Review

Can Melatonin Ameliorate Smoking-Related Cadmium-Induced Decreases in Bone Mineral Density?

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Can Melatonin Ameliorate Smoking-Related Cadmium-Induced Decreases in Bone Mineral Density?

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Abstract: Cadmium, an environmental toxin, is associated with a range of adverse health effects due to increased Reactive Oxygen Species production, including decreased bone mineral density and osteoporosis. Notably, cadmium is found at concentrations 4-5x higher in the blood of smokers versus non-smokers. Experiments performed in human cancer cells indicate that melatonin may directly protect against cadmium-induced tissue damage via regulation of mitochondrial activity. Further, recent evidence has demonstrated that melatonin can improve bone health for individuals with osteoporosis and partially protect against cadmium-associated inhibition of bone repair. Here we review this data and propose supplementation with melatonin as a strategy to protect against the negative impacts of cadmium exposure on bone mineral density within individuals regularly exposed to cadmium via cigarette smoking.

Keywords: cadmium; bone density; cigarette; melatonin; smoking; osteoblast

Introduction

Longitudinal cohort and cross-sectional studies have established that exposure to cadmium, an environmental toxin, is a risk factor for decreased bone mineral density (BMD) and osteoporosis. Experimental support for these epidemiological findings has shown that bone health, as measured by BMD, is highly sensitive to cadmium exposure even at levels as low as 0.3-10 mg/kg body weight (Buha et al., 2019), through both inhibition of the activity of osteoblasts, thus decreasing bone deposition; and stimulation of osteoclast differentiation, resulting in increased resorption and pit formation within bone (X. Chen et al., 2009, 2013; W. Liu et al., 2020; Ou et al., 2021). Ultimately, this results in the characteristic imbalance between bone deposition and resorption typically associated with the onset of osteoporosis in later life (X. Chen et al., 2009, 2013; W. Liu et al., 2020; Ou et al., 2021).

Literature Review

Cadmium exposure via cigarette smoking and the effect on bone health

A 2019 study estimated that there were 1.14 billion cigarette smokers globally, indicating that smoking remains a widespread risk factor to human health (GBD 2019 Tobacco Collaborators, 2021). Amongst the many negative health consequences associated with smoking, it is a known risk factor for osteoporosis and poor bone health, with one meta-analysis estimating the risk of osteoporotic fracture being 32% higher in smoking men and women compared to non-smokers (Kanis et al., 2005). Multiple mechanisms have been proposed that can potentially mediate this association, including the alteration of sex hormones and increased oxidative stress (Al-Bashaireh et al., 2018). Cadmium, an environmental toxin known to promote increased production of Reactive Oxygen Species (ROS) and mitochondrial dysfunction, is present in tobacco at concentrations ranging from 0.5-1.0 g of cadmium per cigarette, and is found at concentrations 4-5x higher in smokers versus non-smokers (Ganguly et al., 2018). Cadmium is known to induce osteoblast dysfunction, specifically through oxidative stress,

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leading to DNA damage, mitochondrial dysfunction, and endoplasmic reticulum stress, ultimately resulting in apoptosis of the osteoblasts and consequently a decrease in bone formation (Ma et al., 2021).

A study by Li and colleagues (Li et al., 2020) investigated how much the smoking-associated risk of osteoporosis was mediated by cadmium. Analysis of retrospective cohort data revealed that each 10-pack year (packs of cigarettes smoked per day, multiplied by the smoking duration, in years) could lead to 1.06 additional hip fractures per 1000 person-years, with 0.67 of this risk due to cadmium from tobacco smoke (Li et al., 2020). These results were supported by Elbeialy & Eldosouky (Elbeialy & Eldosouky, 2018), who found an inverse relationship between serum and urinary cadmium and bone health. Thus, smoking is a known risk factor for poor bone health, with cadmium exposure being a relatively well characterized mediator of this risk. Despite this, no treatment or preventative measure has been identified that specifically mitigates the effects of cigarette smoking-associated cadmium-induced decreases in bone health.

