Research Article **Open Access**

BCHD
Bioactive Compounds in Health and Disease

Association between cadmium and lead in active and passive cigarette smokers with bone mass: a retrospective study

Mahmoud Helmy Elsaied [1], Samir Atef Farid Elmetwally [1], Mokhtar Ahmed Mokhtar Abo-Elfotoh [1], EL-Sayed Hamdey EL-Sayed Gawesh[1*], Ahmed Ibrahim Elshoura[1], Amal Mahmoud Hammad[2], Medhat Mohamed Abdelsalam Darwish[3], Magdy Yousef Elsaied[4], Ahmed Shabaan Abdelmonsef [4], Tarek M Nasrallah [5], Mohamed Abdallah Hassan[6], Nancy Shalaby[7]

[1]Department of Forensic Medicine and Clinical Toxicology, Damietta Faculty of Medicine, Al-Azhar University, Damietta, Egypt; [2]Department of Medical Biochemistry, Damietta Faculty of Medicine, Al-Azhar University, Damietta, Egypt; [3] Department of Medical Biochemistry, Damietta Faculty of Medicine, Al-Azhar University, Damietta, Egypt; [4]Department of Physiology, Damietta Faculty of Medicine, Al-Azhar University, Damietta, Egypt; [5]Department of Rheumatology, Physical Medicine and Rehabilitation, Damietta Faculty of Medicine, Al-Azhar University, Damietta, Egypt; [6]Department of Orthopedic Surgery, Damietta Faculty of Medicine, Al-Azhar University, Damietta, Egypt; [7]Department of Forensic Medicine and Clinical Toxicology, Damietta Faculty of Medicine, Damietta University, Damietta, Egypt

***Corresponding Author:** EL-Sayed Hamdey EL-Sayed Gawesh, Department of Forensic Medicine and Clinical Toxicology, Damietta Faculty of Medicine, Al-Azhar University, Al-Kafrawi St, Damietta, 13759, Egypt

Submission Date: March 26th, 2023; **Acceptance Date:** May 10th, 2023; **Publication Date:** May 11th, 2023

Please cite this article as: Elsaied M. H., Elmetwally S. A. F., Abo-Elfotoh M. A. M., Gawesh E. H. E., Elshoura A. I., Hammad A. M., Darwish M. M. A., Elsaied M. Y., Abdelmonsef A. S., Nasrallah T. M. Hassan M. A., Shalaby N. Association between Cadmium and Lead in Active and Passive Cigarette Smokers with Bone Mass: A retrospective Study. *Bioactive Compounds in Health and Disease* 2023; 6(5): 86-96. DOI: https://www.doi.org/10.31989/bchd.v6i5.1095

ABSTRACT

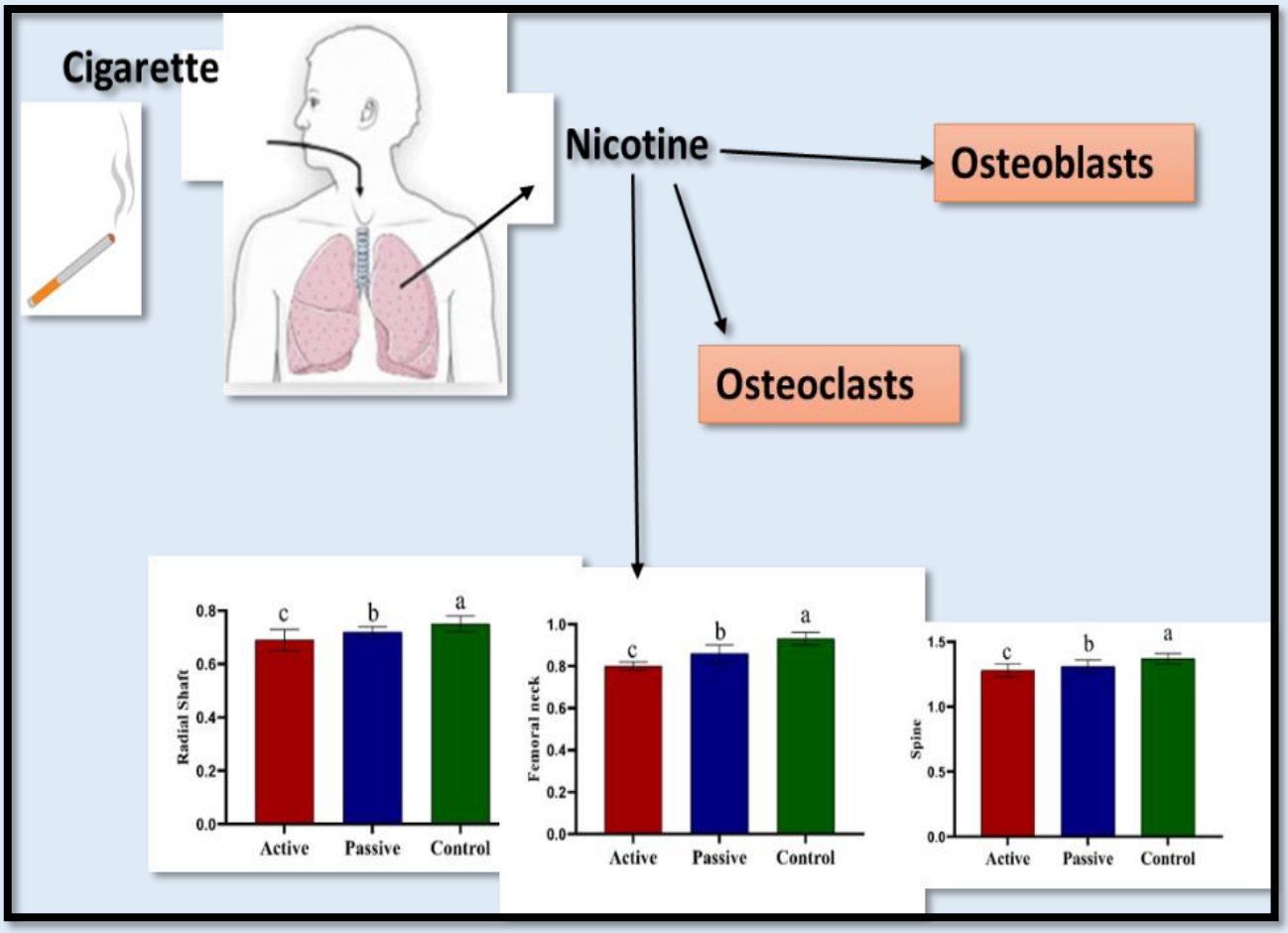
Objective: Cigarette smoking harms all body systems, and its effects are primarily related to nicotine. However, the heavy metal content (mainly lead and cadmium) could add to nicotine's hazardous effects. Thus, the current study aimed to investigate the effect of cigarette smoking content of cadmium and lead on bone mineral density.

