# Effect of Vitamin D<sub>3</sub> and Virgin Coconut Oil on Cartilage Degeneration, Inflammation and Functional Abilities in Early Knee Osteoarthritis

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# ABSTRACT

**Background:** Knee osteoarthritis (KOA) is a chronic, degenerative knee joint disorder associated with inflammation, pain, stiffness, and reduced functional abilities, thereby impacting the patient's quality of life. The prevalence of KOA is growing rapidly in India and around the globe, even in younger populations. Vitamin D plays a crucial role in musculoskeletal health and deficiency of this vitamin is highly prevalent across the age groups impacted by KOA. Consumption of vitamin D rich foods along with functional foods possessing antioxidant and analgesic properties needs to be explored as a potentially novel, low cost dietary strategy for the prevention and management of chronic musculoskeletal disorders such as KOA.

**Objective:** To assess the effect of vitamin  $D_3$  and Virgin Coconut Oil (VCNO) supplementation on vitamin  $D_3$  status, cartilage degeneration, inflammatory status, and functional abilities in early KOA.

**Methods:** One hundred vitamin D deficient, age and gender-matched adults afflicted by early KOA (30-65yrs) were selected purposively from K. J. Somaiya Hospital and Research Centre, Mumbai, India after obtaining ethical clearance from the institute. Informed consent was obtained

from the participants. They were then counselled on making required dietary modifications, with due emphasis on vitamin  $D_3$  rich foods, and were given an at-home KOA exercise program. The participants were divided equally into two experimental groups: E1 (n=50) and E2 (n=50), with equal number of males and females in each group. The groups were either supplemented with Vitamin  $D_3$  alone (group E1) or Vitamin  $D_3 + VCNO$  (group E2). All the participants were assessed for vitamin D status (serum 25 (OH) D levels (CLIA), Vitamin  $D_3$  intake (3-day diet recall and FFQ), pain (VAS), stiffness, functional abilities (WOMAC and 6 MWT), and inflammation (ESR and serum CRP). The cartilage marker (s-COMP (ELISA)) was assessed only in a subset of participants (n =40) from each group, both pre and post intervention. Data were analyzed using SPSS 16.0.

**Results:** A significant rise in Vitamin  $D_3$  intake and serum vitamin  $D_3$  levels (p<.001) was observed post-supplementation in both groups. sCOMP (<.001), ESR, and serum CRP (<.001) were significantly reduced in both the groups, indicating a decline in cartilage degeneration and inflammatory status. VAS score (<.001) was significantly reduced in both groups, indicating reduced pain intensity. Total WOMAC score (p<.001) was significantly reduced, with a highly significant improvement in the distance covered during the 6 MWT (<.001), indicating improved functional abilities. The mean difference of effect in all the above parameters was higher in the vitamin  $D_3$  and VCNO supplementation group (E2) than the group which received vitamin  $D_3$  alone (E1).

**Conclusion:** Supplementation of vitamin  $D_3$  along with VCNO could be an effective strategy for delaying the progression of KOA by reducing cartilage degeneration, inflammation and pain, as well as improving functional abilities. Thus, simultaneous improvement of vitamin  $D_3$  status and oxidative stress should be considered in early KOA management. Non-conventional antioxidant and anti-inflammatory functional foods such as VCNO could be further explored.

**Key words:** Knee osteoarthritis, vitamin D, virgin coconut oil, anti-inflammatory, functional food, functional abilities, cartilage degeneration, inflammation, WOMAC, sCOMP.

# INTRODUCTION

OA is a chronic degenerative joint disease characterized by cartilage degeneration, inflammation, and functional disability. Common symptoms are pain and stiffness which often impact a patient's quality of life [1, 2]. It occurs in the joints of the hands, spine, hips, and knees. Knee osteoarthritis (KOA) prevalence is growing rapidly in India (28.7%) and around the globe in both urban and rural areas. Its incidence is currently higher in women than in men and a rising trend has been observed in younger populations. This change can have a tremendous impact on the functional capacity and productivity of these groups [5]. Progressive KOA shows an increase in bone resorption similar to osteoporosis [6]. Early stages of KOA can be managed by diet and exercise, however, advanced stages require surgical treatment. Appropriate dietary and exercise strategies are low cost, non-pharmacological interventions that could reduce cartilage degeneration and inflammation. Thus the anti-inflammatory and anti-oxidative roles of traditional functional foods need to be explored as a novel approach to the management of chronic musculoskeletal disorders.

Moreover, deficiency of Vitamin D is highly prevalent across the age groups impacted by KOA in India and globally [7, 8]. This indicates a strong need for further research on the role of vitamin D in the management of KOA [9]. Vitamin D contributes to joint health as an anti-inflammatory agent and if oxidative stress were to be controlled simultaneously, patients would likely encounter greater relief from the symptoms of degenerative diseases like KOA. There is a paucity of data on the definitive role of nutrition, specifically the impact of vitamin D and antioxidants, in minimizing osteoarthritis symptoms, thus necessitating evidence-based research [10]. Virgin coconut oil (VCNO) is an emerging functional food that has shown antioxidant effects in rats and which promotes osteoblastic activity, reducing bone resorption [11]. Most VCNO studies are on animal models and hence need to be scaled up to human research. In humans, VCNO could also have antiinflammatory effects and facilitate the absorption of vitamin D in the gut if consumed simultaneously. Hence its role in the management of degenerative bone diseases such as KOA could be further explored. This study was conducted to understand the "Effect of vitamin D<sub>3</sub> and Virgin Coconut oil on cartilage degeneration, inflammatory status, and functional abilities in early knee osteoarthritis." The investigators proposed the following null hypothesis: The intake of Vitamin D<sub>3</sub> and VCNO does not offer any cumulative benefits to KOA patients.