Preventing loss of bone mineral density

Widely used treatments for decreased BMD include anti-resorptive drugs and hormone replacement therapies (HRT). However, both these treatments have significant limitations. For example, anti-resorptive drugs can have significant side effects for the patient including osteonecrosis of the jaw and harsh repression of bone turnover (Brown, 2017; Kennel & Drake, 2009). Furthermore, HRT is used to balance estrogen levels within peri- and post-menopausal women to prevent the rapid bone loss common in post-menopause (Gambacciani & Levancini, 2014). Thus, HRT is not a viable treatment option for men or women pre-menopause. Cigarette smokers are present across the lifespan, found within both sexes, and are often chronic users/consumers. Thus, the existing treatments to improve BMD are insufficient to address the needs of this large and varied population of individuals.

Importantly however, recent data has indicated that melatonin can protect against cadmium-induced oxidative stress and may attenuate the negative effects of cadmium exposure on bone repair (Hyun et al., 2023; Luo et al., 2021). However, the efficacy of melatonin use in cigarette smokers to preserve bone mineral density has not yet been characterized. Given this information, we propose that supplementation with melatonin is an appropriate strategy for the prevention of bone density loss in individuals who are regularly exposed to cadmium by limiting oxidative stress and mitochondrial impairment.

Melatonin & bone health

Melatonin is a hormone synthesized and produced by the pineal gland (Cipolla-Neto & Amaral, 2018). The primary function of melatonin is in regulating the sleep-wake cycle and the modulation of circadian rhythms, and hence is a widely accessible pharmaceutical used to treat sleep-wake disturbances (Geoffroy et al., 2015; Tordjman et al., 2017; Xie et al., 2017).

Recent studies have found that melatonin also positively affects bone homeostasis and has been proposed as a potential treatment for osteoporosis/osteopenia (Amstrup et al., 2015; Wang et al., 2019). Melatonin is known to have strong antioxidant properties, which may in part explain its protective effects on bone (X. Liu et al., 2013; Lu et al., 2021; Tordjman et al., 2017). Melatonin's antioxidant capacities are exerted directly through its ability to scavenge free radicals and indirectly through activating antioxidative enzymes, inhibiting pro-oxidative enzymes, and tempering DNA repair pathways (Galano et al., 2018; Majidinia et al., 2017; Reiter et al., 2010). These mechanisms allow for melatonin to protect against free-radical-associated DNA damage (Galano et al., 2018). Furthermore, melatonin is thought to preserve the antioxidant capacity and bone-formation potential of bone-marrow-derived mesenchymal stem cells (BMMSCs) (W. Chen et al., 2020), in addition to having broader effects on bone, including promotion of osteoblast cell differentiation and type I collagen synthesis, thereby stimulating bone proliferation, and inhibition of bone resorption through the downregulation of RANKL-mediated osteoclast formation and activation (Koyama et al., 2002; Lu et al., 2021; Nakade et al., 1999; Xu et al., 2018). Within human populations, randomized control trials have found that melatonin supplementation is both well-tolerated and effective at improving

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physical symptoms in perimenopausal women with osteoporosis (Kotlarczyk et al., 2012); and was able to increase bone mineral density at the femoral neck in a well-tolerated dose-dependent manner (Amstrup et al., 2015). Melatonin is also thought to be effectively non-toxic and is capable of improving circadian rhythm sleep disorders and poor sleep quality (Amstrup et al., 2015; Zisapel, 2018). Thus, melatonin is a safe, potential therapeutic for osteoporosis that is advantageous over other drugs, such as hormone replacement therapy and anti-resorptive drugs that often have significant side effects.

Melatonin & cadmium

Interestingly, previous observational and experimental studies have noted that cigarette smoking and third-hand smoke is associated with lower serum melatonin levels (Jiang et al., 2021; Ursing et al., 2005). Third-hand smoke is defined as the environmental hazard created via accumulation of second-hand smoke toxins on indoor objects (Jiang et al., 2021). Evidence indicates that polycyclic aromatic hydrocarbons (PAHs) in cigarette smoke can increase the activity of cytochrome P_{450}(CYP)1A2 (Ursing et al., 2005). CYP1A2 is associated with the breakdown of melatonin by the liver, which may explain the observed association between smoking and abnormally decreased serum melatonin (Ursing et al., 2005). However, other studies have found higher circulating daytime levels of melatonin in smokers (Tarquini et al., 1994). Thus, the association between smoking and melatonin levels remains to be clarified.