Subjects and Methods: A retrospective analysis of data from active, passive, and non-smokers (every 70 subjects) was analyzed for patient demographics, laboratory investigation, serum cotinine (as a confirmatory marker of smoking, bone mineral density (BMD), blood and urinary levels of cadmium and lead).

Results: Hemoglobin concentrations and red blood cell count were significantly reduced, while erythrocyte sedimentation rate and liver enzymes were significantly increased in active and passive smokers than non-smokers. Serum cadmium, lead, and cotinine were raised considerably in passive and active than non-smokers (0.47±0.05, 21.94±3.99, 5.35±0.90 in active, 0.32±0.09, 18.91±3.30, and 4.35±0.89 in passive, versus 0.09±0.06, 9.84±2.63, and 1.28±0.21 in the control group, successively). Bone mineral density was reduced in active and passive than non-smokers at the radial shaft, femoral neck, and spine. Cotinine was significantly and proportionately correlated with serum cadmium and lead and inversely correlated with bone mineral density. Furthermore, cadmium and lead were inversely correlated with BMD.

Conclusion: Cigarette smoke was associated with higher concentrations of cadmium, and lead may directly and indirectly share in the harmful effects of smoking on BMD.

Keywords: Bone Mineral Density, Cotinine, Toxic Heavy Metals, Smoking



INTRODUCTION

Cigarette smoking has harmful effects on all body systems. It has about 7,000 harmful chemicals, and evidence shows that tobacco smoking leads to cancer, premature death, and different chronic diseases (e.g., coronary heart disease and chronic obstructive pulmonary disease). In addition, its association with the development of osteoporosis has a growing body of evidence. However, such relationship's magnitude and pathophysiological mechanisms remain unclear [1-3].

The mechanisms of harmful effects of smoking on bone health remain poorly understood due to contradictory results [4]**.** However, these effects are categorized into direct and indirect, as well as many stages of bone formation, and turnover is affected. These include, but have not limited to, the alteration in body weight due to the anorexigenic effect of nicotine [5], hormonal alterations of the parathyroid hormone-vitamin D axis [6, 7], alteration of adrenal hormones [8], sex hormones [3, 9], and increased oxidative stress [10, 11]**.**

Environmental exposure to lead (Pb) and cadmium (Cd) increases the risk of harmful health consequences, including endocrine disruption [12, 13]. Both metals are found in dry tobacco at high concentrations (0.7–3.6 µg/g Cd and 0.4 to 12.2 µg/g Pb) [14, 15]. Therefore, lead and cadmium become components of tobacco smoke when tobacco has been smoked. In addition, 46–60 % lead and 81–90 % cadmium transformed from dry tobacco to the particulate phase of tobacco smoke [16, 17]. This consequently produced an elevated level of Cd and Pb in different tissue samples and the blood of smokers [18-20]. However, the relationship between elevated Pb and Cd levels and bone mineral density was not examined, except in a few animal studies [21-23].

The study aimed to evaluate the potential hazardous effects of cigarette smoke content of cadmium and lead on bone mineral density (BMD).

MATERIALS AND METHODS

The present study included 210 persons selected from Al-Azhar University hospitals during the period from June 2017 through June 2022. They were divided into three groups according to their smoking activity. Group I included 70 active smokers. Group II included 70 passive smokers. Group III included 70 non-smokers. To be included, active and passive smokers must be regularly exposed to at least 10 cigarettes/day for at least 6 consecutive months before inclusion in the study. Both males and females are included regardless of smoking condition (active or passive). Subjects in the control group with cotinine levels ≥ 3 ng/ml were excluded from the study.