# MATERIALS AND METHODS

This research on the effect of Vitamin  $D_3$  and VCNO in early KOA, is an interventional, clinical trial approved by the Medical Ethics Committee of K.J. Somaiya Medical College, Hospital, and Research Centre, Mumbai, India.

*Place of the Study:* The study was conducted at the Department of Nutrition and Dietetics and the Department of Orthopaedics at K.J.Somaiya Hospital and Research Centre.

*Sampling*: One hundred vitamin D deficient (< 20 ng/ml) [12], age and gender-matched early KOA patients (30-65yrs) with symptoms of Kellgren Lawrence (KL) grades I and II [13] were diagnosed by an expert Orthopaedic surgeon and selected purposively from K. J. Somaiya Hospital and Research Centre, Mumbai, India. Pregnancy and lactation, history of arthroscopy, presence of other forms of arthritis, administration of intra-articular steroids within the past 3 months, administration of hyaluronic acid in the last 9 months, the presence of a simultaneous unstable disease (i.e. typhoid, malaria, tuberculosis, jaundice, tumor), cancer, severe renal or hematologic disorder, endocrine/hormonal disorders (thyroid and parathyroid but not diabetes mellitus), cardiac insufficiency, moderate to severe neuropathy, and spondylosis were the conditions considered for exclusion. The patients screened in the bone health camps who fit the inclusion-exclusion criteria were then made aware of the study and its potential benefits, and were given the opportunity to enroll. Those who voluntarily agreed to participate in the study were asked to sign an informed consent form.

**Research Design:** An experimental research design was planned to study the effect of the vitamin  $D_3$  and VCNO supplementation. The selected participants were divided equally into two experimental groups: Experimental Group 1 (E1) (n=50) and Experimental Group 2 (E2) (n=50), each with an equal number of males and females.

Biochemical and Dietary Assessment: A standardized questionnaire was used to record all the baseline data of the participants (sociodemographic information, physical assessment, biochemical assessment, diet history, sun exposure details, and functional abilities). All the participants were assessed for vitamin D status by the CLIA method using the ADVIA centaur XP Vitamin D Total Assay, Immunoassay system (Siemens Healthcare, Erlangen, Germany) [14, 15]. Their vitamin D<sub>3</sub> intake was assessed via a dietary history survey (3-day diet recall and food frequency questionnaire) [16]. Sun exposure data such as timing, duration, frequency, percent skin exposure, skin color, and clothing were also recorded [17, 18]. Other data were recorded such as selfperceived pain score via the visual analogue scale (VAS) [19], stiffness and functional abilities via the Western Ontario McMasters Osteoarthritis (WOMAC) Index [20] and a six minute walk test (6 MWT) [21], inflammation markers (Erythrocyte sedimentation rate, ESR) via the Westergren method [22], and serum C-reactive protein (CRP) via Rhelax slide test [23]. Serum cartilage oligomeric matrix protein (s-COMP), a novel prognostic biomarker and non-aggrecans marker of cartilage degeneration for early diagnosis of KOA patients [24], was analyzed at the National Institute of Immunohaematology (NIIH), ICMR, Mumbai, India, using the Human COMP ELISA Kit-EK0913 (Boster Bio, Fremont, California, USA) [25]. The standard reference values and cutoffs for the above methods are given below (Table 1).

Parameter Markers		Standard Reference Values	Cut-offs	
Vitamin D <sub>3</sub>	Serum 25 (OH) D (ng/ml)	30-70 #	> 30	
Pain score	VAS score (mm)	40-70*	>40	
Functional abilities score	Total WOMAC score	0- 96*	$\leq 59 \text{ (low)}$ 60-80 (moderate) $\geq 81 \text{ (high)}$	
Inflammatory	ESR (mm/hr)	0-20**	< 20	
markers	Serum CRP (mg/dl)	0 -2 **	< 2.0	
Cartilage biomarker (KOA prognostic biomarker) sCOMP (pg/ml)		6000-7000***	<6000	

**Table 1.** Standard Reference Values and Cut-Offs of the Assessed Markers.

# Reference values and cut-offs for serum vitamin D levels, Institute of Medicine guidelines (2010) [12].

\* Reference values and cut-offs for VAS, Cut-off points for pain, Boonstra et al., in 2014 and Total WOMAC scores for early KOA (KL grades I and II) patients, www.rheumatology.com/WOMAC [19, 20]. \* Reference values and cut-offs for ESR and CRP levels, Westregren method, and Rhelax Slide test respectively [22, 23].

\*\*Reference values and cut-offs for sCOMP (Human COMP ELISA, Boster Bio, Fremont, USA) were established during this study at NIIH [24].

*Dietary and Exercise Modifications:* All the participants (n=100) were recommended dietary and exercise modifications with due consideration to their nutritional status and in accordance with ICMR guidelines (2010) [26], which include the management of obesity (small and frequent meals, and a balanced diet) as well as emphasis on the intake of bone micronutrients and anti-inflammatory foods (Table 2).

