

Effect of Cigarette Smoking on Bone Mineral Density among healthy Men

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Abstract: Osteoporosis is a skeletal disorder characterized by low bone mass and loss of bone tissue that may lead to weak and fragile bone. It is affected by numerous factors, including age, dietary factors, lack of exercise, menopause, underweight, excessive alcohol consumption and cigarette smoking. The aim of the present study is to investigate the influence of cigarette smoking on Bone mineral Density (BMD) and examine the relation between cigarette smoking and vitamin D among men. A total of ninety-six males, aged between 32–50 years, were recruited and divided into 4 groups according to their smoking status: non smoker (n=26), light smoker (n=18), moderate smoker (n=26) and heavy smoker (n=26). Participants visited the Center of Excellence for Osteoporosis Research (CEOR) at King Abdul Aziz University and completed a questionnaire about medical history, lifestyle and smoking habits. Written informed consent was obtained from all participating men. Bone density was measured using dual-energy X ray absorptiometry (DXA) at three sites, the lumbar spine (L1-L4), femur neck and total hip. Serum Ca, PO₄, Mg, iPTH, 25-OHD₃, OC and CTx were measured. We found that bone mineral density at femur neck was significantly lower in light and heavy smoker groups compared to non smoker group (P< 0.05). BMD were positively correlated with body mass index (P<0.01) and waist to hip ratio (P<0.01) in heavy smoker group and negatively correlate with number of years smoked (P< 0.05). Nearly 69% of our study population had low serum 25-OHD levels (<25nmol/L). In the heavy smoker group, 80% had vitamin D deficiency (<25 nmol/L) compared with 46% in the non-smoker group. There were a significant negative correlation between serum 25-OHD and number of cigarettes smoked daily (P< 0.05) and PTH level (p<0.01) in heavy smoker group. Smoking was associated with reduced BMD in the heavy smoker group and the smoking duration (years) had a stronger effect on BMD than the number of cigarettes smoked daily.

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1. Introduction

Cigarette smoking is one of the main issues influencing the wellbeing of humans. It is one of the major public health problems in Saudi Arabia and it is increasing at an alarming rate [1]. Smoking is thought to cause low bone density through various pathways. The direct action of smoking on bone cells have not been clearly defined, both stimulation and inhibition of osteoblast formation and activity being reported [2]. Smoking is associated with increased concentrations of free radicals, which may affect bone resorption. Nicotine and the free radicals released act to inhibit the cellular metabolism of osteoblasts and reduce collagen synthesis [3]. There is other finding that nicotine is also toxic to calcitonin [4]. The previous study showed that, increase bone resorption in current smokers may suggest indirectly that osteoblasts are damaged in smokers. Tobacco and smoke constituents may induce the apoptosis of different cells [5]. Smoking damages the endothelial cells of the blood vessels and smokers are more likely

to suffer from peripheral vascular disease, reducing blood supply to the bones [6].

Smoking has been linked to changes in hormones secretion, leading to a decrease PTH level (thus reducing calcium absorption) and estrogen levels as well as to an increase in the level of cortisol and adrenal androgens, changes that have been linked to an increased risk of osteoporosis [7]. The previous studies showed that calcium absorption was lower in smokers as compared with non-smokers and this is attributed to the lower PTH and serum calcitriol levels seen in smokers [8]. The impairment of calcium absorption results in accelerated bone loss [7]. Smoking reduces the level of vitamin D in the body, which is required for good bone health, lower 25-OHD levels in smokers compared with non-smokers have been reported in several studies, but the mechanism of this association is unclear [9]. One possible explanation may be enhanced hepatic metabolism of vitamin D metabolites following induction of liver enzymes due to smoking [10].

Since cigarette smoking has been implicated as a risk factor for osteoporosis, however, most studies of the relationship between smoking and osteoporosis have been carried out in postmenopausal women and there is little information regarding the effect of smoking on bone in men, so this study was designed to investigate the influence of cigarette smoking on BMD and to examine the relation between cigarette smoking and vitamin D among men.