The clinical evaluation was completed by full history taking and clinical examination. Height and weight were documented, and body mass index (BMI) was calculated as body weight (kg) divided by squared height (m^2). The laboratory investigations were performed and documented and included erythrocyte sedimentation rate (ESR), complete blood count (CBC), rheumatoid factor, liver enzymes (serum ALT, AST), serum creatinine, blood urea and fasting and postprandial blood sugar, and serum uric acid.

Specific laboratory tests included the measurement of serum and urinary cadmium and lead. According to Ivanenko, et al., Pb and Cd studies were conducted using a Varian AA-880 Zeeman atomic absorption spectrophotometer connected to a GTA-100 electrothermal atomizer and a programmed sample dispenser [24]. The detection limits of lead were 0.66 µg/l and 0.0025 µg/l for cadmium. In addition, smoking exposure was assessed by measuring serum cotinine levels using a Human Cotinine ELISA Kit (MyBioSource, Inc, USA) according to manufacturer instructions. Values < 3 ng/ml were specific for non-smokers [25].

Dual-energy X-ray absorptiometry (DXA) was used using a Lunar Prodigy densitometer to quantify BMD at the radial shaft, lumbar spine (LS) (L2-L4), and femoral

neck (GE Lunar, WI, USA). (g/cm^2) was used to indicate areal BMD. At the intersection of the proximal two-thirds and the distal one-third, the density of the radial shaft was measured [26].

Statistical data analysis: The social science statistical software was used to code, tabulate, and analyze the obtained data (SPSS version 16, SPSS Inc, Chicago, USA) [27]. First, frequency and percentages were computed for categorical data and groups compared by the Chi-square test. Otherwise, means and standard deviations were calculated as quantitative data, and groups were compared by One-way analysis of variances with the least significant differences to compare two groups. Finally, the correlation between the two parameters was calculated using the Pearson correlation coefficient. Statistics were deemed significant if $p < 0.05$ [28].

RESULTS

Males were predominant overall in the study, and there was a significant increase of males in active than passive and non-smokers (94.3% vs. 20.0% and 42.9%, respectively). The majority of patients were in their fifties. Hemoglobin concentrations and RBCs count were significantly reduced in passive and active smokers than in non-smokers. However, compared to non-smokers, the white blood cell count was considerably higher in active and passive smokers. Active and passive smokers had considerably higher ESR and liver enzymes (ALT and AST) than non-smokers. The serum cadmium and serum lead levels were significantly higher in active passive smokers and in passive smokers than non-smokers. However, urinary levels did not show significant differences. In addition, the subject's age, body mass index platelets, serum uric acid, and serum creatinine did not appear to have substantial differences between groups. The serum cotinine showed a significant increase in activity than passive smokers (5.35±0.90 vs. 4.35±0.89 ng/ml, respectively) and in passive than non-smokers (4.35±0.89 vs. 1.28±0.21 ng/ml, respectively) (Table 1). Bone mineral density showed a statistically significant reduction in active and passive than non-smokers in all measured areas (radial shaft, femoral neck, and lumbar spine) (Fig 1).

There was a significant inverse correlation between cotinine from one side and each hemoglobin concentration, red blood cell count, and BMD of the femoral neck, radial shaft, and lumbar spine. In addition, the correlation was proportional and significant with ESR, liver enzymes, serum cadmium, serum lead concentration, urea, and serum creatinine (Table 2).

In the current work, there was an inverse (negative) correlation between each serum cadmium and serum lead from one side and BMD at the radial shaft, lumbar spine, and femoral neck (Table 3).

Table 1. Patient characteristics and laboratory investigations among study groups

Variable		Active smokers	Passive smokers	Non-smokers	p
Sex (n,%)	Male	66 (94.3%)	14 (20.0%)	30 (42.9%)	***<0.001****
	Female	4 (5.7%)	56 (80.0%)	40 (57.1%)	
Age (years)		45.71±8.35 [a]	45.41±7.80 [a]	45.27±7.17 [a]	0.94
BMI (kg/m^2)		25.79±2.33 a	25.56±2.32 a	51.11±1.94 a	0.19
Hemoglobin		9.23±0.65 b	9.81±0.63 b	10.98±0.94 a	***<0.001****
RBCs x 10^6		3.62±0.37 b	3.94±0.31b	4.34±0.37a	***<0.001****
WBCs x 10^3		6.91±1.84a	5.10±1.07bc	4.58±0.91c	***<0.001****
Platelets x 10^3		251.37±38.04a	258.36±50.08a	252.57±21.67a	0.51
Serum uric acid		5.99±1.03a	5.72±1.13a	5.67±1.29a	0.23
ESR		22.02±4.60a	20.77±3.12b	15.31±2.35c	***<0.001****