*Home Exercise Regimen for Early KOA:* Well-planned home exercise regimens have been reported to be effective in KOA management [27], hence a structured home-based KOA exercise program was advised for these participants. The program included a 3-4-minute warmup of gentle walking, followed by eight exercises: isometric quadriceps exercises, straight leg raise, static hold exercise, isometric hamstring exercise, knee flexion, knee extension, and hip abduction. For each exercise, participants were instructed to perform 10-15 repetitions, 2-3 times daily for the strengthening of quadriceps, hamstrings, vastus medialis, obliques, and tensor fascia lata muscles [28], and were asked to avoid cross legged sitting and squatting postures.

*Dietary Supplementation Protocols:* For the two experimental groups, the recommended supplementation protocols were intended for a period 12 weeks. Details of the implemented dietary supplementation protocols were as follows:

*Vitamin*  $D_3$  *Sources and Supplementation:* The dietary intake of Vitamin  $D_3$  was calculated using the recent Indian food data composition tables for vitamin  $D_3$  [29]. After considering the serum 25 (OH) D levels, dietary vitamin  $D_3$  consumption and sun exposure patterns of both groups, they were provided recommendations on modifying their consumption of vitamin D rich foods and on sun exposure. Additionally, they were supplemented with an injectable form of vitamin D, followed by weekly doses in powder form and daily doses in tablet form. Details of these dosages are given in Table 2.

Duration (Weeks)	Form and dose of vitamin D <sub>3</sub> supplementation as suggested by the expert Orthopedic Surgeon	Suggestions for Dietary Vitamin D3 and calcium intake and sun exposure
1 <sup>st</sup> week	One intramuscular injection of 600,000 IU	sardines, salmon were suggested as per the
2 <sup>nd</sup> to 6 <sup>th</sup> week	Oral supplements (powder) 60,000 IU Vitamin D <sub>3</sub> sachet once a week for 5 weeks	<ul> <li>dietary requirements to meet daily RDA of vitamin D.</li> <li>Calcium rich foods: milk, curd, Bombay duck, green leafy vegetables, dried prunes.</li> </ul>
7 <sup>th</sup> to 12 <sup>th</sup> week	Oral supplements (tablet)–500 mg elemental calcium and 400 IU Vitamin $D_3$ capsule daily	• Sun exposure: suggestion of direct sunlight exposure for 10-15 minutes between 11 am to 2 pm without sunscreen, with arms and face exposed.

Table 2. Vitamin D<sub>3</sub> Supplementation, Dietary and Sun Exposure Recommendations.

# Note: Vitamin  $D_3$  and calcium rich foods list was adapted from Indian Food Composition tables (2017) and vitamin  $D_3$  requirements are in accordance with the RDA for Indians (2010) [29, 30]. The sun exposure window and duration mentioned in the table are in accordance with the geographic location of the tropical country, India [31].

*VCNO supplementation:* Group E2 received 2 teaspoons (i.e. 10 ml) of branded, FSSAI approved VCNO, which is 25% of the recommended allowance of daily visible dietary fat intake in

accordance with the RDA (2010) for Indian adults [30]. This dosage was administered twice daily during a regular meal, as a topping on the food consumed, (i.e. it was not involved in the cooking process) because exposure to high temperatures can cause the degradation of VCNO via oxidation. Participants (n=100) attended monthly follow-ups at the hospital. The follow-ups were planned to assess adherence to suggested diet and exercise changes along with providing reinforced counselling for diet and exercise.

*Post Intervention Reassessment:* After 12 weeks of supplementation, all the participants were reassessed for the various biochemical and functional markers mentioned above.

*Statistical Analysis:* The data were compiled into Microsoft Excel 2009 and analyzed using SPSS 16.0. A paired t-test was used to determine the effect of intervention on the selected parameters measured for the subjects of each experimental group. An independent sample t-test (unpaired t-test) was used to ascertain which of the 2 supplementation regimens was more effective in altering the selected parameters of early KOA management.

#### RESULTS

The results obtained after statistical analysis of the collected data are presented in the form of tables and graphical figures. To understand the effect of supplementation, the pre-post supplementation data within each of the experimental groups were analyzed. Prior to supplementation, all the participants had low vitamin D status.

The present study findings revealed a highly significant rise in dietary vitamin  $D_3$  intake and serum vitamin  $D_3$  levels (p<.001) in both the groups—Vitamin  $D_3$  (E1) and Vitamin  $D_3$  and VCNO (E2) supplementation groups (Table 3). This indicates the effectiveness of the administered dosage of vitamin D in improving vitamin D status of the early KOA participants who were deficient in vitamin  $D_3$  before intervention.

The results were further analyzed to understand any difference between the effectiveness of the two interventions in altering the selected parameters. This analysis is presented in Figures 1, 2, 3.

Positive effects on cartilage degeneration, inflammation, and functional abilities were seen in both experimental groups (Figures 1-3). Further, to understand the level of significance in the effectiveness of the two supplementation protocols, an unpaired t-test was applied to the mean difference of pre and post-supplementation values for various parameters. The results are presented in Table 4.