2. Subjects and Methods

A total of 96 volunteers males who visited Centre of Excellence for Osteoporosis Research, King Abdul Aziz university, Jeddah, Saudi Arabia, aged between 32–50 years, were divided into 4 groups according to their smoking situations: non smoker (n=26), light smoker (n=18), moderate smoker (n=26) and heavy smoker (n=26). The heavy smoker group was composed of subjects who smoked over 20 cigarettes a day. The moderate smoker group was composed of subjects who smoked 11–20 cigarettes a day. The light smoker group was composed of subjects who smoked under 10 cigarettes a day [11].

All subjects were clinically examined and completed a validated standardized (CEOR) questionnaire. A questionnaire covering data on Age, bodyweight, height, body mass index (BMI), waist to hip ratio (WHR), medical history, life style, smoking habits, and the use of vitamins and medications. For each subject, the biochemical markers for liver, kidney, and some endocrinal glands functions were investigated to confirm that any individual incorporated in this study was healthy. Men who are suffering from diabetes mellitus, chronic disorders of liver and kidney were excluded from this study. Men who receiving calcium/vitamin supplements were also excluded.

Bone Mineral Density measurements (BMD)

Bone mineral density of the anteroposterior lumbar spine (L1-L4), mean of right and left femoral neck and mean of right and left total hip were determined by using dual-energy X-ray absorptiometry (DXA) using (LUNAR Prodigy Model, USA). Based on WHO criteria all subjects with T-score < -2.5 were diagnosed as osteoporotic patients, while T-score between -1 and -2.5 classified as having osteopenia and a T-score > -1 is considered normal.

Biochemical parameters measurement

Fasting blood and urine samples were collected under standardized conditions. Serum and urine samples were stored at -80°C within 30 min

after centrifugation at $3000\times g$ for 10 min until analysis. Serum Ca, Mg and PO_4 were measured using VITROS 250 Chemistry System Autoanalyzer (Ortho-Clinical Diagnostics — Johnson & Johnson Co., USA). Serum 25(OH)D and intact PTH were measured by direct competitive and a direct sandwich chemiluminescence immunoassays using LIASON autoanalyzer, respectively (DiaSorin Inc, Stillwater, MN, USA). The serum bone turnover markers including osteocalcin (OC) and Carboxy terminal cross-linking telopeptide of type I collagen (CTX) were measured using Elecsys and cobas immunoassay analyzer (Roche Diagnostics GmbH, D-68298 Mannheim, Germany).

Statistical analysis

Analyses were performed with PASW (Predictive Analytics Software version 18.0 for Windows, 2009). ANOVA analysis (One-Way Analysis of Variance) was carried out to identify any significant differences among the 4 groups: non-smoker, light smoker, moderate smoker and heavy smoker for different variables. Bonferroni Post Hoc test was used when significance tests were made between groups. Result that was not normally distributed was analysis by nonparametric test. Associations between continuous variables were examined using Pearson correlation coefficients.

3. Results

The study was carried out on ninety six healthy men classified into four groups according to the smoking status. Groups were non-smoker, light smoker, moderate smoker, and heavy smoker. Table 1 represent a comparison of age, BMI, WHR, cigarette duration, BMD values at the lumbar spine (L1-L4), femoral neck and total hip, s-Ca, s-Mg, s- PO_4 , s-iPTH, s-25-OHD₃, s-OC and s-CTx between groups. A significant difference was found in WHR between groups, moderate smoker group showed a significant decrease in WHR compared to non smoker group ($P < 0.05$). A statistical significant difference was found between the studied groups as regard to BMD at femur neck. By using the bonferroni post hoc test, BMD at femur neck BMD was significantly lower in light and heavy smoker groups compared to non-smoker group ($P < 0.05$). No significant differences were found in age, waist, BMI, s-Ca, s-Mg, s- PO_4 , s-iPTH, s-25-OHD₃, s-OC and s-CTx among the groups.

In heavy smoker group a significant positive correlation was found between BMI and BMD at lumbar spine (L1-L4) ($r=0.739$, $P < 0.01$), femur neck ($r=0.777$, $P < 0.01$) and total hip ($r=0.825$, $P < 0.01$). Also significant positive correlation was found between WHR and BMD at

lumbar spine (L1-L4) ($r=0.460^*$, $P<0.05$), femur neck ($r=0.512^{**}$, $P<0.01$) and total hip ($r=0.534^{**}$, $P<0.01$).