Notably, a recent study by Hyun and colleagues [5] has revealed that melatonin directly counteracts the effect of cadmium on ROS levels. This is because melatonin enhanced the expression of mitochondrially-localized signal transducer and activator of transcription 3 (mitoSTAT3) that is reduced by cadmium exposure (Hyun et al., 2023). MitoSTAT3 is thought to play an important role in the modulation of the electron transport chain, ROS homeostasis, transcription of mitochondrial DNA , ATP production, and apoptosis (Hyun et al., 2023). Thus, regulating the levels of mitoSTAT3 with melatonin could attenuate ROS damage and mitochondrial dysfunction caused by cadmium (Hyun et al., 2023). In support, Luo et al (Luo et al., 2021) determined that pre-treatment with melatonin helped to maintain the integrity of the mitochondrial structure of BMMSCs and decrease DNA damage caused by cadmium within these cells, thereby protecting them against apoptosis. The authors conclude that melatonin may help to prevent cadmium-associated premature aging and apoptosis of BMMSCs (Luo et al., 2021). BMMSCs can differentiate into osteoblasts, meaning that if melatonin can prevent cadmium-associated apoptosis of these cells, an increase in osteoblast numbers may result (Hu et al., 2018). These findings suggest that increasing or maintaining the number of functional osteoblasts through preventative melatonin supplementation may help to rebalance bone deposition and resorption, thereby suppressing cadmium-associated loss of bone mineral density.

Thus, the prevention of cadmium-associated damage may be a mechanism by which melatonin can improve bone mineral density and osteoporosis outcomes within populations regularly exposed to cadmium, including smokers. However, the clinical relevance of melatonin supplementation in this population has yet to be explored. Given melatonin supplementation's well-tolerated nature and its potential to protect against cadmium-related exposure, examining this link may provide a new avenue for treating and preventing bone-mineral loss disorders such as osteoporosis within individuals who are exposed to cadmium.

Testing the strategy

This strategy could be tested within randomized control trials that compare the effect of melatonin supplementation on bone mineral density, and fracture rate, across cigarette smokers of all ages. Additional consideration may be given to matched case-control trials that consider inclusion criteria such as age, number of cigarettes smoked per week, serum cadmium levels, or basal melatonin levels, given that endogenous melatonin levels can differ widely between individuals (Burgess & Fogg, 2008). As melatonin has been shown to help prevent age-related osteoporosis, these results could also be compared to the effectiveness of melatonin in preventing bone mineral density loss in age-matched non-smoking individuals.

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According to our hypothesis, the loss of BMD over time within cigarette smokers supplemented with melatonin should be less pronounced than that of smokers who did not supplement for melatonin.

Conclusion

Cadmium, an environmental toxin known to cause decreased bone mineral density, is introduced into the body through cigarette smoking among other methods. To improve health outcomes for cigarette smokers, the authors propose the use of melatonin as a supplement. Melatonin is a widely used and well-tolerated over-the-counter pharmaceutical commonly utilized in the modulation of sleep-wake disorders. Importantly, new research has revealed that melatonin may be capable of attenuating ROS damage and mitochondrial dysfunction associated with cadmium exposure, as well as preventing apoptosis of BMMSC's. Importantly, melatonin supplementation would be a cost-effective and widely accessible treatment to prevent bone loss in smokers. Evidence supporting this strategy would present an option to improve health outcomes for millions of cigarette smokers globally and may overall reduce the burden of fractures related to loss of bone-mineral density. Thus, the proposal that supplementation with melatonin will prevent bone density loss in individuals who are regularly exposed to cadmium is one that should be considered for further clinical research.