Variable	Active smokers	Passive smokers	Non-smokers	p
SGOT	35.45±8.56a	33.05±7.74b	21.92±5.96c	***<0.001****
SGPT	32.31±9.49a	31.94±8.38b	20.21±9.97c	***<0.001****
Serum urea	29.45±9.18a	27.04±7.58b	21.01±4.94c	***<0.001****
Creatinine	0.67±0.16a	0.68±0.17a	0.62±0.18a	0.10
Serum Cadmium (µg/l)	0.47±0.05a	0.32±0.09b	0.09±0.06c	***<0.001****
Urinary cadmium (µg/l)	0.032±0.009a	0.037±0.016a	0.032±0.026a	0.23
Serum lead (µg/l)	21.94±3.99a	18.91±3.30b	9.84±2.63c	***<0.001****
Urinary lead (µg/l)	0.50±0.67a	0.37±0.52a	0.34±0.56a	0.24
Cotinine (ng/ml)	5.35±0.90a	4.35±0.89b	1.28±0.21c	***<0.001****

The results are expressed as a mean value ± standard error of the mean. Values with a different lowercase letter in superscript within the same column are significantly different from each other according to the Fisher test at p<0.001. *=Highly significant at p-value <0.001.

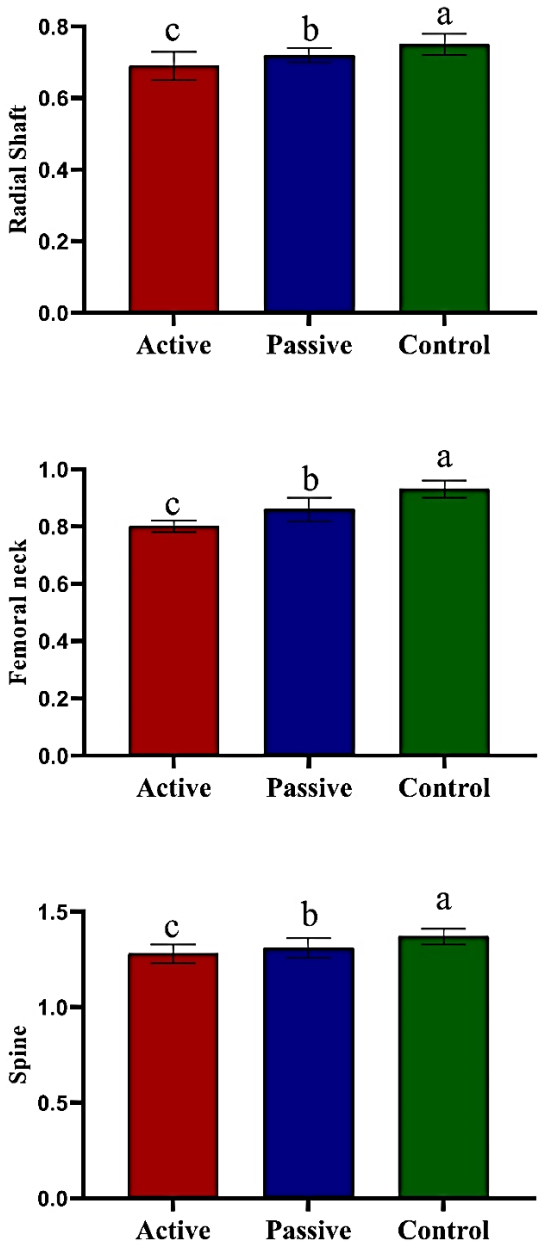


Figure 1. Comparison between studied groups regarding bone mineral density (g/cm^2)

Table 2. Correlation between serum cotinine and different variables.

	Cotinine	
	r	p
Age	0.029	0.675
BMI	0.082	0.237
Hemoglobin	***-0.640***	***<0.001***
RBCs	***-0.588***	***<0.001***
WBCs	***0.440***	***<0.001***
Platelets	0.015	0.831
Serum uric acid	0.040	0.560
ESR_1	***0.562***	***<0.001***
SGOT	***0.597***	***<0.001***
SGPT	***0.546***	***<0.001***
Urea	***0.396***	***<0.001***
Serum cadmium	***0.859***	***<0.001***
Urinary cadmium	0.039	0.577
Serum lead	***0.781***	***<0.001***
Urinary lead	0.082	0.239
Radial shaft	***-0.577***	***<0.001***
Femoral neck	***-0.806***	***<0.001***
Spine	***-0.677***	***<0.001***

r: Pearson correlation, p: p value at 0.001

Table 3. Correlation between bone mineral density and serum or urinary cadmium and lead in all studied cases.

