Article

Role of Jumpstart Nutrition®, a Dietary Supplement, to Ameliorate Calcium-to-Phosphorus Ratio and Parathyroid Hormone of Patients with Osteoarthritis

Apurba Ganguly

Department of Research and Development, OPTM Research Institute, 145 Rashbehari Avenue, Kolkata 700029, India; apurbaganguly15@gmail.com; Tel.: +91-983-038-9616

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Abstract: The aim of this study was to use Jumpstart Nutrition® bone supplementing combination with vitamin-K$_2$ and coenzyme-Q$_{10}$ characterized by an innovative delivery system that improves bioavailability of calcium-to-phosphorus ratio (CPR) and parathyroid hormone (PTH) in the management of osteoarthritis (OA). This eight-week registry included 108 patients treated for symptomatic OA confirmed with radiological images. On top of that, 63 patients used Jumpstart Nutrition® supplement, mainly prepared with calcium, phosphorus, coenzyme-Q$_{10}$, vitamin-K$_2$, vitamin-D$_2$, vitamin-C, folic acid, curcumin and boswellic acids. Rescue medication was also recommended. Patients’ pain and functional capacity through outcome measures—knee-injury osteoarthritis outcome scale (KOOS) and Karnofsky performance scale (KPS), biomarkers such as levels of CPR, PTH and 25-hydroxy-vitamin-D were evaluated for the groups with and without supplement using appropriate kits. After eight weeks, the levels of CPR and PTH were all significantly improved ($p < 0.001$), fewer subjects had to use rescue medication ($p < 0.05$) and variation of pain and functional capacity under KOOS and KPS ($p < 0.05$) of the patients in the supplement group compared to controls. This registry study indicates that Jumpstart Nutrition® can be used safely for effective management of OA patients for the amelioration of CPR, PTH and functional activities confirmed with biomarkers and radiological images correlated with the Kellgren-Lawrance scale.

Keywords: osteoarthritis; vitamin-K$_2$; coenzyme-Q$_{10}$; calcium-to-phosphorus ratio; parathyroid hormone

1. Introduction

Osteoarthritis (OA) is an inflammatory disease that involves painful bone degeneration, muscles and connective tissues damage, which occurs around the world [1–4]. According to the Global Burden of Disease Study reports of the World Health Organization (WHO), nearly 130 million people will suffer from OA worldwide by 2050 and the number of women will be almost double that of the number of men aged over 60 years, constituting a significant social burden [5]. Several biochemical parameters are altered abnormally in various tissues, viz. blood, cartilages, bones and synovial fluid, during osteoarthritis [6–9].

Osteoarthritis is generally managed with the help of palliative measures and symptomatic medications that focus on the reduction and control of symptoms including lifestyle changes, weight control, exercise, administration of anti-inflammatories and analgesics along with calcium and vitamin D supplements to control vitamin and mineral deficiencies during OA [10,11].

Calcium and phosphorus are the most and second most abundant micronutrients that regulate neuromuscular function and skeletal mineralization in the body [12].