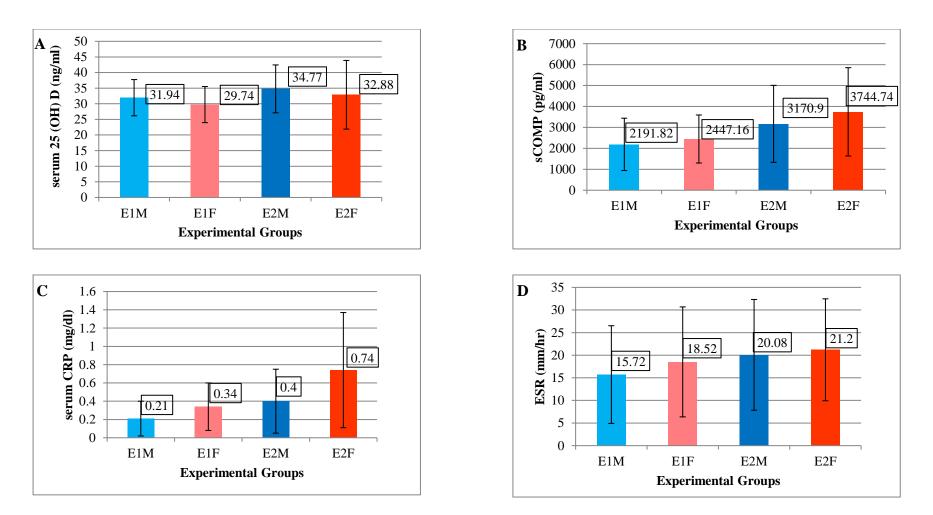
Though a highly significant rise in serum 25 (OH) vitamin D was observed postsupplementation in both groups, the mean difference between the groups was not significant.

Prior to supplementation with vitamin  $D_3$ , progressive cartilage degeneration along with increasing severity of KOA was observed in the patients, supported by significantly higher (p<.05) mean sCOMP levels in grade II than in grade I patients. But, post-supplementation, serum CRP levels and ESR status showed highly significant reductions in participants of both genders of both the groups (t=6.471, p<.001 and t=7.614, p<.001) (Table 3). A declining trend was observed in the serum inflammatory markers, ESR and CRP, in both experimental groups (Figure 1C and 1D).

**Table 3.** Vitamin D Status of the Participants (n=100).

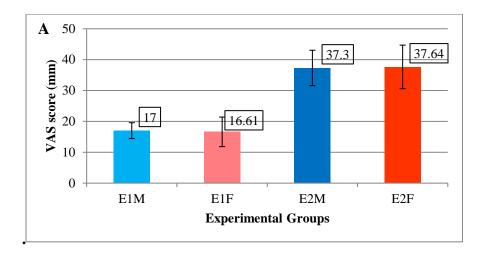
		E1 (Vitamin D <sub>3</sub> supplementation) (n=50)				E2 (Vitamin D <sub>3</sub> +VCNO supplementation) (n=50)				
Parameters	Gender	Pre	Post	t value	p value	Gender	Pre	Post	t value p valu	
		Mean ± SD	Mean ± SD				Mean ± SD	Mean ± SD		p value
Serum 25 (OH) D levels (ng/dl)	Males (n=25)	14.02 ± 3.57	46.04± 9.236	-27.50***	.000	Males (n=25)	12.96 ± 3.17	47.73 ± 10.76	-22.66***	.000
	Females (n=25)	13.93 ± 3.60	43.67 ± 9.10	-25.72***	.000	Females (n=25)	12.81 ± 3.37	45.68 ± 14.07	-14.94***	.000
Dietary Vitamin D <sub>3</sub> intake (IU)	Males (n=25)	27.24 ± 22.59	49.15 ± 28.04	-5.54***	.000	Males (n=25)	22.61 ± 17.24	36.44 ± 22.88	77***	.000
	Females (n=25)	16.07 ± 14.24	34.62 ± 20.26	-6.09***	.000	Females (n=25)	23.32 ± 18.37	39.33 ± 21.16	-6.71***	.000

#Note : (\*\*p<.01;\*\*\*p<.001); (#E1-vitamin D<sub>3</sub> supplementation ; E2-vitamin D<sub>3</sub> and VCNO supplementation).



**Figure 1.** Post-supplementation effects and the mean reduction of A) serum 25 (OH) D levels, B) sCOMP levels (cartilage marker), C) CRP levels (inflammatory marker), D) ESR levels (inflammatory marker) of the participants.

# E1M-Experimental Group 1 Males, E1F-Experimental Group 1 Females; E2M-Experimental Group 2 Males, E2F-Experimental Group 2 Females.



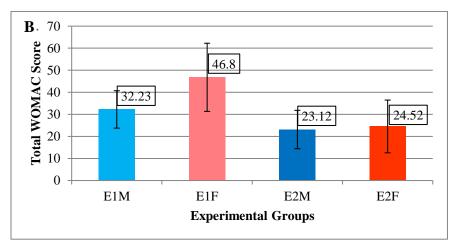


Figure 2. Mean reduction of VAS score and Total WOMAC score upon supplementation.

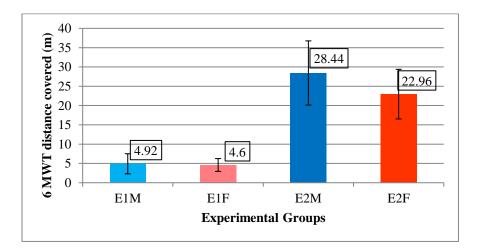


Figure 3. Mean improvement of distance covered during 6MWT upon supplementation.

# E1M–Experimental Group 1 Males, E1F–Experimental Group 1 Females; E2M–Experimental Group 2 Males, E2F–Experimental Group 2 Females.