	Serum cadmium		Urinary cadmium		Serum lead		Urinary lead	
	r	p	r	p	r	p	r	p
Serum cadmium	-------	-------	0.053	0.446	0.794	<0.001	0.075	0.280
Urinary cadmium	0.053	0.446	-------	-------	-0.014	0.835	0.016	0.814
Serum lead	0.794	<0.001	0.014	0.835	-------	-------	0.068	0.326
Urinary lead	0.075	0.280	0.016	0.814	0.068	0.326	-----	------
Radial shaft	-0.552	<0.001	0.073	0.294	-0.616	<0.001	-0.124	0.072
Femoral neck	-0.810	<0.001	0.001	0.997	-0.697	<0.001	-0.117	0.092
Spine	-0.641	<0.001	0.114	0.098	-0.608	<0.001	-0.066	0.343

r: Pearson correlation, p: p-value at 0.001

DISCUSSION

This study aims to evaluate the effect of cigarette smoking content of cadmium and lead on BMD. It included three equal groups (every 70 subjects) of active, passive, and non-smokers. Serum cadmium and lead levels were significantly higher in active and passive smokers than in non-smokers. However, bone mineral density was significantly reduced in all measured areas. The inverse correlation between Cd and Pb and BMD from the other confirmed the association. Serum cotinine levels were significantly and proportionately correlated with serum cadmium and lead while inversely correlated with bone mineral density.

These results align with Hou, et al. [29], who reported that serum cotinine and smoking had a harmful impact on the bone in the form of increased cases of osteoporosis and osteopenia. This association remains constant after the adjustment of confounding factors. Serum cotinine and the frequency of bone diseases were positively and nonlinearly correlated. Fang, et al. also reported that high serum cotinine levels significantly reduced lumbar BMD participants aged 30 years or older [30].

Other studies also reported an association between smoking and osteoporosis [31]. However, Lorentzon, et al. did not find significant differences between non-smokers and smokers regarding the distribution of volumetric BMD [32]. Marques, et al. found significant bone mineral density differences in elderly subjects [33]. These suggested that smoking may accelerate the process of aging-induced osteopenia. Chang, et al. [34] reported that many studies had shown a significant association between cigarette smoking and reduced fracture healing power impairment and BMD. Yet, the underlying processes are still not completely known. The presence of nicotine was blamed for the harmful consequences. Unfortunately, cigarettes include a variety of chemicals that are harmful to bone health [35]. Here, we could confirm the harmful effects of nicotine by the significant increase its metabolite cotinine and added effects by the increased levels of cadmium and lead. However, we cannot abolish the effects of other substances. The many toxic compounds in cigarettes make it impossible to test every substance. However, the positive correlation between cotinine and cadmium and lead confirms their participation in the harmful effects of smoking. Fernández-Torres, et al. reviewed the evidence of the association between cigarette-Cd content and the development of joint diseases [36]. They stated that cigarette-Cd might lead to osteoarthritis, osteoporosis, and rheumatoid arthritis. They explained that the higher concentration of Cd triggers oxidative stress and low-grade inflammation. In addition, the reduction in antioxidant enzymes favors bone resorption. Elonheimo, et al. hypothesized a connection between osteoporosis and blood concentrations of industrial chemicals such as phthalates, poly-fluoro-alkyl compounds, and heavy metals like cadmium and lead [37].

In an interesting study, Ananda Jayalal, et al. estimated the lead-bone content of victims who died due to chronic kidney disease of uncertain etiology and found a significant increase in bone-lead concentration than those who died from non-chronic kidney disease [38]. However, cadmium and mercury did not significantly differ between cases and controls. They suggested that the marked deterioration of renal function could be related to the progressive increase of lead concentration that could not be diagnosed during life. Jalili, et al. stated that there is a biological plausibility explaining the possible association between heavy metal exposure and the risk of osteoporosis or osteopenia [39]; it was inconclusive. Thus, they performed a systematic review to evaluate this association. Their results revealed that cadmium exposure was associated with a raised risk of

osteopenia or osteoporosis. However, the effect of lead was significant in men, not in women. They confirmed that the fact detected in the current study indicated that blood levels of heavy metals, not urinary values, were associated with the raised risk of osteoporosis or osteopenia. However, they reported heterogeneity of studies that present a limitation of generalization of results.

Current results align with Law and Hackshaw [40], who noted a significant reduction of bone mass in active than in non-smokers. The review by Wong, et al. [41] revealed that the effects of smoking on bone mass are dose dependent. The present research confirms this by finding an adverse relationship between cotinine and BMD. Brzóska and Moniuszko-Jakoniuk [42] discovered a substantial inverse relationship between Cd and BMD. Järup and Åkesson also found a negative relationship between forearm BMD and Cd level [43]. Gao et al. discovered a substantial loss in BMD of the lumbar spine and femur in smoke-exposed rats compared to non-exposed rats in an animal investigation [44]. César-Neto, et al. [45] showed a reduction in bone density in subjects exposed to cigarette smoke inhalation (passive smokers). Gao, et al. confirmed the effect of passive smoking on the bone of female rats with an increased risk of fractures [44].

CONCLUSION

In short, the results of the present study proved the harmful effects of smoking on bone mineral density, and it may be related to the direct or indirect effects of higher concentrations of cadmium and lead. However, one limitation of the present study is the small number of investigated cases. Thus, it is recommended to design future wide-scale studies to elucidate the potential mechanisms of hazardous effects of cadmium and lead content of smoking on bone health.