Our bones contain nearly 99% calcium, which regulates vascular contraction, vasodilatation, glandular secretion, muscular contraction, glycogen metabolism, neurotransmission and, finally, the
maintenance of bone health and mineralization [13–16]. At the same time, 85% of phosphorus contained in the bones and teeth facilitates mineral metabolism, cellular signal transduction, the exchange of energy and, along with calcium, bone development [13–16]. A high level of phosphates, a form of phosphorus, in the blood is called hyperphosphatemia and hypophosphatemia occurs when the level of phosphates is too low in the blood. These symptoms include joint pain, muscle pain, muscle weakness, fatigue and low energy levels. The remaining 1% of the calcium is extracellular and plays a role in nerve conduction, muscle contraction, blood clotting and immune system activation [13–17]. Therefore, a decrease in blood calcium level causes bone deformation, renal disease and hypoparathyroidism, among other problems [18], and the estimated blood calcium-to-phosphorus ratio (CPR) is known to be a suitable marker during bone formation [14,19]. The ratio between the calcium and phosphorus in bone formation and their interaction during absorption and metabolism and the relationship between the consumption of these nutrients as supplements is the main objective of this study and their ratio is suggested to be maintained as 1.4:1.0 [20,21]. Kemi et al. has emphasized that a lower calcium-to-phosphorus ratio affects serum parathyroid hormone concentration and calcium metabolism in healthy people with adequate calcium intake [20]. Parathyroid hormone (PTH) is an 84-amino-acid peptide secreted by two pairs of parathyroid glands located adjacent to the back of the thyroid gland in the neck. PTH has two major mechanisms for promoting the absorption of calcium—by increasing Ca^{2+} reabsorption in the kidneys and by stimulating the activation of vitamin D, when the concentration of Ca^{2+} drops, the parathyroid gland releases PTH to help bring the Ca^{2+} concentration backup [22,23].

Although the effects of vitamin D metabolites on bone are complicated, it causes bone resorption by mature osteoclasts and regulates bone proteins. Vitamin D is a secosterol hormone that is present in humans in an endogenous (vitamin D_3) and exogenous (vitamin D_2) form at a ratio of approximately 2:1 [23,24]. Vitamin D_3 (cholecalciferol) is synthesized in the skin under the influence of ultraviolet radiation. Vitamin D_3 is also available in oral supplements and injection. Vitamin D_2 (ergocalciferol) is produced by ultraviolet irradiation of the plant sterol ergosterol and is available through the diet. Both forms of vitamin D require further metabolism to be activated and their respective metabolism is indistinguishable [23,24]. The Institute of Medicine (IOM) (Tribhuvan University, Kathmandu, Nepal) has suggested that a 25(OH) vitamin D >20 ng/mL is adequate [25], while The Endocrine Society suggests that >30 ng/mL is optimal [26]. The IOM suggests that supplements of 600–800 IU daily will produce adequate levels in most adults, with an upper safe dose of 4000 IU daily [26]. Therefore, 8 µg of vitamin D has been mixed in the present supplement per serving dose of 25 g.

Besides vitamin D, two other vitamins, namely vitamin-K_2, known as the coagulation vitamin [27], and coenzyme-Q_{10} are absolutely required to bind calcium to make the skeleton stronger and less susceptible to fracture [28] and for muscle dysfunction associated with structural and alterations of skeletal muscle mitochondria, which controls the metabolism of reactive oxygen species (ROS), Ca^{2+} homeostasis and apoptosis [29].

Moreover, oral supplement of oleo-gum resin of *Boswellia serrata* (also called Indian frankincense or Salai guggal) and curcumin have been used as Ayurvedic medicine in India for centuries as a remedy for the treatment of chronic inflammatory diseases, including osteoarthritis and chronic bowel diseases [30–33].

At the same time, the bioactive substances of whey/soy protein such as hormones, growth factors and cytokines regulate cell growth in both normal cells and tumor cells by suppressing proliferation or enhancing apoptosis [34,35].

Several bone supplements available in the market add calcium and vitamin D, or folic acid, or a number of amino acids, or with hydrolyzed amino collagen, or with glucosamine. A bone supplement with vitamin-K_2, coenzyme-Q_{10}, boswellic acids and nano-curcumin (a powerful antioxidant) in addition to proportionate calcium, phosphorus, vitamin C mixed with protein powers of soy and whey is a first time endeavor to control CPR and PTH and to reduce high inflammatory and oxidative values in the management of OA.
The aim of the present supplement registry study was to conduct a pilot assessment for the improvement of bioavailability of calcium-to-phosphorus ratio (CPR), parathyroid hormone (PTH) and vitamin D in the management of OA.