Parameters	Gender	E1 (Mean ± SD)	E2 (Mean ± SD)	t value	p value
Serum 25 (OH)	Male (n=25)	$31.94 \pm 5.81$	34.77 ± 7.67	-1.48	.15
D (mg/dl)	Female(n=25)	$29.74 \pm 5.78$	32.88 ± 11.00	-1.26	.21
Serum CRP (mg/dl)	Male (n=25)	.21 ± .19	.40 ± .35	-3.25**	.002
	Female(n=25)	.34 ± .26	.74 ± .63	-2.96**	.006
ESR(mm/hr)	Male (n=25)	$15.72 \pm 10.80$	20.08 ± 12.25	-1.34	.19
	Female(n=25)	$18.52 \pm 12.16$	21.20 ± 11.27	81	.22
sCOMP (pg/ml)	Male (n=25)	2191.82± 1249.57	3170.9 ± 1837.95	-5.37***	.000
	Female (n=25)	2447.16 ±1146.91	3744.74 ± 2108.61	-7.02***	.000
6MWT distance	Male (n=25)	$4.92\pm2.60$	$28.44 \pm 8.32$	-13.49***	.000
(m)	Female(n=25)	$4.60 \pm 1.66$	$22.96 \pm 6.43$	-13.83***	.000
VAS score (mm)	Male (n=25)	$17.00\pm2.57$	$37.30 \pm 5.74$	-16.15***	.000
	Female(n=25)	16.61 ±4.79	37.64 ± 7.05	-12.35***	.000
Total WOMAC score	Male (n=25)	8.48 ± 22.23	23.12 ±8.70	-9.61***	.000
	Female (n=25)	$46.80 \pm 15.45$	$24.52 \pm 11.95$	-8.46***	.000

Table 4. Mean Difference in the Effectiveness of Su	upplementation on Various Parameters.
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# Note: (\*\*p<.01; \*\*\*p<.001); (#E1, vitamin D<sub>3</sub> supplementation ; E2 vitamin D<sub>3</sub> and VCNO supplementation).

sCOMP (<.001), ESR, and serum CRP (<.001) reduced significantly in both the groups, indicating a decline in cartilage degeneration and inflammatory status. However, the reduction in the ESR and CRP was lower with vitamin  $D_3$  supplementation alone than with vitamin  $D_3$  and VCNO supplementation. Similarly, a higher reduction in mean sCOMP levels was observed with the double supplementation received by experimental group 2 (Figure 1B).

VAS score (<.001) significantly reduced in both groups, indicating reduced self-perceived pain intensity. Improvements in functional abilities and pain (as indicated by the increased 6-MWT and reduced VAS and Total WOMAC scores) were higher with vitamin  $D_3$  and VCNO supplementation than with vitamin  $D_3$  supplementation alone (Figures 2-3).

The results presented in Table-3 revealed a significant improvement in distance covered during the 6MWT (p<.001), and a significant reduction in VAS score (p<.001) and total WOMAC score (p<.001) in both the experimental groups, with the improvement being higher in E2,

indicating a synergistic effect between vitamin  $D_3$  and VCNO on pain and functional abilities in early KOA patients. Interestingly, the mean distance covered during 6-MWT was higher and mean Total WOMAC score was lower in male participants compared to female participants in both groups. Total WOMAC score (p<.001) significantly reduced, with a highly significant improvement in the distance covered during the 6 MWT (<.001) more in female participants than in male in both the experimental groups indicating improved functional abilities.

Vitamin D<sub>3</sub> and VCNO supplementation also showed a positive effect on all the parameters studied including serum CRP (p<.001), sCOMP (p<.001), VAS score (p<.001), total WOMAC (p<.001), and 6 MWT distance (p<.001) for both the genders. But the effect on serum vitamin D levels and ESR was not significant (Table 3). The mean difference of effect in all the above parameters was higher in the vitamin D<sub>3</sub> and VCNO supplementation group (E2) than the group which received vitamin D<sub>3</sub> alone (E1).

#### DISCUSSION

*Vitamin D Supplementation in Early KOA:* Vitamin D supplementation improved the distance covered during the 6MWT and WOMAC score, and reduced VAS score with an improvement in serum vitamin D levels in early KOA [32]. The administered doses of calciferol (bolus dose of 60,000 IU/day for 10 days, followed by 6,000 IU/month for six months) have been reported to reduce pain and improve WOMAC score, demonstrating the clinical benefit of vitamin D<sub>3</sub> supplementation in early KOA patients [33]. A meta-analysis of 4 randomized control trials revealed that daily vitamin D supplementation of more than 2000 IU in early KOA patients significantly improved pain and function scores but not tibial cartilage loss and incidence of adverse events [34]. These researchers advocated a need for more research studies with higher doses and unique combination of forms to support the role of vitamin D supplementation in attenuating KOA advancement.

*VCNO supplementation in bone health:* Researchers have reported several health benefits of VCNO such as a role in weight management through the reduction of visceral adiposity (since its SFA content is majorly composed of lauric acid (48%-56%) and it is a rich source of MCTs [35, 36, 37]. Also, the antioxidant polyphenol fraction of VCNO reduces the oxidative stress in chronic diseases [38, 39]. Researchers have confirmed the role of VCNO in reducing bone loss and osteoporosis progression in rats, but its efficacy in human bone health still needs to be tested [40, 41]. VCNO, being rich in polyphenols and medium-chain fatty acids, acted as an anti-stress functional oil at the dose of 10 ml/kg-body-weight in mice, reducing immobility time, restoring oxidative stress post force-swim test, raising brain antioxidant levels, decreasing brain 5-hydroxytryptamine levels, decreasing the weight of the adrenal glands, and lowering serum cholesterol, triglyceride, glucose, and corticosterone levels [42]. VCNO supplementation also improved the functional status and QOL of cancer patients [43]. However, all these studies were based on rat models, the information on the effect of VCNO on functional abilities in bone health of humans is not available so far. Hence, this present study could be considered as the first attempt to ascertain the effect of VCNO on pain and functional abilities of KOA patients.