Author Contributions: Conceptualization, MHE, SAFE, MAMA, EHEG, AIE, AMH, MMAD, MYE, ASA, NES, TMN, and MAH; methodology, MHE, SAFE, MAMA, EHEG, AIE, AMH, MMAD, MYE, ASA, NES, TMN, and MAH; data curation, MHE, SAFE, MAMA, EHEG, AIE, AMH, MMAD, MYE, ASA, NES, TMN, and MAH; writing—original draft preparation, MHE, SAFE, MAMA, EHEG, AIE, AMH, MMAD, MYE, ASA, NES, TMN, and MAH; writing—review and editing, MHE, SAFE, MAMA, EHEG, AIE, AMH, MMAD, MYE, ASA, NES, TMN, and MAH. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

Funding: This research received no external funding.

REFERENCES

1. Ratajczak AE, Szymczak-Tomczak A, Rychter AM, Zawada A, Dobrowolska A, Krela-Kaźmierczak I: Impact of Cigarette Smoking on the Risk of Osteoporosis in Inflammatory Bowel Diseases. J Clin Med 2021, 10: 1515. DOI: https://doi.org/10.3390/jcm10071515
2. Al-Bashaireh AM, Haddad LG, Weaver M, Chengguo X, Kelly DL, Yoon S, The Effect of Tobacco Smoking on Bone Mass: An Overview of Pathophysiologic Mechanisms, J Osteoporosis 2018, 2018: 1-17. DOI: https://doi.org/10.1155/2018/1206235
3. Rabie ASI, Salah H, Said AS, Shaaban AH, Abdou LM, Khalil DM, Kharaba Z, Afifi H, Sofy MR, Youssef EM, Clinical Consequences for Individuals Treated with Tocilizumab for Serious COVID-19 Infection. Healthcare, 2023, 607.
4. Cusano NE, Skeletal Effects of Smoking, Curr Osteopor Rep 2015, 13: 302-309. DOI: https://doi.org/10.1007/s11914-015-0278-8
5. Mineur YS, Abizaid A, Rao Y, Salas R, DiLeone RJ, Gündisch D, Diano S, De Biasi M, Horvath TL, Gao X-B, Picciotto MR, Nicotine Decreases Food Intake Through Activation of POMC Neurons, Science 2011, 332: 1330-1332. DOI: https://doi.org/10.1126/science.1201889

6. Kassi E, Stavropoulos S, Kokkoris P, Galanos A, Moutsatsou P, Dimas C, Papatheodorou A, Zafeiris C, Lyritis G, Smoking is a significant determinant of low serum vitamin D in young and middle-aged healthy males, Hormones 2014. DOI: https://doi.org/10.14310/horm.2002.1521
7. Kargin NC, Marakoglu K, Unlu A, Kebapcilar L, Korucu EN, Comparison of bone turnover markers between male smoker and non-smoker, Acta Medica Mediter 2016, 32: 317-323. DOI: http://dx.doi.org/10.19193/0393-6384_2016_2_47
8. Tilbrook AJ, Wagenmaker ER, Karsch FJ, Clarke IJ, Breen KM, Oakley AE, Cortisol Reduces Gonadotropin-Releasing Hormone Pulse Frequency in Follicular Phase Ewes: Influence of Ovarian Steroids, Endocrinology 2009, 150: 341-349. DOI: https://doi.org/10.1210/en.2008-0587
9. Wang W, Yang X, Liang J, Liao M, Zhang H, Qin X, Mo L, Lv W, Mo Z, Cigarette smoking has a positive and independent effect on testosterone levels, Hormones 2013, 12: 567-577. DOI: https://doi.org/10.14310/horm.2002.1445
10. Callaway DA, Jiang JX, Reactive oxygen species and oxidative stress in osteoclastogenesis, skeletal aging and bone diseases, J Bone Mineral Metab 2015, 33: 359-370. DOI: https://doi.org/10.1007/s00774-015-0656-4
11. El-Sheshtawy HS, Mahdy HM, Sofy AR, Sofy MR, Production of biosurfactant by Bacillus megaterium and its correlation with lipid peroxidation of Lactuca sativa. Egypt J Petro 2022, 31: 1-6. DOI: https://doi.org/10.1016/j.ejpe.2022.03.001
