



HASIL VALIDASI DAN PENILAIAN
KARYA ILMIAH DOSEN FAKULTAS KEDOKTERAN GIGI UNIVERSITAS AIRLANGGA
(MEDIA PUBLIKASI KARYA ILMIAH : JURNAL ILMIAH INTERNASIONAL)

Nomor : 60 / UN3.1.2. S.9 / PJ / 2023

A. Identitas Karya Ilmiah

- Judul Jurnal Ilmiah (Artikel) : The Effect of Uv-B Exposure and Vitamin K2 on the Bone Formation in Covid-19 Pandemic Era
Jumlah penulis : 11 (sebelas) orang
Status Pengusul : Penulis Ke-2
Identitas Jurnal Ilmiah : a. Nama Jurnal : Journal of International Dental and Medical Research
b. Nomor ISSN: 1309-100X
c. Volume, Nomor, bulan, tahun : Vol. 14, No. 3, tahun 2021
d. Penerbit : Ektodermal Displazi Grubu
e. DOI artikel :
f. Alamat web Jurnal : <http://www.jidmr.com/journal/contents-of-jidmr-2021-vol-14-no-3/>
g. Terindeks di Scimagojr/Thomson Reuter ISI Knowledge atau di :
Q3 (SJR) : 0,21, H.Indeks : 14, Coverage : 2009- on going

- B. Kategori Publikasi Jurnal Ilmiah : Jurnal Ilmiah Internasional Bereputasi (SJR > 0,10)
(beri ✓ pada kategori yang tepat) : Jurnal Ilmiah Internasional Bereputasi (SJR < 0,10)
 Jurnal Ilmiah International terindeks di Web of science clarivate analytics / kelompok emerging sources citation indeks (tidak terindeks SJR)

C. Hasil Validasi Ketua Departemen

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Surabaya, 1 Maret 2023

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Nama : Prof. Dr. Soegeng Wahlujo, drg, MKes,
SpKGA., Supsp KKA(K)

NIP : 1954111019810301003

* Coret salah satu

Form Penilaian Kualitas Karil dan Kesesuaian Bidang Ilmu

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A	Identitas Karya Ilmiah	
1	Judul : The Effect of Uv-B Exposure and Vitamin K2 on the Bone Formation in Covid-19 Pandemic Era	
2	Nama Penulis : 1. Sindy Cornelia Nelwan, 2. Udijanto Tedjosasongko, 3. Satiti Kuntari, 4. Haryono Utomo, 5. Tania Saskianti, 6. Mega Moeharyono Puteri, 7. Devi Dharmawan, 8. Yufita Fitriani, 9. Pradita Agung Kurnia, 10. Amalia Ramadhani Mufida, 11. Nadhia Zahria Fajrin	
3	Nama Jurnal : Journal of International Dental and Medical Research	
B	Peng-index : Scimago Q3 SJR 0,21 (2021)	
C	Relevansi kompetensi dosen dengan substansi karya ilmiah	<ol style="list-style-type: none"> 1. Artikel ini membahas tentang Pengaruh Paparan Uv-B dan Vitamin K2 Terhadap Pembentukan Tulang di Era Pandemi Covid-19 salah satunya adalah kekurangan vitamin D sebagai malnutrisi, Pandemi Covid-19 memaksa semua aktivitas dilakukan dari rumah (misalnya, belajar dan bekerja dari rumah). Berkurangnya aktivitas di luar rumah menyebabkan paparan UV-B yang lebih sedikit. 2. Artikel ini sesuai dengan bidang keahlian pengusul yaitu Cariology. 3. Tidak ada keterkaitan dengan naskah Disertasi pengusul yang berjudul : Initial acquisition and transmission of Mutans Streptococci in children at day nursery
D	Kesesuaian antara lingkup / subjek area jurnal dengan karya ilmiah yang diusulkan	<ol style="list-style-type: none"> 1. Alamat Web Jurnal : http://www.jidmr.com/journal/contents-of-jidmr-2021-vol-14-no-3/ (Web Journal) http://www.jidmr.com/journal/wp-content/uploads/2021/10/23-D21_1493_Sindy_Cornelia_Nelwan_Indonesiac.pdf (alamat pdf) 2. Kebenaran ISSN/ISBN : 1309-100X 3. Termasuk "Predatory" tidak (jurnal; penerbit) : tidak masuk pada predatory 4. Syarat komposisi Editor Board : lebih dari 20 Negara 5. Syarat kontributor penulis artikel : Penulis kedua 6. Keberkalaan penerbitan : 4 terbitan pertahun 7. Subjek area dan katagori jurnal : dental and medical research
E	Kepastian tidak ada pelanggaran integritas akademik	<ol style="list-style-type: none"> 1. Indikasi plagiasi (lihat check similarity) : 19% 2. Fabrikasi : tidak ada 3. Falsifikasi : tidak ada 4. Praktek kepalsuan : tidak ada
Nilai pengusul (penulis pertama dan corespondensi 60%) =		
Nilai pengusul (penulis pertama / penulis corespondensi masing - masing 40%)		
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Penilai Angka Kredit I