2. Materials and Methods

2.1. Recruitment of Patients

This eight-week registry included 243 patients, aged 40 to 75 years old, who were treated at Organic Phyto Therapeutic Method (OPTM) Health Care (P) Ltd. (a government registered clinic with license number 34218956, Kolkata, West Bengal, India) from November 2018 to January 2019. The OPTM Research Institute Ethics Committee has evaluated and approved the present study protocol. The institute is also registered with the government. An Institutional Review Board approved the consent form for the physical examinations, blood sample collections and radiological images, which was required for the study, and it was signed by all patients in the first phase of the screening procedure.

2.2. Exclusion Criteria

One hundred and 35 of 243 patients with concomitant diseases or risk conditions requiring drug treatment, severe metabolic disorders, drugs/alcohol addiction and/or psychiatric diseases, oncological conditions, pregnant, planned conception, multiple drug dependence, a history of cancer including carcinomatosis and granulocytic leukemia, a history of chronic liver, kidney and heart diseases, and also patients who did not agree to a physical evaluation and/or attend weekly follow-up visits were all included in the exclusion criteria.

2.3. Study Design

After evaluating the exclusion criteria, 63 of the remaining 108 patients suffering with acute OA, who had elevated levels of CPR and PTH confirmed with radiological images and biochemical parameters, were treated with Jumpstart Nutrition® bone supplement and the remaining 45 subjects were treated without supplements and considered as control subjects. The baseline demographic characteristics of all patients are presented in Table 1. The gender-wise classifications for both the groups are shown in Figure 1.

Table 1. Demographic data and baseline characteristics of the study subjects.

| Characteristic                  | Control Group |           | Experimental Group |           |
|--------------------------------|---------------|-----------|---------------------|-----------|
|                                | Female        | Male      | Female              | Male      |
| No of subjects (%)             | 27 (60.00)    | 18 (40.00)| 39 (61.90)          | 24 (38.10)|
| Mean age (SD) in years         | 60.89 (11.37) | 61.38 (10.21)| 60.78 (11.13) | 61.12 (13.68)|
| Mean weight (SD) in kg         | 71.71 (5.05)  | 71.32 (5.11)| 71.67 (6.12)      | 70.77 (6.78)|
| Mean height (SD) in meter      | 1.55 (0.92)   | 1.58 (0.88)| 1.56 (0.93)        | 1.57 (0.98)|
| Mean BMI (SD) in kg/m²         | 29.85 (6.94)  | 28.57 (6.64)| 29.43 (7.11)      | 28.71 (6.32)|
| Mean symptom duration in years | 6.89 (1.90)   | 7.78 (1.78)| 6.12 (2.11)        | 7.26 (2.01)|
| Indian ethnic group (%)        |               |           |                     |           |
| Bengali                        | 8 (29.63)     | 5 (27.78) | 14 (35.90)          | 6 (25.00)|
| Gujarati                       | 3 (11.11)     | 2 (11.11) | 6 (15.38)           | 3 (12.50)|
| Marwaree                       | 5 (18.52)     | 2 (11.11) | 4 (10.26)           | 4 (16.67)|
| Marathi                        | 3 (11.11)     | 2 (11.11) | 3 (7.69)            | 3 (12.50)|
Table 1. Cont.