Table 2 demonstrates the relationship between smoking and BMD values at the lumbar spine (L1-L4), femur neck and total hip. By using person correlation, a significant negative relation was found between BMD at lumbar spine (L1-L4) and total hip with numbers of years smoked. No statistical significant relation was found between number of cigarettes smoked daily and measurements of BMD at three sites. A significant negative correlation ($r= -0.404^*$, $P<0.05$) was found between number of cigarette smoked daily and 25-OHD in heavy smoker group. A significant negative correlation were found between s-PTH

level and serum 25-OHD₃ level in non smoker ($r=-0.541^{***}$, $P<0.01$) and heavy smoker group (-0.599^{**} , $P<0.01$).

As shown in Table 3 adequate serum 25(OH) D levels (> 75 nmol/L) were 7.7% of non smoker group and 3.8% of moderate smoker group. Vitamin D insufficiency (≥ 50 to ≤ 75 nmol/L) was 3.8% in non smoker, moderate smoker and heavy smoker group. Moderate to severe vitamin D deficiency as defined by serum 25(OH) D levels < 25 nmol/L was observe in all groups. We perceive that 80.8% of heavy smoker group, 73% of moderate smoker group, 66.7% of light smoker group and 46% of non smoker had moderate to severe vitamin D deficiency.

Table 1: Comparison of age, BMI, waist, WHR, cigarette duration, BMD values at the lumbar spine (L1-L4), femoral neck and total hip, s-Ca, s- Mg, s- PO₄, s-iPTH, s-25-OHD₃, s-OC and s-CTX in non smoker, light smoker, moderate smoker and heavy smoker groups

Variables	Non smoker (n=26)	Light smoker (<10 Cig/day)	Moderate smoker(11-20 Cig/day)	Heavy smoker (> 20 Cig/day)	ANOVA (P-value)
Age	40.46 ± 4.66	37.83 ± 4.58	39.12 ± 4.29	39.31 ± 5.48	0.396
Waist(cm)	96.84 ± 11.56	93.00 ± 9.64	86.88 ± 15.98	95.42 ± 19.45	0.095
WHR	0.91 ± 0.06	0.89 ± 0.05	0.84 ± 0.12	0.92 ± 0.12	0.025
Smoking duration	0	13.78 ± 5.02	17.77 ± 7.12	17.07 ± 6.22	0.157
Femur neck BMD(g/cm ²)	1.07 ± 0.11	0.96 ± 0.23	1.00 ± 0.11	0.97 ± 0.12	0.041
Total hip BMD(g/cm ²)	1.08 ± 0.10	1.04 ± 0.12	0.99 ± 0.22	1.00 ± 0.14	0.120
s-Ca (mmol/L)	2.56 ± 0.24	2.61 ± 0.50	2.49 ± 0.17	2.51 ± 0.19	0.489
s - Mg (mmol/L)	0.78 ± 0.065	0.77 ± 0.06	0.78 ± 0.088	0.78 ± 0.06	0.857
s -PO ₄ (mmol/L)	1.22 ± 0.18	1.24 ± 0.22	1.26 ± 0.22	1.32 ± 0.16	0.238
s-iPTH (pmol/L)	9.08 ± 4.36	7.79 ± 4.22	8.14 ± 3.57	7.65 ± 3.31	0.560
s-25-OHD ₃ (nmol/L)	27.16 ± 19.12	23.79 ± 9.98	23.84 ± 17.04	18.41 ± 10.46	0.225
s-OC (ng/ml)	18.71 ± 5.77	20.75 ± 6.7	23.63 ± 7.31	20.79 ± 6.84	0.072
s-CTX (pg/ml)	427.9 ± 194.6	462.59 ± 200.2	455.7 ± 168.74	406.83 ± 191.45	0.723

Data are presented as mean ± SD

P value is significant at < 0.05 , Ca(calcium),Mg(magnesium),PO₄(inorganic phosphate) PTH (parathyroid hormone), 25-OHD₃(25- hydroxyvitamin D),OC(Osteocalcin) and CTX(Carboxy-terminal cross-linking telopeptide of type I collagen). n= number of subjects

Table 2 Correlation between bone mineral density and smoking

	(L1-L4) BMD	T-score	Femur (neck) BMD	T-score	Total hip BMD	T-score
Cigarette Smoked (no/day)	-0.002-	-0.167-	0.080	-0.075-	-0.047-	0.002
SmokingDuration (Year)	-0.181-	-0.248-*	-0.099-	-0.007-	-0.267-*	-0.167-

*. Correlation is significant at $P<0.05$ no/day (number of cigarette smoked per day)

Table 3. Percentage values of serum 25-OHD according to smoking status.