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References

Al-Bashaireh, A. M., Haddad, L. G., Weaver, M., Chengguo, X., Kelly, D. L., & Yoon, S. (2018). The Effect of Tobacco Smoking on Bone Mass: An Overview of Pathophysiologic Mechanisms. *Journal of Osteoporosis*, *2018*, 1206235. https://doi.org/10.1155/2018/1206235

Amstrup, A. K., Sikjaer, T., Heickendorff, L., Mosekilde, L., & Rejnmark, L. (2015). Melatonin improves bone mineral density at the femoral neck in postmenopausal women with osteopenia: A randomized controlled trial. *Journal of Pineal Research*, *59*(2), 221–229. https://doi.org/10.1111/jpi.12252

Brown, J. P. (2017). Antiresorptives: Safety Concerns—Clinical Perspective. *Toxicologic Pathology*, *45*(7), 859–863. https://doi.org/10.1177/0192623317737066

Buha, A., Jugdaohsingh, R., Matovic, V., Bulat, Z., Antonijevic, B., Kerns, J. G., Goodship, A., Hart, A., & Powell, J. J. (2019). Bone mineral health is sensitively related to environmental cadmium exposure- experimental and human data. *Environmental Research*, *176*, 108539. https://doi.org/10.1016/j.envres.2019.108539

Burgess, H. J., & Fogg, L. F. (2008). Individual Differences in the Amount and Timing of Salivary Melatonin Secretion. *PLoS ONE*, *3*(8), e3055. https://doi.org/10.1371/journal.pone.0003055

Chen, W., Chen, X., Chen, A. C., Shi, Q., Pan, G., Pei, M., Yang, H., Liu, T., & He, F. (2020). Melatonin restores the osteoporosis-impaired osteogenic potential of bone marrow mesenchymal stem cells by preserving SIRT1-mediated intracellular antioxidant properties. *Free Radical Biology and Medicine*, *146*, 92–106. https://doi.org/10.1016/j.freeradbiomed.2019.10.412

Chen, X., Wang, G., Li, X., Gan, C., Zhu, G., Jin, T., & Wang, Z. (2013). Environmental level of cadmium exposure stimulates osteoclasts formation in male rats. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association*, *60*, 530–535. https://doi.org/10.1016/j.fct.2013.08.017

Chen, X., Zhu, G., Gu, S., Jin, T., & Shao, C. (2009). Effects of cadmium on osteoblasts and osteoclasts in vitro. *Environmental Toxicology and Pharmacology*, *28*(2), 232–236. https://doi.org/10.1016/j.etap.2009.04.010

Cipolla-Neto, J., & Amaral, F. G. do. (2018). Melatonin as a Hormone: New Physiological and Clinical Insights. *Endocrine Reviews*, *39*(6), 990–1028. https://doi.org/10.1210/er.2018-00084

Elbeialy, A. A., & Eldosouky, H. A. (2018). AB0984 Cadmium toxicity as a probable cause of smoking induced bone loss. *Annals of the Rheumatic Diseases*, *77*(Suppl 2), 1614–1614. https://doi.org/10.1136/annrheumdis-2018-eular.3369

Galano, A., Tan, D.-X., & Reiter, R. J. (2018). Melatonin: A Versatile Protector against Oxidative DNA Damage. *Molecules : A Journal of Synthetic Chemistry and Natural Product Chemistry*, *23*(3), 530. https://doi.org/10.3390/molecules23030530

Gambacciani, M., & Levancini, M. (2014). Hormone replacement therapy and the prevention of postmenopausal osteoporosis. *Przegla d Menopauzalny = Menopause Review*, *13*(4), 213–220. https://doi.org/10.5114/pm.2014.44996

Ganguly, K., Levänen, B., Palmberg, L., Åkesson, A., & Lindén, A. (2018). Cadmium in tobacco smokers: A neglected link to lung disease? *European Respiratory Review: An Official Journal of the European Respiratory Society*, *27*(147), 170122. https://doi.org/10.1183/16000617.0122-2017

GBD 2019 Tobacco Collaborators. (2021). Spatial, temporal, and demographic patterns in prevalence of smoking tobacco use and attributable disease burden in 204 countries and territories, 1990-2019: A systematic analysis from the Global Burden of Disease Study 2019. *Lancet (London, England)*, *397*(10292), 2337–2360. https://doi.org/10.1016/S0140-6736(21)01169-7

Geoffroy, P. A., Etain, B., Franchi, J.-A. M., Bellivier, F., & Ritter, P. (2015). Melatonin and Melatonin Agonists as Adjunctive Treatments in Bipolar Disorders. *Current Pharmaceutical Design*, *21*(23), 3352–3358. https://doi.org/10.2174/1381612821666150619093448