| Characteristic                          | Control Group | Experimental Group |
|----------------------------------------|---------------|-------------------|
| Tamil                                  | 2 (7.41)      | 2 (5.13)          |
| Punjabi                                | 2 (7.41)      | 4 (10.26)         |
| Shindhi                                | 1 (3.70)      | 3 (7.69)          |
| North East India                       | 3 (11.11)     | 3 (7.69)          |
| **Dietary habits (%)**                 |               |                   |
| Vegetarian                             | 12 (44.44)    | 28 (71.79)        |
| Non-vegetarian                         | 15 (55.56)    | 10 (28.21)        |
| **Other habits (%)**                   |               |                   |
| Smoking                                | 3 (11.11)     | 2 (5.13)          |
| Drinking alcohol                       | 7 (25.93)     | 6 (15.38)         |
| Drinking tea and coffee                | 12 (44.44)    | 18 (46.15)        |
| Chewing tobacco                        | 2 (7.41)      | 3 (7.69)          |
| **Analysis of radiological reports (%)**|               |                   |
| KOA in right knee with osteophytes     | 10 (37.04)    | 12 (30.77)        |
| KOA in left knee with osteophytes      | 17 (62.96)    | 27 (69.23)        |
| **Work status (%)**                    |               |                   |
| Employed fulltime                      | 2 (7.41)      | 3 (7.69)          |
| Employed part time                     | 1 (3.70)      | 2 (5.13)          |
| Housewife / Home-maker                 | 20 (74.07)    | 27 (69.23)        |
| Retired                                | 2 (7.41)      | 4 (10.26)         |
| Self employed                          | 2 (7.41)      | 3 (7.69)          |
| **Marital status (%)**                 |               |                   |
| Single                                 | 2 (7.41)      | 2 (5.13)          |
| Married                                | 17 (62.96)    | 28 (71.79)        |
| Separated                              | 2 (7.41)      | 2 (5.13)          |
| Divorced                               | 1 (3.70)      | 2 (5.13)          |
| Widowed                                | 5 (18.52)     | 5 (12.82)         |
| **Multiple complaints or comorbidities (%)** |       |                   |
| Constipation                           | 17 (62.96)    | 27 (69.23)        |
| Acidity and reflux                     | 12 (44.44)    | 16 (41.03)        |
| Insomnia                               | 10 (37.04)    | 18 (46.15)        |
| Varicose veins                         | 8 (29.63)     | 14 (35.90)        |
| Urinary incontinence                   | 13 (48.15)    | 19 (48.72)        |
| Crepitus during knee flexion           | 14 (51.85)    | 21 (53.85)        |
| Morning stiffness (<30 min.)           | 17 (62.96)    | 18 (46.15)        |
| **Measures taken to diminish pain and inflammation (%)** | | |
| Kneecap uses                           | 24 (88.89)    | 34 (87.18)        |
| Lumbar belt uses                       | 5 (18.52)     | 4 (10.26)         |
| Paracetamol and NSAID use              | 26 (96.30)    | 37 (94.87)        |
| Arthrocentesis (four months ago)       | 11 (40.74)    | 8 (20.51)         |
| Use of hyaluronic acid injection       | 8 (29.63)     | 6 (15.38)         |
Table 1. Cont.

| Characteristic                        | Control Group | Experimental Group |
|---------------------------------------|---------------|--------------------|
| Massage with herbal or other gels     | 18 (66.67)    | 20 (111.11)        |
|                                       | 25 (64.10)    | 17 (70.83)         |
| Homeopathic treatment                 | 19 (70.37)    | 16 (88.89)         |
|                                       | 19 (48.72)    | 18 (75.00)         |
| Ayurvedic treatment                   | 21 (77.78)    | 17 (94.44)         |
|                                       | 20 (51.28)    | 20 (83.33)         |
| Stick/walker use                      | 18 (66.67)    | 11 (61.11)         |
|                                       | 18 (46.15)    | 10 (41.67)         |

Supplements taken to reduce pain or improve fitness (%)

|                                     | Control Group | Experimental Group |
|-------------------------------------|---------------|--------------------|
| Calcium with vitamin D              | 11 (40.74)    | 10 (55.56)         |
|                                     | 32 (82.05)    | 19 (79.17)         |
| Vitamin D injection                 | 8 (29.63)     | 7 (38.89)          |
|                                     | 14 (35.90)    | 6 (25.00)          |
| Glucosamine                         | 6 (22.22)     | 8 (44.44)          |
|                                     | 9 (23.08)     | 4 (16.67)          |
| Glucosamine and chondroitin         | 5 (18.52)     | 4 (22.22)          |
|                                     | 7 (17.95)     | 3 (12.50)          |

Figure 1. Gender-wise allocation of 63 experimental subjects and 45 control subjects.