Serum 25-OHD level (cut offs) (nmol/L)	Non smoker (n=26)	Light smoker (<10 Cig/day) (n=18)	Moderate smoker (11-20 Cig/day) (n=26)	Heavy smoker (>20 Cig/day) (n=26)
<12.5	11.5% (3/26)	16.7% (3/18)	11.5% (3/26)	38.5% (10/26)
12.5-24.9	34.6% (9/26)	50.0% (9/18)	61.5% (16/26)	42.3% (11/26)
25 - 49.9	42.3% (11/26)	33.3% (6/18)	19.2% (5/26)	15.4% (4/26)
50-74.9	3.8% (1/26)	—	3.8% (1/26)	3.8% (1/26)
>75	7.7% (2/26)	—	3.8% (1/26)	—

4. Discussion

Cigarette smoking was first identified as a risk factor for osteoporosis more than 20 years ago [9]. In this study we assessed the BMD in 96 men, classified into 4 groups non, light, moderate and heavy cigarette smokers. The prevalence of osteopenia in the study groups at the lumbar spine was 5.6% in light smoker, 7.7% in moderate and heavy smoker groups. Similarly at the femur neck site, osteopenia was observed in 5.6% in light smokers, 7.7% in moderate smokers and 15.4% in heavy smokers, respectively. In heavy smoker group 3.8% had osteoporosis as indicated by lumbar spine (L1-L4) and total hip but in non smoker group the BMD values were normal at three sites. A statistical significant difference was found as regard to BMD at femur neck. Light and heavy smoker group had a significantly lower femur neck BMD compared to non smoker group. In agreement with our results, previous study reported that smokers had significantly lower BMD than non-smokers [13]. Other study reported that BMD measurements of the lumbar vertebra in male smokers were significantly lower than those in non-smokers [14]. Recent study were found that smoking was a negative predictor for BMD among males [15]. In contrast to our finding, the study that evaluated the BMD in 161 subjects which they divided into 4 groups according to their smoking classes: non-smoker, light, moderate and heavy smokers, they found that BMD values were not significantly different among the groups [11]. Thus, the disagreement of these results may be due to the characteristics of the study population where they were young adult males who have a relatively short history of cigarette smoking. In this study, a significant negative relation were found between number of years smoked and BMD values at lumbar spine (L1-L4) and total hip but no statistical significant relations were found between numbers of cigarettes smoked daily and BMD. This is in agreement with previous study in which they noted that, for BMD values at the hip in smokers of both genders there was a significant negative relation with numbers of years smoked [16]. Smoking could

reduce BMD either through changes in body mass or by direct effects on the skeleton [17]. However, we found a significant positive relationship between BMD at lumbar spine (L1-L4), femur neck, total hip and BMI in heavy smoker group which have a high percentage of normal BMI (23.3%) than other groups. The osteoporotic participant was in heavy smoker group and had lower BMI. Smokers tend to be thinner and have lower body fat compared with non-smokers. The mechanism for this association remains unclear, but appetite suppression may be responsible [3]. This is in agreement with other study in which they found that smokers have lower body fat compared with non-smokers [18]. The lower body weight in smokers compared with nonsmokers was also found in previous study [17]. In a study on Moroccan men low BMI scores were associated with BMD loss and this association was stronger with hip BMD than with lumbar spine [19]. Recent study showed that the changes in BMI was positively correlated with percent changes in BMD at the spine, total hip and femoral neck in elderly Lebanese men and women [20].

In epidemiologic studies, the WHR is widely used as an index of regional adipose tissue distribution [21]. There are few reports about the influence of WHR on BMD in men. In our study a significant positive correlation was found between BMD and WHR in light and heavy smoker groups. Men with highest WHR tended to have higher BMD. Various studies indicate that fat mass may have beneficial effects on increasing bone mass [21]. In agreement to our results, a study showed that abdominal fat weight and WHR were positively and significantly associated with bone mass [21]. It's been reported that WHR was inversely related to bone mass in Polish men [22]. These conflicting results may be partially due to differences related to sex, sample size, study design and analysis methods, and population structure.