*Effect of Vitamin D and VCNO Supplementation on Vitamin D Status, Cartilage Degeneration, Inflammation and Functional Abilities in Early KOA Management:* KOA is a chronic degenerative joint disease. Its pathophysiology is initiated with cartilage degeneration in the knee joints. Low vitamin D status increases the risk for OA, resulting in reduced cartilage volume. The role of active vitamin D in reducing articular cartilage degeneration by triggering chondrocyte hypertrophy has been proven [44]. Recent research reported that vitamin D supplementation significantly improved serum 25(OH)D levels, PTH, severity grade, and pain score of Indian OA patients who previously had low vitamin D status [45]. The present study on early KOA Indian patients confirmed these findings indicating a positive effect of Vitamin D supplementation in the management of KOA. Though the role of vitamin D<sub>3</sub> in bone health is well established, it's anti-inflammatory effect needs to be further explored.

Recently, in KOA pathophysiology research, the focus has been shifted from mere cartilage degeneration towards the concept of a whole joint disease, with due emphasis on the chronic inflammation triggered by the metabolic alterations secondary to synovitis in the knee joint [46]. VCNO's anti-arthritic effect has been reported in arthritis induced rats. This effect is due to the bioactive compounds (i.e., the polyphenol fraction isolated from VCNO) which exhibited antioxidant and anti-inflammatory effects [47]. Researchers have reported the anti-inflammatory effects of vitamin D<sub>3</sub> as well as the anti-inflammatory and analgesic effects of VCNO but have indicated a strong need for more evidence based research on these within the frame of OA management [48, 49]. sCOMP levels serve as a prognostic biomarker of cartilage degeneration in KOA at earlier stages and they are directly proportional to cartilage degeneration [50]. Vitamin  $D_3$ supplementation in healthy women was effective in maintaining lower CRP levels [51]. KOA is a chronic form of arthritis, wherein afflicted individuals experience pain and stiffness in the knee joints, affecting daily function, which impacts their quality of life. Hence, supplementation was also assessed for its effect on self-perceived pain (VAS score), stiffness, and functional abilities (WOMAC score and 6-MWT). Supplementation was found to have a positive impact through a coincidence with declining trend in pain intensity and significant improvements in functional abilities. The findings of the present study confirmed this declining trend in cartilage degeneration and inflammatory markers as well as improvement in functional abilities in early KOA patients, post intervention. It also revealed the improved effectiveness of vitamin D<sub>3</sub> and VCNO supplementation in reducing inflammation and thereby, cartilage degeneration, indicating an antiinflammatory and anti-arthritic effect. Based on the above results, the null hypothesis proposed by the investigators that the intake of VCNO & Vitamin D does not offer any cumulative benefits to early KOA patients has been rejected.

#### CONCLUSION

Vitamin  $D_3$  and VCNO supplementation could be a beneficial strategy in delaying KOA progression by improving vitamin D status; reducing cartilage degeneration, inflammation and pain; and improving functional abilities. Simultaneous improvement of vitamin  $D_3$  status and reduction of cartilage degeneration, inflammation and oxidative stress should be considered crucial to early KOA management. Non-conventional antioxidant and anti-inflammatory nutrients and functional foods such as vitamin  $D_3$  and VCNO could be further explored.

**List of Abbreviations:** KOA, Knee Osteoarthritis; KL grading, Kellgren Lawrence grading; VCNO, Virgin Coconut oil; serum 25 (OH) D; serum 25 – hydroxy D; sCOMP, serum cartilage oligomeric matrix protein; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; VAS visual analogue scale, WOMAC Western Ontario Mcmasters osteoarthritis index; ELISA, Enzyme linked Immunosorbent Assay; 6-MWT, six minute walk test; QOL, Quality of life.

**Competing Interests:** There are no conflicts of interest to declare.

**Author's Contributions:** This work is a part of a PhD research project of Monal Velangi. The execution of the study, data collection, statistical analysis and manuscript preparation were all done by her. Subhadra Mandalika, who served in the capacity of research guide, provided guidance for the project documentation. Surendra Shukla, as the Orthopaedic surgeon, provided medical expertise in disease diagnosis. Vandana Pradhan, as the expert Scientist in Clinical Immunology, helped in the sCOMP analysis at NIIH.