12. Yang AM, Lo K, Zheng TZ, Yang JL, Bai YN, Feng YQ, Cheng N, Liu SM, Environmental heavy metals and cardiovascular diseases: Status and future direction, Chron Dis Translational Med 2020, 6: 251-259. DOI: https://doi.org/10.1016/j.cdtm.2020.02.005
13. Wu J-X, Lau ATY, Xu Y-M, Indoor Secondary Pollutants Cannot Be Ignored: Third-Hand Smoke, Toxics 2022, 10: 363. DOI: https://doi.org/10.3390/toxics10070363
14. Piadé JJ, Jaccard G, Dolka C, Belushkin M, Wajrock S, Differences in cadmium transfer from tobacco to cigarette smoke, compared to arsenic or lead, Toxicolo Rep 2015, 2: 12-26. DOI: https://doi.org/10.1016/j.toxrep.2014.11.005
15. Caruso R, O'Connor R, Stephens W, Cummings K, Fong G, Toxic Metal Concentrations in Cigarettes Obtained from U.S. Smokers in 2009: Results from the International Tobacco Control (ITC) United States Survey Cohort, Inter J Env Res Public Health 2013, 11: 202-217. DOI: https://doi.org/10.3390/ijerph110100202
16. Pinto E, Cruz M, Ramos P, Santos A, Almeida A, Metals transfer from tobacco to cigarette smoke: Evidences in smokers' lung tissue, J Hazard Mater 2017, 325: 31-35. DOI: https://doi.org/10.1016/j.jhazmat.2016.11.069
17. Martirosyan D, Lampert T, Ekblad M, Classification and regulation of functional food proposed by the Functional Food Center, Func Food Sci 2022, 2: 25-46. DOI: http://dx.doi.org/10.31989/ffs.v2i2.890
18. Richter PA, Bishop EE, Wang J, Kaufmann R, Trends in Tobacco Smoke Exposure and Blood Lead Levels Among Youths and Adults in the United States: The National Health and Nutrition Examination Survey, 1999–2008, Preventing Chron Dis 2013, 10. DOI: https://doi.org/10.5888/pcd10.130056
19. Tsai J, Homa DM, Gentzke AS, Mahoney M, Sharapova SR, Sosnoff CS, Caron KT, Wang L, Melstrom PC, Trivers KF, Exposure to Secondhand Smoke Among Nonsmokers — United States, 1988–2014, MMWR. Morb Mortal Weekly Rep2018, 67: 1342-1346. DOI: https://doi.org/10.15585/mmwr.mm6748a3
20. Martirosyan D, Ekblad M, Functional foods classification system: exemplifying through analysis of bioactive compounds, Func Food Sci 2022, 2: 94-123. DOI: http://dx.doi.org/10.31989/ffs.v2i4.919
21. Ko CH, Chan RLY, Siu WS, Shum WT, Leung PC, Zhang L, Cho CH, Deteriorating Effect on Bone Metabolism and Microstructure by Passive Cigarette Smoking Through Dual Actions on Osteoblast and Osteoclast, Calcified Tissue Inter 2015, 96: 389-400. DOI: https://doi.org/10.1007/s00223-015-9966-8
22. Kim KH, Lee CM, Park SM, Cho B, Chang Y, Park SG, Lee K, Secondhand smoke exposure and osteoporosis in never-smoking postmenopausal women: the Fourth Korea National Health and Nutrition Examination Survey, Osteopor Inter 2012, 24: 523-532. DOI: https://doi.org/10.1007/s00198-012-1987-9
23. Mirmiranpour H, Ashoori MR, Mikaeili AS, Pezeshki S, Serani A, Boez A, Martirosyan D, The effect of squalene on proteinuria in patients with type 2 diabetes mellitus, Bio Compounds Health Dis 2022, 5: 117-135. DOI: http://dx.doi.org/10.31989/ffs.v2i7.949
24. Ivanenko NB, Solovyev ND, Ivanenko AA, Ganeev AA, Application of Zeeman Graphite Furnace Atomic Absorption Spectrometry with High-Frequency Modulation Polarization for the Direct Determination of Aluminum, Beryllium,