2.4. Evaluation of Knee-Injury Osteoarthritis Outcome Scale

Pain and disability are common factors in any OA patient. Therefore, the knee-injury osteoarthritis outcome scale (KOOS) developed by Roos et al. [36] to assess the patient’s opinion about their knee pain and associated problems as an instrument. All the scoring data under KOOS were evaluated for each patient at the baseline and at the end of the eight-week treatment for both the groups. Their mean, standard deviation, and percentage of improvement and declination were also graphically evaluated separately by gender for both the groups.

2.5. Evaluation of Karnofsky Performance Scale

The Karnofsky performance scale (KPS) was used to determine a patient’s prognosis to carry out daily activities and it ranges from zero to 100 [37]. A higher score shows the patient is in a better position to carry out daily activities. The mean, standard deviation, and the percentage of improvement and declination of performance were separately evaluated at the end of the registry period compared to the baseline activities for both the groups.

2.6. Evaluation of Biochemical Parameters

From each subject of the with and without supplementary groups, 5 mL of blood sample was collected in a vial (vial containing ethylenediaminetetraacetic acid, EDTA). Then blood samples were
centrifuged at 1000×g for 10 min at 4 °C (Cryo Scientific Systems Pvt. Ltd., Chennai, Tamil Nadu, India) to obtain serum. Finally, the serum was used to analyze calcium, phosphorus, PTH hormone, and 25-hydroxy vitamin-D for both the groups. A kit Calcium AS FS was used to carry out the quantitative assessment of calcium (mg/dL) by the method of the photometric test using Arsenazo III (DiaSys Diagnostic Systems GmbH, Holzheim, Germany) at a wavelength of 650 nm. The kit was developed from the methods of Michaylova and Ilkova [38] and Bauer [39]. The quantitative assessment of phosphorus (mg/dL) was carried out using the photometric test (DiaSys Diagnostic Systems GmbH) at a wavelength of 340 nm. The kit was developed from the methods of Thomas [18]. A kit Intact-PTH ELISA (enzyme-linked immunosorbent assay) (Biomerica Inc., Irvine, CA, USA, Ref 7022) was used to carry out the quantitative assessment of PTH (pg/mL) as an immunoassay. The kit was developed from the methods of Raisz et al. [40] Mallette [41] and Kruger et al. [42]. Using an ELISA kit, 25-hydroxy vitamin D was measured; this method first described by Engvall in 1972 [43]. Each test for each patient was rechecked by the BS-240 Mindray fully automated biochemistry analyzer before reporting the final test results for both the groups. The mean, standard deviation, and their mean differences (MDs), 95% confidence intervals (CIs), p-values, odds ratio and their 95% CIs and coefficient of variances were evaluated for each patient at the baseline and at the end of the eight-week registry period.

2.7. Evaluation of Pearson’s Correlation Coefficients between Two Biomarkers

To determine the predictive values of each abnormal level of biomarker at the baseline compared to the level at the end of eight-week registry period, Pearson’s correlation coefficients were evaluated with their p-values.

2.8. Evaluation of Radiological Images with the Kellgren-Lawrance Grading Scale

Radiological images for both knee joints of 108 patients for both the groups were evaluated at the baseline and at the end of the eight-week registry periods. The anterior-posterior (AP) views of the knee joints of two female and two male patients of both groups were separately evaluated. The Kellgren and Lawrance (KL) system is a common method of classifying the severity of knee osteoarthritis, developed by Kellgren et al. in 1957 [44]. The KL classification was originally described using anterior-posterior (AP) knee radiographs based on the five grades.