In the present study, high prevalence of vitamin D deficiency as defined by serum 25-OHD levels <50 nmol/L (<20 ng/ml), was common in Saudi men. Nearly 93.8% of our study population

had low serum 25-OHD levels (<50nmol/L). The main risk factors for vitamin D deficiency were obesity, poor sun exposure, higher WHR [23]. Furthermore, sedentary lifestyle, smoking, and inadequate vitamin D supplementation are independent predictors of severe to moderate vitamin D deficiency [24]. In our study, Serum 25-OHD levels were lower in heavy smokers compared with non-smokers, 80.8% of heavy smokers had vitamin D deficiency (<25nmol/L) compared with 46% in non-smokers. A significant negative relation was found between numbers of cigarettes smoked daily and 25-OHD in heavy smoker group. The present results are in agreement with a study in which they found that serum 25-OHD levels correlated inversely to number of cigarettes currently smoked per day [17]. Earlier study showed that; the current smokers had lowered 25-OHD concentrations than never smokers [18]. Similar results have been previously described in smoker [13]. The former study have been reported that smoking has a significant effect on vitamin D metabolism and reduces serum levels of 25-OHD. However, the mechanisms underlying low 25-OHD levels in smokers are not clear [9]. Increased activity of liver enzymes induced by cigarette smoking is one potential explanation for the reduced vitamin D level [17].

Vitamin D is an important determinant of serum PTH levels [25]. A deficit of vitamin D would be expected to impair calcium absorption efficiency and lead to rise in PTH production [26]. In non-smoker 46% have vitamin D deficiency (<25nmol/L) and 61.5% have higher PTH level but in heavy smoker group 80.8% (<25nmol/L) have vitamin D deficiency and 53.3% have higher PTH level. This might be confirming our observation that there was a significant negative association of serum 25-OHD with PTH level in non- and heavy smoker groups. This inverse relationship has been described by others [24, 20]. In Saudi men, Icelandic men and Lebanese men and women respectively.

Conclusion and Recommendations

We conclude that, smoking have a negative effect on bone health. It seems to be associated with a reduced BMD at femur neck and reduced vitamin D level in heavy smoker group. We found that, the extent of the smoking effect was strongly associated with the duration of smoking than the quantity of cigarette smoked. In the future, more research is needed to understand the biologic mechanism that result in lower BMD among smoker men. The effects of smoking on sex steroid hormones, powerful determinants of bone loss are needed to be investigated in further studies. Also the sample size

must be taken into consideration as the study leading to further community based studies.

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References

1. Hashim T. Smoking habits of students in College Of Applied Medical Science. Saudi Medical Journal 2000; 21(1): 76-80.
2. Compston, J. Smoking and the Skeleton, J Clin Endocrinol Metab 2007; 92(2):428-429
3. Wong P, Christie J, Wark J. The effects of smoking on bone health. Clinical Science 2007; 113: 233-241
4. Gaston M, Simpson A. Inhibition of fracture healing. J Bone Joint Surg Br 2007; 89 (12): 1553-60.
5. Bagchi M, Balmoori J, Bagchi D, Ray S, Kuszynski C, Stohs S. Smokeless tobacco, oxidative stress, apoptosis, and antioxidants in human oral keratinocytes. Free Radical Biol Med 1999; 26: 992-1000
6. Vestergaard P, Mosekilde L. Fracture risk associated with smoking: a meta-analysis. J Intern Med 2003; 254(6):572-583.
7. Kapoor D, Jones T. Smoking and hormones in health and endocrine disorders. Eur J Endocrinol 2005; 152(4):491- 499
8. Jorde R, Saleh F, Figenschau Y, Kamycheva E, Haug E, Sundsfjord J. Serum parathyroid hormone levels in smokers and nonsmokers. The fifth Tromso study. European Journal of Endocrinology, 2004;152: 1-8.
9. Brot C, Jorgensen N, Sorensen O. The influence of smoking on vitamin D status and calcium metabolism. Euro J of Clini.Nutri 1999; 53: 920-926
10. Jones G, Scott F. A cross-sectional study of smoking and bone mineral density in premenopausal parous women: effect of body mass index, breastfeeding, and sports participation. J. Bone Miner. Res. 1999; 14: 1628-1633
11. Bae Y, Cho H, Kim M. Nutrient intake and bone health status of Korean male college