Nama :
NIP :
Bidang Ilmu :

The Effect of Uv-B Exposure and Vitamin K2 on the Bone Formation in Covid-19 Pandemic Era

Sindy Cornelia Nelwan¹, Udijanto Tedjosasongko¹, Satiti Kuntari¹, Haryono Utomo²,
Tania Saskianti¹, Mega Moeharyono Puteri¹, Devi Dharmawan¹, Yufita Fitriani¹, Pradita Agung Kurnia¹,
Amalia Ramadhani Mufida¹, Nadhia Zahria Fajrin¹

1. Department of Pediatric Dentistry, Faculty of Dental Medicine, Airlangga University, Indonesia.
2. Department of Dental Forensic, Faculty of Dental Medicine, Airlangga University, Indonesia.

Abstract

Vitamin D deficiency is malnutrition that occurs in developed and developing countries, which worsened by Covid-19 pandemic. Covid-19 pandemic forces all activities doing from home (e.g., study and work from home). Reduced activity outside the home causes less UV-B exposure that beneficial for vitamin D synthesis endogen (>80%). The role of UV-B lamp considered more effective than the sun exposure with unstable intensity. The use of a UV-B lamp is one of new alternative to overcome vitamin D deficiency. Except the UV-B, the vitamin D3 and K2 are also effective in activating vitamin D. It claimed that combination of vitamin D3 and K2 is more effective in activating active vitamin D than single dose of the vitamin itself thus enhancing bone formation markers within the serum.

In vivo experimental study conducted in 96 *rattus novergicus*. The total sample was 96, consisting of 48 BALP serum level groups and 48 OCN serum level groups. The sample divided into six groups, consisting of one control group and five experimental groups (UV-B, Vitamin D3, and K2).

A significant difference was found. ANOVA and paired t-test used to compare the results of each group. An increase was found in all BALP and OCN serum level groups, compared to the control group. A significant difference was found in all BALP and OCN serum level groups ($P < 0.005$).

Combination of 290-315 nm of UV-B, vitamin D3, and K2 supplements improve bone formation marker production in the serum (BALP and OCN)

Experimental article (J Int Dent Med Res 2021; 14(3): 1008-1013)

Keywords: UV-B radiation, vitamin K2, bone formation marker, Covid-19.

Received date: 21 April 2021

Accept date: 18 June 2021

Introduction

A pandemic is a disease that has disseminated worldwide over a certain —usually short period of time. In other words, a pandemic is an epidemic that has spread over multiple continents and spread over the world. The most recent global pandemic is the well-known COVID-19, caused by the coronavirus, which started as an epidemic in Wuhan, Hubei, China. Beginning in December 2019, and spreading to the world within months.¹ The coronavirus

disease 2019 (COVID-19) as a global pandemic disease that announcement by the World Health Organization is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It belongs to the subfamily β -coronaviruses and shares 79.5% of the genetic sequence of SARS-CoV. The clinical disease caused by SARS-CoV-2 is called Coronavirus disease 2019 (COVID-19).^{2,3} COVID-19 pandemic that was declared by WHO on the 11th of March 2020 with a pattern of rapid infection spread and had an impact on decreasing community activities. As a result of the COVID-19 pandemic, schools from kindergarten to college and offices was closed. The community carries out all their activities from home so that physical activities outside the home become less than usual. Work and study activities from home lead to reduce exposure to sunlight (UV-B) in the community, especially children, which can lead to reduce vitamin D in the body which obtained from the synthesis of