Cadmium, Chromium, Mercury, Manganese, Nickel, Lead, and Thallium in Human Blood, Arch Envir Contamin Toxicol 2012, 63: 299-308. DOI: https://doi.org/10.1007/s00244-012-9784-1

25. Benowitz NL, Bernert JT, Caraballo RS, Holiday DB, Wang J, Optimal Serum Cotinine Levels for Distinguishing Cigarette Smokers and Nonsmokers Within Different Racial/Ethnic Groups in the United States Between 1999 and 2004, Amer J Epidemiol 2009, 169: 236-248. DOI: https://doi.org/10.1093/aje/kwn301
26. Bergh C, Söderpalm A-C, Brisby H, Preoperative dual-energy X-ray absorptiometry and FRAX in patients with lumbar spinal stenosis, J Orthopaedic Surgery Res 2018, 13: 253. DOI: https://doi.org/10.1186/s13018-018-0964-1
27. Sofy MR, Mancy AG, Alnaggar AEAM, Refaey EE, Mohamed HI, Elnosary ME, Sofy AR, A polishing the harmful effects of Broad Bean Mottle Virus infecting broad bean plants by enhancing the immunity using different potassium concentrations, Notu Botan Horti Agro Cluj-Napoc*a* 2022, 50: 12654.
28. Dawood MF, Abu-Elsaoud AM, Sofy MR, Mohamed HI, Soliman MH, Appraisal of kinetin spraying strategy to alleviate the harmful effects of UVC stress on tomato plants, Environ Sci Poll Res 2022, 29: 52378-52398. DOI: https://doi.org/10.1007%2Fs11356-022-19378-6
29. Hou W, Chen S, Zhu C, Gu Y, Zhu L, Zhou Z, Associations between smoke exposure and osteoporosis or osteopenia in a US NHANES population of elderly individuals, Front Endocrinol 2023, 14: 1074574. DOI: https://doi.org/10.3389/fendo.2023.1074574
30. Fang J-G, Wang D-J, Yang H-Y, Zhang H, Tong J-Y, Lin Z-J, Pal China S, Association between Serum Cotinine Levels and Bone Mineral Density: An Analysis of the National Health and Nutrition Examination Survey (NHANES), Int J Endocrinol 2022, 2022: 1-6. DOI: https://doi.org/10.1155/2022/6830705
31. Agarwal S, Germosen C, Kil N, Bucovsky M, Colon I, Williams J, Cusano N, Walker M, Smoking Is Associated with Sex-Specific Effects on Bone Microstructure in Older Men and Women, J Clinical Densitom 2021, 24: 341-350. DOI: https://doi.org/10.1016/j.jocd.2020.07.002
32. Lorentzon M, Swanson C, Andersson N, Mellström D, Ohlsson C, Free Testosterone Is a Positive, Whereas Free Estradiol Is a Negative, Predictor of Cortical Bone Size in Young Swedish Men: The GOOD Study, J Bone Mineral Res 2005, 20: 1334-1341. DOI: https://doi.org/10.1359/JBMR.050404
33. Marques EA, Elbejjani M, Gudnason V, Sigurdsson G, Lang T, Sigurdsson S, Aspelund T, Siggeirsdottir K, Launer L, Eiriksdottir G, Harris TB, Cigarette smoking and hip volumetric bone mineral density and cortical volume loss in older adults: The AGES-Reykjavik study, Bone 2018, 108: 186-192. DOI: https://doi.org/10.1016/j.bone.2018.01.014
34. Chang C-J, Jou IM, Wu T-T, Su F-C, Tai T-W, Cigarette smoke inhalation impairs angiogenesis in early bone healing processes and delays fracture union, Bone Joint Res 2020, 9: 99-107. DOI: https://doi.org/10.1302/2046-3758.93.BJR-2019-0089.R1
35. Aspera-Werz RH, Ehnert S, Heid D, Zhu S, Chen T, Braun B, Sreekumar V, Arnscheidt C, Nussler AK, Nicotine and Cotinine Inhibit Catalase and Glutathione Reductase Activity Contributing to the Impaired Osteogenesis of SCP-1 Cells Exposed to Cigarette Smoke, Oxidative Med Cellular Long 2018, 2018: 1-13. DOI: https://doi.org/10.1155/2018/3172480
36. Fernández-Torres J, Zamudio-Cuevas Y, Martínez-Nava GA, Aztatzi-Aguilar OG, Sierra-Vargas MP, Lozada-Pérez CA, Suárez-Ahedo C, Landa-Solís C, Olivos-Meza A, Del Razo LM, Camacho-Rea MC, Martínez-Flores K, Impact of Cadmium Mediated by Tobacco Use in Musculoskeletal Diseases, Biolog Trace Element Res 2021, 200: 2008-2015. DOI: https://doi.org/10.1007/s12011-021-02814-y
37. Elonheimo H, Lange R, Tolonen H, Kolossa-Gehring M, Environmental Substances Associated with Osteoporosis–A Scoping Review, Inter J Environ Res Public Health 2021, 18: 738. DOI: https://doi.org/10.3390/ijerph18020738
38. Ananda Jayalal TB, Mahawithanage STC, Senanayaka SMHM, Dassanayaka PB, Evidence of selected nephrotoxic elements in Sri Lankan human autopsy bone samples of patients with CKDu and controls, BMC Nephrol 2020, 21: 384. https://doi.org/10.1186/s12882-020-02049-4
39. Jalili C, Kazemi M, Taheri E, Mohammadi H, Boozari B, Hadi A, Moradi S, Exposure to heavy metals and the risk of osteopenia or osteoporosis: a systematic review and meta-analysis, Osteopo Inter 2020, 31: 1671-1682. DOI: https://doi.org/10.1007/s00198-020-05429-6
40. Law MR, Hackshaw AK, A meta-analysis of cigarette smoking, bone mineral density and risk of hip fracture:

recognition of a major effect, Bmj 1997, 315: 841-846. DOI: https://doi.org/10.1136/bmj.315.7112.841

41. Wong P Peter KK, Christie J J, Wark John D, The effects of smoking on bone health, Clinical Sci 2007, 113: 233-241. DOI: https://doi.org/10.1042/CS20060173
42. Brzóska MM, Moniuszko-Jakoniuk J, Bone metabolism of male rats chronically exposed to cadmium, Toxicol Appl Pharmacol2005, 207: 195-211. DOI: https://doi.org/10.1016/j.taap.2005.01.003
43. Järup L, Åkesson A, Current status of cadmium as an environmental health problem, Toxicol Appl Pharmacol 2009, 238: 201-208. DOI: https://doi.org/10.1016/j.taap.2009.04.020
44. Gao S-g, Li K-h, Xu M, Jiang W, Shen H, Luo W, Xu W-s, Tian J, Lei G-h, Bone turnover in passive smoking female rat: relationships to change in bone mineral density, BMC Musculoskeletal Disorders 2011, 12. DOI: https://doi.org/10.1186/1471-2474-12-131
45. César-Neto JB, Benatti BB, Manzi FR, Sallum EA, Sallum AW, Nociti Junior FH, The influence of cigarette smoke inhalation on bone density: a radiographic study in rats, Braz Oral Res 2005, 19: 47-51. DOI: https://doi.org/10.1590/S1806-83242005000100009