- students as related to smoking situations. *Nutrition Research and Practice* 2008; 2(3): 184-190
12. Chin J, Venner P, Lacombe L, Vinholes J, Prelevic G. Osteoporosis in men. *J. R Soc Med* 2001; 94:620-623.
 13. Supervía A, Nogués X, Enjuanes A, Vila J, Mellibovsky L, Serrano S, Aubía J, Díez-Pérez A. Effect of smoking and smoking cessation on bone mass, bone remodeling, vitamin D, PTH and sex hormones. *J Musculoskelet Neuronal Interact* 2006; 6:234-241.
 14. Lorentzon M, Mellström D, Haug E, Claes Ohlsson C. Smoking Is Associated with Lower Bone Mineral Density and Reduced Cortical Thickness in Young Men. *J Clin Endocrinol Metab* 2007; 92(2):497-503
 15. Karadavut, K., Kutsa, Y., Çakci, A., Hekimoglu, B. and Basarn, A. Predictors of Bone Mineral Density in Men. *Fýrat University Medical Journal of Health Sciences* 2011; 25 (1): 33 – 38
 16. Sneve M, Emaus N, Joakimsen R, Jorde R. The association between serum parathyroid hormone and bone mineral density, and the impact of smoking: the Tromsø Study. *Europ J of Endocrin* 2008; 158:401– 409
 17. Hermann A, Brot C, Gram J, Kolthoff N, Mosekilde L. Premenopausal smoking and bone density in 2,015 perimenopausal women. *J Bone Miner Res* 2000; 15:780-787.
 18. Szulc P, Garnero P, Claustrat B, Marchand F, Duboeuf F, Delmas P. Increased bone resorption in moderate smokers with low body weight: The Minos Study. *J Clin Endocrinol Metab* 2002; 87: 666-674.
 19. El Maghraoui A, Ghazi M, Gassim S, Ghozlani I, Mounach A, Rezqi A, Dehhaoui M. Risk factor of osteoporosis in healthy Moroccan men. *BMC Musculoskeletal Disorders* 2010;11:148
 20. Arabi A, Baddoura R, El-Rassi R, El-Hajj G. PTH level but not 25 (OH) vitamin D level predicts bone loss rates in the elderly. *Osteoporos Int* 2012; 23(3):971-80.
 21. Zhao L, Jiang H, Papasian C, Maulik D, Drees B, Hamilton J, Deng H. Correlation of Obesity and Osteoporosis: Effect of Fat Mass on the Determination of Osteoporosis. *J Bone Miner Res* 2008; 23:17–29.
 22. Jankowska E, Rogucka E, Medras M. Are general obesity and visceral adiposity in men linked to reduced bone mineral content resulting from normal ageing? A population based study. *Andrologia*. 2001; 33(6): 384-389
 23. Ardawi M, Qari M, Rouzi A, Maimani A, Raddadi R. Vitamin D status in relation to obesity, bone mineral density, bone turnover markers and vitamin D receptor genotypes in healthy Saudi pre- and postmenopausal women. *Osteoporosis Int*. 2011;22(2):463-75.
 24. Ardawi M, Sibiany A, Bakhsh T, Qari M, Maimani A. High prevalence of vitamin D deficiency among healthy Saudi Arabian men: relationship to bone mineral density, parathyroid hormone, bone turnover markers and lifestyle factors *Osteoporosis Int* 2012; 23: 675–686.
 25. Snijder M, van Dam R, Visser M, Deeg D, Dekker J, Bouter L, Seidell J, Lips P. Adiposity in relation to vitamin D status and parathyroid hormone levels: a population-based study in older men and women. *J Clin Endocrinol Metab* 2005; 90(7):4119-23.
 26. Heaney R. The Vitamin D requirement in health and disease. *J. Steroid Biochem Mol Biol* 2005; 97(1–2):13–9.

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