2.9. Management of Supplement Studies

The formulation of the Jumpstart Nutrition® bone supplement has been scientifically evaluated by Nanophyto wellness Pvt Ltd, Kolkata, India. Food Safety and Standards Authority of India (FSSAI) has strictly scrutinized the pharmacokinetics of the ingredients used the supplement along with their dietary reference intakes (DRIs) as recommended by the Food and Nutrition Board of the Institute of Medicine, National Academy of Sciences (Washington, DC, USA). Finally, they have issued a license bearing the number 10018031002579 to the company under the Food Safety and Standards Act, 2006. Twenty-five grams of supplement was to be consumed, mixed with cold/warm water, preferably in the afternoon. The ingredients used in the supplement are: i) 737 mg of minerals composed of calcium, phosphorus, and iron in the ratio 5:4:0.21; ii) 125.13 mg of vitamins containing 100 mg coenzyme-Q₁₀, 25 mg vitamin-C, 100 µg folic acid, 20 µg vitamin-K₂, and 8 µg vitamin-D₂; and iii) 275 mg of other phytonutrients such as boswellic acids and curcumin in the ratio 8:3 mixed with protein powers of soy and whey in the ratio 3:7, based on Recommended Dietary Allowance (RDA) guidelines.

The minerals were collected from Mitushi Biopharma (Ahmedabad, Gujarat, India), vitamins were collected from Herbo Nutra (Razapur Khurd, New Delhi) and other phytonutrients were collected from Sami Labs Ltd (Bangalore, India), and protein powders were collected from Kiwi Nutritech (Chennai, India).

The aim of the supplement studies is to define the field of active evaluation of supplements and the possible activation of proper balancing of the levels of calcium, phosphorus, vitamin-D, parathyroid hormone or of other management plans.
The following rules were adopted for the use of supplements in this study:

- As the supplements are not drugs, they are not prescribed but are recommended for the improvement of the levels of calcium, phosphorus, vitamin D, and parathyroid hormone during the management of OA.
- The supplement is used in addition to the best management/care, if available, based on appropriate international guidelines.
- There is no interference with any other treatment or preventive measure while using the supplement.
- A register is always maintained for the evaluation of these studies.
- The supplement is not very costly and easily available on the market without prescription.
- The patients considered as experimental subjects were those who have consumed the supplement continuously for eight weeks as per register.
- A possible placebo effect is explained, and no placebo is used.
- Safety and tolerability were strictly evaluated.

2.10. External Study Reviewers

All results and data before and after the treatment were evaluated by an external reviewing panel, not in contact with the registry patients.

2.11. Statistical Analysis

Continuous variables, such as serum CPR, PTH, and vitamin D levels, in the OA cohort are expressed as the mean, standard deviation (SD), and 95% CIs of differences and the experimental and control groups were compared using the Mann-Whitney U test, as the data do not follow a normal distribution. Non-parametric odds ratios (ORs) for CPR, PTH, and vitamin D, and their 95% CIs were performed to evaluate the accuracy of various measurements in predicting the experimental group compared to the control group. The statistical software IBM SPSS (version 20) was used for all statistical analyses. An alpha level of 5% was established, that is, a p-value less than 0.05 was considered statistically significant.

3. Results

3.1. Enrolment and Baseline Characteristics of Patients

Table 1 shows the baseline characteristic features of the total of 108 patients who were included in the study. All the patients were suffering with OA and had elevated levels of CPR, and PTH and a diminished level of vitamin D, along with bone erosion and skeletal muscle damaged confirmed by biochemical parameters and radiological images. Of these, 63 patients used Jumpstart Nutrition® bone supplement. Gender-wise analysis of all the patients is shown in Figure 1.

3.2. Biochemical Parameters

The mean levels of CPR, and PTH at the end of eight-week of treatment with the supplement were all highly significant (p < 0.05), except the level of PTH for men and the vitamin D levels of all patients, when compared to the baseline separately by gender (Figures 2–4). All the biochemical levels of control subjects without supplement were not significant compared to the control (Figures 5–7).
Figure 2. Levels of Calcium-to-Phosphorus ratio of 63 experimental subjects, female ($n = 39$) and male ($n = 24$), before and after eight-week using supplement (*$p < 0.0001$).