Hu, L., Yin, C., Zhao, F., Ali, A., Ma, J., & Qian, A. (2018). Mesenchymal Stem Cells: Cell Fate Decision to Osteoblast or Adipocyte and Application in Osteoporosis Treatment. *International Journal of Molecular Sciences*, *19*(2), 360. https://doi.org/10.3390/ijms19020360

Hyun, M., Kim, H., Kim, J., Lee, J., Lee, H. J., Rathor, L., Meier, J., Larner, A., Lee, S. M., Moon, Y., Choi, J., Han, S. M., & Heo, J.-D. (2023). Melatonin protects against cadmium-induced oxidative stress via mitochondrial STAT3 signaling in human prostate stromal cells. *Communications Biology*, *6*(1), Article 1. https://doi.org/10.1038/s42003-023-04533-7

Jiang, W., Wu, H., Yu, X., Wang, Y., Gu, W., Wei, W., Li, B., Jiang, X., Wang, Y., Hou, W., Dong, Q., Yan, X., Li, Y., Sun, C., & Han, T. (2021). Third-hand smoke exposure is associated with abnormal serum melatonin level via hypomethylation of CYP1A2 promoter: Evidence from human and animal studies. *Environmental Pollution*, *277*, 116669. https://doi.org/10.1016/j.envpol.2021.116669

Kanis, J. A., Borgstrom, F., De Laet, C., Johansson, H., Johnell, O., Jonsson, B., Oden, A., Zethraeus, N., Pfleger, B., & Khaltaev, N. (2005). Assessment of fracture risk. *Osteoporosis International: A Journal Established as Result of Cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA*, *16*(6), 581–589. https://doi.org/10.1007/s00198-004-1780-5

Kennel, K. A., & Drake, M. T. (2009). Adverse Effects of Bisphosphonates: Implications for Osteoporosis Management. *Mayo Clinic Proceedings*, *84*(7), 632–638.

Kotlarczyk, M. P., Lassila, H. C., O'Neil, C. K., D'Amico, F., Enderby, L. T., Witt-Enderby, P. A., & Balk, J. L. (2012). Melatonin osteoporosis prevention study (MOPS): A randomized, double-blind, placebo-controlled study examining the effects of melatonin on bone health and quality of life in perimenopausal women. *Journal of Pineal Research*, *52*(4), 414–426. https://doi.org/10.1111/j.1600-079X.2011.00956.x

Koyama, H., Nakade, O., Takada, Y., Kaku, T., & Lau, K. H. W. (2002). Melatonin at pharmacologic doses increases bone mass by suppressing resorption through down-regulation of the RANKL-mediated osteoclast formation and activation. *Journal of Bone and Mineral Research: The Official Journal of the American Society for Bone and Mineral Research*, *17*(7), 1219–1229. https://doi.org/10.1359/jbmr.2002.17.7.1219

Li, H., Wallin, M., Barregard, L., Sallsten, G., Lundh, T., Ohlsson, C., Mellström, D., & Andersson, E. M. (2020). Smoking-Induced Risk of Osteoporosis Is Partly Mediated by Cadmium From Tobacco Smoke: The MrOS Sweden Study. *Journal of Bone and Mineral Research*, *35*(8), 1424–1429. https://doi.org/10.1002/jbmr.4014

Liu, W., Le, C. C., Wang, D., Ran, D., Wang, Y., Zhao, H., Gu, J., Zou, H., Yuan, Y., Bian, J., & Liu, Z. (2020). Ca2+/CaM/CaMK signaling is involved in cadmium-induced osteoclast differentiation. *Toxicology*, *441*, 152520. https://doi.org/10.1016/j.tox.2020.152520

Liu, X., Gong, Y., Xiong, K., Ye, Y., Xiong, Y., Zhuang, Z., Luo, Y., Jiang, Q., & He, F. (2013). Melatonin mediates protective effects on inflammatory response induced by interleukin-1 beta in human mesenchymal stem cells. *Journal of Pineal Research*, *55*(1), 14–25. https://doi.org/10.1111/jpi.12045

Lu, X., Yu, S., Chen, G., Zheng, W., Peng, J., Huang, X., & Chen, L. (2021). Insight into the roles of melatonin in bone tissue and bone-related diseases (Review). *International Journal of Molecular Medicine*, *47*(5), 82. https://doi.org/10.3892/ijmm.2021.4915