*Corresponding author:

Sindy Cornelia Nelwan
Department of Pediatric Dentistry,
Faculty of Dentistry, Airlangga University,
Jl. Prof. Dr. Moestopo No 47
Surabaya-Indonesia
E-mail : sindy-c-n@fkg.unair.ac.id

vitamin D3 in the skin with the initiation of UV-B exposure (>80%).^{3,4}

Ultraviolet-B from sunlight involved in vitamin D synthesis, which is essential in maintaining health. Most in vivo vitamin D synthesis occurs via exposure to sunlight, whereas a small part obtained through diet. Therefore, appropriate exposure to sunlight recommended to satisfy vitamin D need. However, vitamin D deficiency still become a serious issue to many people. To resolve this problem, the study of artificial lighting which can offer exposure of UV-B light with similar characteristic of sunlight.

Vitamin D production in human skin based on sun exposure depends on the position of the sun, or the zenith angle. The amount of UV radiation reaching the earth's surface increases when the zenith angle decreases. Moreover, the zenith angle depends on the latitude, season, and time of the day. As a result, the optimal times for vitamin D production in human skin between 10 AM-3PM. The frequency, season, latitude, altitude, and weather conditions can influences the cutaneous production of vitamin D3. It is difficult to obtain an adequate amount of vitamin D3 from sun exposure without some guidance. Furthermore, it is difficult to spend outdoor activities between 10 AM and 3 PM because of working time. Therefore, UV-B lamp as an alternative for vitamin D synthesis. UV-B lamp can also use for treating and preventing recurrent vitamin D deficiency in patients whom unable to absorb vitamin D through their gastrointestinal tract due to lipid malabsorption.⁵

Human body has two major sources of vitamin D synthesis, UV-B exposure for skin and dietary supplements. Bone and tooth development needs micronutrients such as calcium, magnesium, phosphor, fluoride, and vitamin D to increase their strength.⁶ Inactive vitamin D (7-dehydrocholesterol) with 290-315 nm UV-B formed pre-vitamin D3 (25 dihydroxycholecalciferol) which could be hydrolyzed and changed into an active form of 1.25 dihydroxycholecalciferol known as calcitriol. Active form of vitamin D (1.25 dihydroxycholecalciferol or calcitriol) has biological effect on vitamin D receptors (VDR) in osteoblast.^{7,8}

Vitamin D deficiency is a malnutrition issue that occurs in both developing and developed countries, which worsened by the

Covid-19 pandemic. Covid-19 pandemic has changed several life aspects due to the virus, some restrictions are applied. Countries around the world were applied physical distancing policies. Accordingly, social interaction, learning activities were carried out from home.⁹

According to American Academy of Pediatric (AAP), vitamin D deficiency (less than 20 ng/mL) known as hypovitaminosis D. Hypovitaminosis D resulted from either insufficient UV-B exposure or intestinal absorption. Hypovitaminosis D on bone is manifested by the decrease in calcium and phosphor absorption, causing the parathyroid gland produce parathyroid hormone (PTH) that expected to recover the calcium level. The increasing of PTH results in calcium reabsorption and phosphor excretion was lead the body to produce osteoclast and reduce bone mineralization. If this condition remains for weeks or months, this condition lead to stunting, rickets, and muscle weakness that makes children difficult to stand and walk.^{10,11}

Before the Covid-19 pandemic, it is reported that Southeast Asian citizens suffered from vitamin D deficiency, which indicated by the level of 25-hydroxyvitamin D [25 (OH) D] that is less than 50 nmol/L (20 ng/ mL)¹². **Based on previous study by Fitrah Ernawati et al, vitamin D level in 2 - 13 years old children in Indonesia showed that vitamin D status, especially in school-aged children should be supervised.**¹³

