearm endothelial function and bone mineral loss in postmenopausal women. Atherosclerosis. 2004 Oct;176(2):387-92.
22. Lynn H, Kwok T, Wong SY, Woo J, Leung PC. Angiotensin converting enzyme inhibitor use is associated with higher bone mineral density in elderly Chinese. Bone. 2006 Apr;38(4):584-8.
23. Lavoie JL, Sigmund CD. Minireview: overview of the renin-angiotensin system-an endocrine and paracrine system. Endocrinology. 2003 Jun;144(6):2179-83.
24. Sakai K, Agassandian K, Morimoto S, Sinnayah P, Cassell MD, Davisson RL, et al. Local production of angiotensin II in the subfornical organ causes elevated drinking. J Clin Invest. 2007 Apr;117(4):1088-95.
25. Shimizu H, Nakagami H, Osako M, Hanayama R, Kunugiza Y, Kizawa T, et al. Angiotensin II accelerates osteoporosis by activating osteoclasts. FASEB J. 2008 Jul;22(7):2465-75.
26. Izu Y, Mizoguchi F, Kawamata A, Hayata T, Nakamoto T, Nakashima K, et al. Angiotensin II type 2 receptor blockade increases bone mass. J Biol Chem. 2009 Feb; 284(8):4857-64.
27. Gebru Y, Diao TY, Pan H, Mukwaya E, Zhang Y. Potential of RAS Inhibition to Improve Metabolic Bone Disorders. Biomed Res Int. 2013 Jul; 2013:932691.
28. Yang M, Xia C, Song Y, Zhao X, Wong MS, Zhang Y. Impairing effects of angiotensin-converting enzyme inhibitor Captopril on bone of normal mice. Eur J Pharmacol. 2016 Jan;771:40-7.
29. Zhang Y, Wang L, Song Y, Zhao X, Wong MS, Zhang W. Renin inhibitor aliskiren exerts beneficial effect on trabecular bone by regulating skeletal renin-angiotensin system and kallikrein-kinin system in ovariectomized mice. Osteoporos Int. 2016 Mar;27(3):1083-92.
30. Kwok T, Leung J, Zhang YF, Bauer D, Ensrud KE, Barrett-Connor E, et al. Does the use of ACE inhibitors or angiotensin receptor blockers affect bone loss in older men? Osteoporos Int. 2012 Aug;23(8):2159-67.
31. Diao TY, Pan H, Gu SS, Chen X, Zhang FY, Wong MS, et al. Effects of angiotensin-converting enzyme inhibitor, captopril, on bone of mice with streptozotocin-induced type 1 diabetes. J Bone Miner Metab. 2014 May;32(3):261-70.
32. Zhang YF, Qin L, Leung PC, Kwok TC. The effect of angiotensin-converting enzyme inhibitor use on bone loss in elderly Chinese. J Bone Miner Metab. 2012;30:666-73.
33. Liu YY, Yao WM, Wu T, Xu BL, Chen F, Cui L. Captopril improves osteopenia in ovariectomized rats and promotes bone formation in osteoblasts. J Bone Miner Metab. 2011 Mar; 29 (2):149-58.
34. Mirhashemi AH, Ahmad Akhoundi MS, Sheikhzadeh S, Momeni N, Dehpour A, Alaeddini M, et al. Effect of fluoxetine consumption on orthodontic tooth movement in rats. J Dent (Tehran). 2015 Dec;12(12):882-9.
35. Talaeipour AR, Shirazi M, Kheirandish Y, Delrobaie A, Jafari F, Dehpour AR. Densitometric evaluation of skull and jaw bones after administration of thyroid hormones in rats. Dentomaxillofac Radiol. 2005 Nov;34(6):332-6.
36. Bartzela T, Türp JC, Motschall E, Maltha JC. Medication effects on the rate of orthodontic tooth movement: a systematic literature review. Am J Orthod Dentofacial Orthop. 2009 Jan;135(1):16-26.
37. Wiens M, Etminan M, Gill SS, Takkouche B. Effects of antihypertensive drug treatments on fracture outcomes: a meta analysis of observational studies. J Intern Med. 2006 Oct;260(4):350-62.
38. Nakagami H, Osako MK, Shimizu H, Hanayama R, Morishita R. Potential contribution of action of renin angiotensin system to bone metabolism. Curr Hypertens Rev. 2007 May; 3(2): 129-32.
39. Rejnmark L, Vestergaard P, Mosekilde L. Treatment with beta-blockers, ACE inhibitors, and calcium-channel blockers is associated with a reduced fracture risk: a nationwide case-control study. J Hypertens 2006 Mar;24(3):581-9.

40. Perez-Castrillon JL, Silva J, Justo I, Sanz A, Martin-Luquero M, Igea R, et al. Effect of quinapril, quinapril-hydrochlorothiazide, and enalapril on the bone mass of hypertensive subjects: relationship with angiotensin converting enzyme polymorphisms. Am J Hypertens. 2003 Jun;16(6):453–9.
41. Perez-Castrillon JL, Justo I, Silva J, Sanz A, Martin-Escudero JC, Igea R, et al. Relationship between bone mineral density and angiotensin converting enzyme polymorphism in hypertensive postmenopausal women. Am J Hypertens. 2003 Mar;16 (3):233-5.
42. Woods D, Onambele G, Woledge R, Skelton D, Bruce S, Humphries SE, et al. Angiotensin-I converting enzyme genotype-dependent benefit from hormone replacement therapy in isometric muscle strength and bone mineral density. J Clin Endocrinol Metab. 2001 May;86(5):2200-4.
43. Stahl RA, Paravicini M, Schollmeyer P. Angiotensin II stimulation of prostaglandin E2 and 6-keto-F1α formation by isolated human glomeruli. Kidney Int,1984 Jul;26(1):30-4.
44. Barr MJ, Cohen MMJ. ACE inhibitor fetopathy and hypocalvaria: the kidney–skull connection. Teratology. 1991 Nov;44(5):485-95.
45. Henriksen K, Tanko LB, Qvist P, Delmas PD, Christiansen C, Karsdal MA. Assessment of osteoclast number and function: application in the development of new and improved treatment modalities for bone diseases. Osteoporos Int. 2007 May;18(5):681-5.