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## REFERENCES

- 1. Bhatia D, Bejarano T, Novo M: Current interventions in the management of knee osteoarthritis. Journal of Pharmacy and Bioallied sciences 2013, 5(1):30-38
- Duncan K: Medical Nutrition Therapy for Rheumatic Disease, Mahan LK and Stump SE. KRAUSE'S Food & Nutrition Therapy, International Edition, Saunders Elsevier, Canada, 2008, 12ed:1042.
- 3. Pal CP, Singh P, Chaturvedi S, Pruthi KK, Vij A: Epidemiology of knee osteoarthritis in India and related factors. Indian journal of orthopaedics 2016, 50(5):518.
- 4. Litwic A, Edwards MH, Dennison EM, Cooper C: Epidemiology and burden of osteoarthritis. Br Med Bull 2013, 105:185-199. doi: 10.1093/bmb/lds038
- 5. Wallace IJ, Worthington S, Felson DT, Jurmain RD, Wren KT, Maijanen H, Woods RJ, et al.: Knee osteoarthritis has doubled in prevalence since the mid-20th century. Proceedings of the National Academy of Sciences 2017, 114(35):9332-9336.
- 6. Bettica P, Cline G, Hart DJ, Meyer J, Spector TD: Evidence for Increased Bone Resorption in Patients with Progressive Knee Osteoarthritis Longitudinal Results From the Chingford Study. Arthritis and Rheumatism 2002, 46(12):3178–3184.
- 7. Goswami R, Mishra SK, Kochupillai N: Prevalence & potential significance of vitamin D deficiency in Asian Indians. Indian J Med Res 2008, 127:229-238.
- 8. Shivane VK, Sarathi V, Bandgar T, Menon P, Shah NS: High prevalence of hypovitaminosis D in young healthy adults from the western part of India. Postgrad Med J. 2011, 87(1030):514-8.
- 9. Goula T, Kouskoukis A, Drosos G, Tselepis AS, Ververidis A, Valkanis C, Zisimopoulos A, et al.: Vitamin D status in patients with knee or hip osteoarthritis in a

Mediterranean country. J OrthopTraumatol 2015, 16(1):35-39. doi: 10.1007/s10195-014-0322-y.

- Hassan B, Hany A, Khaled Z, Nouran A, Thomas B: Probing The Relation Between Vitamin D Deficiency and Progression of Medial Femoro-tibialOsteoarthitis of the Knee. Current Rheumatology Reviews 2017, 13(1):65-71(7).
- 11. Nevin, KG, Rajamohan T: Virgin coconut oil supplemented diet increases the antioxidant status in rats. Food Chemistry 2006, 99(2):260-266. doi: 10.1016/j.foodchem.2005.06.056
- 12. Institute of Medicine, Food and Nutrition Board: Dietary Reference Intakes for Calcium and Vitamin D. Washington, DC: National Academy Press; 2010.
- 13. Kellgren JH, Lawrence JC: Radiological assessment of osteo-arthrosis. Ann. Rheum. Dis 1957, 16:494.
- 14. Wang C, Wu J, Zong C, Xu J, Ju HX: Chemiluminescent Immunoassay and its Applications. Chinese Journal of Analytical Chemistry 2012, 40(1):3-10.
- 15. Centaur XP Immunoassay System Siemens Healthineers [https://www.healthcare. siemens.co.in/immunoassay/systems/advia-centaur-xp] Retrieved May 12, 2014
- Jelliffe DB: The assessment of the nutritional status of the community. Geneva Switzerland World Health Organization 1966, World Health Organization Monograph Series No. 53:50-84.
- 17. McCarty CA: Sunlight exposure assessment: can we accurately assess vitamin D exposure from sunlight questionnaires? The American Journal of Clinical Nutrition 2008, Volume 87, Issue 4:1097S–1101S.
- Vitamin D Council, News and Research[https://www.vitamindcouncil.org/] Retrieved December 31, 2013
- Boonstra AM, Schiphorst Preuper HR, Balk GA, Stewart RE: Cut-off points for mild, moderate, and severe pain on the visual analogue scale for pain in patients with chronic musculoskeletal pain. Pain 2014, 155(12):2545-2550.
- 20. Clinician Research- American College of Rheumatology [https://www.rheumatology. org/I-Am-A/Rheumatologist/Research/ClinicianResearchers/Western-Ontario-McMaster-Universities-Osteoarthritis-Index-WOMAC] Retrieved July 12, 2013
- 21. Enright PL: The six-minute walk test. Respiratory care 2003, 48(8):783-785.
- 22. Westergren A: Diagnostic tests: the erythrocyte sedimentation rate range and limitations of the technique. Mar 1957, 3(1):20-5.
- 23. Rhelax-CRP Slide test for C-Reactive Protein [http://www.tulipgroup.com/Tulip\_New/ html/pack\_inserts/Rhelax%20CRP.pdf] Retrieved August 4, 2013.
- 24. Attur M, Krasnokutsky-Samuels S, Samuels J, Abramson SB: Prognostic biomarkers in osteoarthritis. CurrOpinRheumatol 2013, 25(1):136-144.
- 25. BosterBio, Human COMP Elisa, Kit [https://www.bosterbio.com/human-comppicokine-trade-elisa-kit-ek0913-boster.html] Retrieved August 25, 2015
- 26. Indian Council of Medical Research: Nutrient requirements and recommended dietary allowances for Indians. A report of the expert group of the Indian Council of Medical Research NIN 2010, 50:127.