Chronic vitamin D deficiency may become a factor that modulates skeletal defects in children, such as micrognathia, microgenia, and retrogenia. Studies on patients with crowding due to narrow dental arch often found lower vitamin D concentration levels. Vitamin D plays important role in bone and teeth metabolism and the immune system^{14,15}. In addition to vitamin D, vitamin K is also important in mineralization process by increasing osteoblast activity in producing alkaline phosphatase (ALP) and Osteocalcin (OCN). ALP is membrane bound glycoprotein found on growing cells, physiologically occurs during bone formation in developmental stages.¹⁶ The absence of heart disease and the cardiac enzyme is within the normal level, ALP is considered to represent Bone-Specific Alkaline Phosphatase (BALP). During the bone growth period in children and adolescents, BALP dominates and contributes up

to 90% of the total of Alkaline Phosphatase.^{17,18} Nutritional adequacy is crucial in the mineralization process, the role of vitamin D3 and K2 supplements were expected to support the mineralization process^{19,20}. Osteocalcin (OCN) is a proteinous noncollagen calcium ion binder, which is also known as γ -carboxy-glutamic acid protein (bone-Glaprotein).²¹

The mineralization process was examined by bone formation markers, including BALP and OCN level. In mineralization process, BALP functions was prepare the alkalic conditions within formed osteoid tissue, allowing calcium easily deposited to the tissue, OCN stimulate matrix deposition and calcium transport to the bone^{18,22}.

Materials and methods

The study design was in vivo experimental laboratory study, we are using BALP and OCN serum level were carried out from 96 white rats (*Rattus novergicus*), the inclusion criteria were 50-200 grams of male Wistar rats (2-3 months old), generally in good condition, adapted for 1 week. We are using 1000 IU of Vitamin D3 (Doctor's Best, US) and 45 mcg of Vitamin K2 (Doctor's Best, US) supplements that were given to the rats once a day. Besides giving the vitamin, we are also giving the rats 290-315 nm UV-B exposure (Phillips) for 25 minutes, three times a week. These 96 white rats (*Rattus novergicus*) divided into six experimental groups:

- Group I : control group
- Group II : Vitamins K2 and D3 supplements.
- Group III : UV-B exposure
- Group IV : UV-B exposure and vitamin K2 supplement.
- Group V : Vitamins D3 and K2 supplements.
- Group VI : UV-B exposure, and vitamins D3 and K2 supplements.

Procedure

Serum were taken using an anesthetic procedure. They were taken at the day before the intervention was given (pre-test), post-treatment value of BALP level was taken at the day-14, while that of OCN level was taken at the day-21. BALP and OCN serums level measured using the enzyme-linked immunosorbent assay

(ELISA) test (Bioenzy, Germany).

Research Ethics

An approval issued for this study by Commission of Ethical Clearance, Faculty of Dentistry Universitas Airlangga numbered 370 / HRECC.FOOM / VIII / 2020.

Results

Anova test resulted significant difference for post-treatments groups in BALP and OCN level ($P < 0.05$). Paired t-test resulted the significance between pre and post treatment groups. On BALP groups (after 14-days treatments) and OCN groups (after 21-days treatment) there was significant difference ($P < 0.05$) was found between pre and post treatment groups. The highest level of BALP and OCN serum was found in Group VI and the lowest level was found in the control group I (figure 1 and 2).

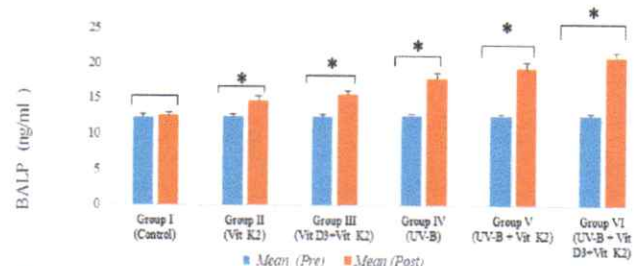


Figure 1. BALP level, a significant difference was found between the number of BALP before and after treatment as shown by paired t-test value ($\text{sig} < 0.05$). The statistical analysis resulted the highest value of produced BALP was obtained from group VI (UV+ Vitamin D3+K2), followed by group V (UV + vitamin K2), Group IV (UV), Group III (Without UV + Vitamin D3+K2), Group II (Without UV + Vitamin K2) and Group I (Control Group).