Luo, H., Gu, R., Ouyang, H., Wang, L., Shi, S., Ji, Y., Bao, B., Liao, G., & Xu, B. (2021). Cadmium exposure induces osteoporosis through cellular senescence, associated with activation of NF-κB pathway and mitochondrial dysfunction. *Environmental Pollution*, *290*, 118043. https://doi.org/10.1016/j.envpol.2021.118043

Ma, Y., Ran, D., Shi, X., Zhao, H., & Liu, Z. (2021). Cadmium toxicity: A role in bone cell function and teeth development. *The Science of the Total Environment*, *769*, 144646. https://doi.org/10.1016/j.scitotenv.2020.144646

Majidinia, M., Sadeghpour, A., Mehrzadi, S., Reiter, R. J., Khatami, N., & Yousefi, B. (2017). Melatonin: A pleiotropic molecule that modulates DNA damage response and repair pathways. *Journal of Pineal Research*, *63*(1). https://doi.org/10.1111/jpi.12416

Nakade, O., Koyama, H., Ariji, H., Yajima, A., & Kaku, T. (1999). Melatonin stimulates proliferation and type I collagen synthesis in human bone cells in vitro. *Journal of Pineal Research*, *27*(2), 106–110. https://doi.org/10.1111/j.1600-079x.1999.tb00603.x

Ou, L., Wang, H., Wu, Z., Wang, P., Yang, L., Li, X., Sun, K., Zhu, X., & Zhang, R. (2021). Effects of cadmium on osteoblast cell line: Exportin 1 accumulation, p-JNK activation, DNA damage and cell apoptosis. *Ecotoxicology and Environmental Safety*, *208*, 111668. https://doi.org/10.1016/j.ecoenv.2020.111668

Reiter, R. J., Manchester, L. C., & Tan, D.-X. (2010). Neurotoxins: Free radical mechanisms and melatonin protection. *Current Neuropharmacology*, *8*(3), 194–210. https://doi.org/10.2174/157015910792246236

Tarquini, B., Perfetto, F., Poli, R., & Tarquini, R. (1994). Daytime Circulating Melatonin Levels in Smokers. *Tumori Journal*, *80*(3), 229–232. https://doi.org/10.1177/030089169408000313

Tordjman, S., Chokron, S., Delorme, R., Charrier, A., Bellissant, E., Jaafari, N., & Fougerou, C. (2017). Melatonin: Pharmacology, Functions and Therapeutic Benefits. *Current Neuropharmacology*, *15*(3), 434–443. https://doi.org/10.2174/1570159X14666161228122115

Ursing, C., Bahr, C. von, Brismar, K., & Röjdmark, S. (2005). Influence of cigarette smoking on melatonin levels in man. *European Journal of Clinical Pharmacology*, *61*(3), 197–201. https://doi.org/10.1007/s00228-005-0908-7

Wang, X., Liang, T., Zhu, Y., Qiu, J., Qiu, X., Lian, C., Gao, B., Peng, Y., Liang, A., Zhou, H., Yang, X., Liao, Z., Li, Y., Xu, C., Su, P., & Huang, D. (2019). Melatonin prevents bone destruction in mice with retinoic acid–induced osteoporosis. *Molecular Medicine*, *25*(1), 43. https://doi.org/10.1186/s10020-019-0107-0

Xie, Z., Chen, F., Li, W. A., Geng, X., Li, C., Meng, X., Feng, Y., Liu, W., & Yu, F. (2017). A review of sleep disorders and melatonin. *Neurological Research*, *39*(6), 559–565. https://doi.org/10.1080/01616412.2017.1315864

Xu, L., Zhang, L., Wang, Z., Li, C., Li, S., Li, L., Fan, Q., & Zheng, L. (2018). Melatonin Suppresses Estrogen Deficiency-Induced Osteoporosis and Promotes Osteoblastogenesis by Inactivating the NLRP3 Inflammasome. *Calcified Tissue International*, *103*(4), 400–410. https://doi.org/10.1007/s00223-018-0428-y

Zisapel, N. (2018). New perspectives on the role of melatonin in human sleep, circadian rhythms and their regulation. *British Journal of Pharmacology*, *175*(16), 3190–3199. https://doi.org/10.1111/bph.14116