- 27. Thomas KS, Muir KR, Doherty M, Jones AC, O'reilly SC, Bassey EJ: Home based exercise programme for knee pain and knee osteoarthritis: randomised controlled trial. Bmj 2002, 325(7367):752.
- 28. Knee Conditioning Program- Orthoinfo [https://orthoinfo.aaos.org/en/recovery/kneeconditioning-program] Retrieved August 20, 2015
- 29. Longvah, T, A<u>n</u>anta<u>n</u>, I, Bhaskarachary K, and Venkaiah K: Indian food composition tables. National Institute of Nutrition, Indian Council of Medical Research.1sted 2017, 63-90;113-168.
- 30. Harinarayan, CV, Holick MF, Prasad UV, Vani PS, Himabindu, G: Vitamin D status and sun exposure in India. Dermato-Endocrinology 2013, 5(1):130–141.
- Indian Council of Medical Research: Nutrient requirements and recommended dietary allowances for Indians. A report of the expert group of the Indian Council of Medical Research 2010, NIN:93-108, 234-236.
- 32. Manoy P, Yuktanandana P, Tanavalee A, Anomasiri W, Ngarmukos S, Tanpowpong T, Honsawek S: Vitamin D Supplementation Improves Quality of Life and Physical Performance in Osteoarthritis Patients. Nutrients 2017, 9(8).
- 33. Sanghi D, Srivastava R, Agarwal S, Natu S, Singh A, Avasthi S: Role Of Vitamin D In Osteoarthritis Knee: A Six Month Double Blind, Randomized, Placebo Control [abstract]. Abstracts of the 2013 World Congress on Osteoarthritis Osteoarthritis And Cartilage 2013, 19(1):S36.
- 34. Gao XR, Chen YS, Deng W: The effect of vitamin D supplementation on knee osteoarthritis: A meta-analysis of randomized controlled trials. Int J Surg 2017, 46:14-20.
- 35. Liau KM, Lee YY, Chen CK, Rasool, AH: An open-label pilot study to assess the efficacy and safety of virgin coconut oil in reducing visceral adiposity. ISRN Pharmacol 2011, 949686.
- 36. Orsavova J, Misurcova L, Ambrozova JV, Vicha R, Mlcek J: Fatty Acids Composition of Vegetable Oils and Its Contribution to Dietary Energy Intake and Dependence of Cardiovascular Mortality on Dietary Intake of Fatty Acids. Int J Mol Sci 2015, 16(6):12871-12890.
- 37. Kumar PP, Krishna AG: Physicochemical characteristics of commercial coconut oils produced in India. Grasas y Aceites 2015, 66(1):062.
- 38. Famurewa AC, Ekeleme-Egedigwe CA, Nwali SC, Agbo NN, Obi JN, Ezechukwu GC. Dietary Supplementation with Virgin Coconut Oil Improves Lipid Profile and Hepatic Antioxidant Status and Has Potential Benefits on Cardiovascular Risk Indices in Normal Rats. J Diet Suppl 2018, 15(3):330-342.
- 39. Iranloye B, Oludare G, Olubiyi, M: Anti-diabetic and antioxidant effects of virgin coconut oil in alloxan induced diabetic male Sprague Dawley rats. Journal of Diabetes Mellitus 2013, 03(04):221-226.
- 40. Hayatullina Z, Muhammad N, Mohamed N, Soelaiman IN: Virgin coconut oil supplementation prevents bone loss in osteoporosis rat model. Evid Based Complement Alternat Med 2012, 237236.

- 41. Abujazia MA, Muhammad N, Shuid AN, Soelaiman IN: The Effects of Virgin Coconut Oil on Bone Oxidative Status in Ovariectomised Rat.Evidence-Based Complementary and Alternative Medicine 2012, 525079.
- 42. Yeap SK, Beh BK, Ali NM, Yusof HM, Ho WY, Koh SP, Alitheen NB, et al.: Antistress and antioxidant effects of virgin coconut oil in vivo. Exp Ther Med 2015, 9(1):39-42.
- 43. Law KS, Azman N, Omar EA, Musa MY, Yusoff NM, Sulaiman SA, Hussain NHN: The effects of virgin coconut oil (VCO) as supplementation on quality of life (QOL) among breast cancer patients. Lipids in health and disease 2014, 13(1):139.
- 44. Garfinkel RJ, Dilisio MF, Agrawal DK. Vitamin D and Its Effects on Articular Cartilage and Osteoarthritis. Orthop J Sports Med 2017, 5(6):2325967117711376.
- 45. Thomas S, Browne H, Mobasheri A, Rayman MP: What is the evidence for a role for diet and nutrition in osteoarthritis? Rheumatology (Oxford) 2018, 57(suppl 4):iv61-iv74.
- 46. Wang X, Hunter D, Xu J, Ding C: Metabolic triggered inflammation in osteoarthritis. Osteoarthritis Cartilage 2015, 23(1):22-30.
- 47. Vysakh A, Ratheesh M, Rajmohanan TP, Pramod C, Premlal S, Sibi PI: Polyphenolics isolated from virgin coconut oil inhibits adjuvant induced arthritis in rats through antioxidant and anti-inflammatory action. International immunopharmacology 2014, 20(1):124-130.
- 48. Mousa A, Misso M, Teede H, Scragg R, de Courten B: Effect of vitamin D supplementation on inflammation: protocol for a systematic review. BMJ Open 2016, 6(4):e010804.
- 49. Intahphuak S, Khonsung P, Panthong A: Anti-inflammatory, analgesic, and antipyretic activities of virgin coconut oil. Pharm Biol 2010, 48(2):151-157.
- 50. Bai B, Li Y: Combined detection of serum CTX-II and COMP concentrations in osteoarthritis model rabbits: an effective technique for early diagnosis and estimation of disease severity. Journal of orthopaedic surgery and research 2016, 11(1):149.
- 51. Azizieh F, Alyahya KO, Raghupathy R: Association between levels of vitamin D and inflammatory markers in healthy women. J Inflamm Res 2016, 9:51-57.