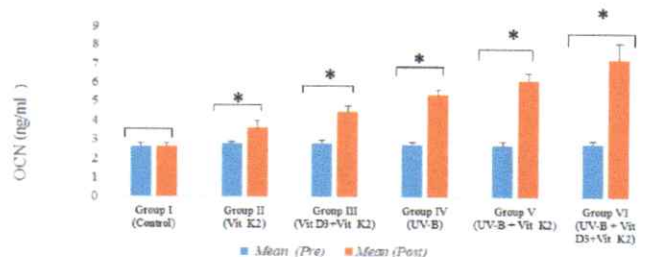


Figure 2. OCN level, a significant difference was found between the number of OCN before and after treatment as shown by paired t-test value ($\text{sig} < 0.05$). The statistical analysis resulted the

highest value of produced OCN was obtained from group VI (UV+ Vitamin D3+K2), followed by group V (UV + vitamin K2), Group IV (UV), Group III (Without UV + Vitamin D3+K2), Group II (Without UV + Vitamin K2) and Group I (Control Group).

Note: symbol (*) intergroup significance.

Discussion

Nutrition plays important role in determining oral health which can affects teeth and oral structure.²³ Vitamin, mineral, and other nutritions are important for the development, growth, maintenance, and recovery of teeth and oral structures. In addition to calcium and vitamin D, the most recent study shows that the combination of calcium, vitamin D3 affects bone metabolism, especially in increasing bone mass density.²⁴ Bone quality can be established based on its size and shape, remodeling process, mineral composition, and collagen content. In remodeling process, the cells playing a role include progenitor cells, osteoblast, osteocytes, and osteoclasts.²⁵

The bone remodeling process is usually measured by bone marker. The homeostasis of the bone matrix can reached by the balance of inflammatory factors.²⁶ Bone apposition marker results from the product of collagen biosynthesis (e.g., propeptides type I) or bone/osteoblast-related protein/enzyme such as ALP and OCN.^{18,27,28} ALP is synthesized by osteoblast which involved in the calcification of bone matrix and mineralization.¹⁶ The absence of heart disease and the cardiac enzyme is within the normal level, ALP is considered to represent Bone-specific Alkaline Phosphatase (BALP). During the bone growth period in children and adolescents, BALP dominates and contributes up to 90% of the total of Alkaline Phosphatase^{17,18} In calcium kinetics assessment on bone formation markers, OCN within the serum is correlated with the bone formation and the number of osteoblasts. Another study shows that OCN was the most abundant protein, which originates from new bone formation.²⁹

BALP is a specific enzyme produced by osteoblast that can decrease the production of inorganic pyrophosphate, and mineralization inhibiting compound.²⁸ In addition to BALP, OCN is one of the most abundant non-collagen proteins that affect mineral matrix deposits in the

process of mineralization. Osteocalcin (OC) is a proteinous noncollagen calcium ion binder, which is also known as γ -carboxy-glutamic acid protein (bone-Glaprotein). It is produced by both osteoblasts and odontoblasts.²¹ OCN regulates calcium ions in the hydroxyapatite (HA) mineral formation. OCN organizes bone extracellular matrix, modulates hydroxyapatite size, and forms a complex with collagen through osteopontin matrix protein, thus increasing the amount of extracellular matrix²⁷ Bone matrix mineralization is the last process of osteogenesis. This process depends on physicochemical and biochemical process that allow Hydroxyapatite (HA) deposition in the extracellular matrix. Mineralization is a second stage process that begins in 5-10 days after organic matrix deposition. When the mineral deposition reaches 70 percentage, the mineralization process running slow and the second stage begins, i.e., maturation process from HA mineral content.

Mineralization of HA crystal begins in matrix vesicles (MV) produced by osteoblast and grows through the plasma membrane through the extracellular matrix. HA crystal grows through the MV membrane and reaches extracellular space. An isolated MV consists of various enzymes, and ALP is the main enzyme. ALP is concentrated to the outer surface of the MV membrane, attached to a bilayer lipid membrane, and bonded to glycosylphosphatidylinositol (GPI) receptor.³⁰

Deposition of HA in MV begins with the accumulation of phosphate by the activity of PHOSPHO1 on phosphoetanolamina (PEA) and phosphocholine. Inorganic phosphate is produced extravascularly from the Inorganic pyrophosphate (PPi) and ATP solution due to the enzymatic action of BALP. BALP and nucleotide pyrophosphatase/phosphodiesterase. PPi emerged outside MV as the inhibitor of potential precipitation and HA crystal growth and prevent free phosphate (Pi) bounded with calcium.³⁰

Nowadays, children activities are identical to the use of technology, such as online games.³¹ Online games make children spend their times indoors without sunlight exposure. Besides, during the covid-19 pandemic where mobility restriction is crucial, individuals are not exposed to sunlight frequently, otherwise the largest source of vitamin D (80% of sources of vitamin D)^{32,33}

In the present study, the important component of bone and tooth mineralization was

obtained through intake of UV-B exposure, optimal doses of vitamin D3 and K2. Based on the result of the study, it was found that the combination of UV-B exposure and oral supplementation of vitamin D3 and K2 exhibited the most optimal result in the level of BALP and OCN, the most important elements in bone and teeth mineralization. The previous study by Ballegoijen et al. showed that the combination of vitamin D and K supplements is more effective in maintaining bone and cardiovascular health, compared to a single vitamin. In this study, bone health was indicated by the serum level of BALP. Vitamin D3 functions change the concentration of vitamin K-dependent protein and to induce in vitro bone formation through osteoblastic specific expression stimulation. Vitamin K2 is required in the carboxylation or activation process of vitamin-k dependent. Vitamin D3 induces the concentration of vitamin K-dependent protein, resulting in osteoblastic specific expression, OCN and matrix Gla protein.³⁴

Vitamin D3, K1, and K2 (MK-4 and MK-7) have osteoconductive effect, anti-inflammation, and antioxidant. These vitamins can prevent Reactive Oxidative Stress (ROS) production.³⁵ It was found that the increase in ROS production directly affects the biological activity of osteoblast through inhibition of runx2 phosphorylation, a process responsible for collagen type I transcription, osteopontin, osteoprotegerin, sialoprotein, OCN, and BALP. A study by Abrozewicz et al showed that vitamin D3 and K2 protect osteoblast from ROS and were useful for tissue proliferation, differentiation, and mineralization. This study explains that vitamin K strengthens the proliferation activity of osteoblast (measured DNA concentration), with MK-7 as the most significant type of vitamin K2 that enhances osteoblast proliferation, either when combined with vitamin D3 or not.³⁵

Vitamin D3 promote osteoblast differentiation, as shown by the increase in specific marker expressions, such as run 2 (RUNX2)-related transcription factor, type I collagen, BALP, and OCN. The study shows that, unlike vitamin D3, vitamin K2 does not affect osteoblast differentiation. However, the combination of vitamin K2 and D3 can significantly increase osteoblast differentiation.³⁵

In this study, it was found that BALP level was formed more significant in the group with UV-B exposed compared to the group that was

only treated with vitamin D3 and K2 supplementation. This research supports that the majority (80%) of vitamin D is obtained through pre-vitamin D (7 dehydrocholesterol) activation through sunlight exposure and the rest of it is obtained through diet intake.⁴ The majority of vitamin D production (80%) is obtained through sensitization from UV-B exposure, which can stimulate osteoblast differentiation in the BALP formation. This is also in line with Mustafa and Abulmeaty's (2016) study, which found that sunlight exposure brings a more significant effect on bone formation structure and homeostasis, compared to vitamin D dietary supplementation. This is more significantly affect in rats of lacking vitamin D than in rats with adequate vitamin D.³⁶

The role of BALP in the mineralization process is to prepare alkalic condition within the formed osteoid tissue, it was done by suppressing the number of mineralization inhibitor, i.e., inorganic pyrophosphate (PPI) and changing it into inorganic phosphate (Pi), allowing calcium to be easily deposited within the tissue.¹⁸ The role of osteocalcin in the mineralization process is to bind calcium and prepare the framework for calcium deposit within the matrix. In an x-ray crystallography study, the form of OCN consists of a 3-dimensional structure containing three Gla residues. These three Gla residues interact with calcium in the Hydroxyapatite bond, which takes a role in mineralization stage maturation. The increase in OCN occurs along with hydroxyapatite mineral deposition during the maturation process.^{29,37} Calcium phosphate is a bond in the form of hydroxyapatite crystal and based on the law of mass action, the crystal deposits within bone and teeth. The increasing enzyme within the tissue creates an alkalic condition that stimulates the deposition of calcium ion, thus increasing the quality of bone mineralization.³⁰

Conclusions

Combination of 290-315 nm of UV-B, vitamin D3, and K2 supplements improve bone formation marker production in the serum (BALP and OCN).

Declaration of Interest

The authors report no conflict of interest.

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