Figure 3. Levels of Parathyroid hormone of 63 experimental subjects, female ($n = 39$) and male ($n = 24$), before and after eight-week using supplement (*$p < 0.001$, *$p = 0.08$).
The correlation coefficients of the experimental subjects with supplements were substantially reduced compared to the control subjects without supplements.

Table 2 shows the levels of correlation coefficients between the ratio of calcium-to-phosphorus at the baseline (CPR⁺) and at the end of eight-week treatment with supplement (CPR⁻), were negatively correlated but not significant (p = 0.286 for females and p = 0.346 for males) while that of the levels of PTH and vitamin D were all highly significant (p < 0.01) for both female-only and male-only. The correlation coefficients between all the biochemical parameters of the control subjects at the baseline and at the end of eight-weeks without supplement for female-only patients and male-only patients were not significant.

Table 2. Analysis of correlation coefficients of biomarkers between baseline and end of eight-week treatment with supplement.

| Comparison of Two Biomarkers | Female (n = 39) | Male (n = 24) |
|------------------------------|-----------------|---------------|
|                              | r-Value | p-Value   | r-Value | p-Value   |
| CPR⁺-Vs-CPR⁻                  | -0.175  | 0.286     | -0.201  | 0.346     |
| PTH⁻-Vs-PTH⁺                  | 0.496   | 0.001     | 0.664   | 0.0004    |
| Vit.D⁻-Vs-Vit.D⁺              | 0.786   | 0.000     | 0.390   | 0.000     |

CPR⁺: level of calcium-to-phosphorus ratio at the baseline; CPR⁻: level of calcium-to-phosphorus ratio at the end of eight-week with supplement; PTH⁺: level of parathyroid hormone at baseline; PTH⁻: Level of parathyroid hormone at the end of eight-week with supplement; Vit.D⁺: level of vitamin D at baseline; Vit.D⁻: Level of vitamin D at the end of eight-week with supplement; n = number of subjects.

Table 3 shows that the percentage of co-efficient of variances for all the studied biochemical parameters of the experimental subjects with supplements were substantially reduced compared to the control subjects without supplements.
3.3. Pain, Stiffness, Performance Parameters

The mean levels of improvements on performance of daily activities under KPS and of the five separately scored subscales under KOOS knee survey after the eight-week treatment with supplements for female-only and male-only patients were all highly significant ($p < 0.05$) compared to the control (Figures 8 and 9). The mean levels of deterioration after eight-weeks without supplements for female-only and male-only patients was not at all significant (Figures 9 and 10).

![Figure 5](image_url) Levels of Calcium-to-Phosphorus ratio of 45 control subjects, female ($n = 27$) and male ($n = 18$), before and after eight-week without using supplement ($^{a}p = 0.74$, $^{b}p = 0.79$).

![Figure 6](image_url) Levels of parathyroid hormone of 45 control subjects, female ($n = 27$) and male ($n = 18$), before and after eight-week without using supplement ($^{a}p = 0.95$, $^{b}p = 0.91$).

Table 3. Analysis of coefficient of variance of control and experimental groups.

| Biomarker | Control Group without Supplement ($n = 45$) | Experimental Group with Supplement ($n = 63$) |
|-----------|--------------------------------------------|---------------------------------------------|
|           | Baseline | After Eight-Week | Baseline | After Eight-Week |
|           | Female   | Male        | Female   | Male        | Female   | Male        | Female   | Male        |
| CPR (%)   | 26.73     | 17.6       | 20.58    | 19.31      | 6.91       | 6.5        | 9.75     | 7.41        |
| PTH (%)   | 41.67     | 60.72      | 41.49    | 58.31      | 41.6       | 40.83      | 20.53    | 18.36       |
| Vitamin D (%) | 43.13   | 49.37       | 43.45    | 49.67      | 43.6       | 30.89      | 33.12    | 29.35       |

CPR = Calcium-to-phosphorus ratio; PTH = Parathyroid hormone; $n$ = number of subjects.
**Figure 7.** Levels of Vitamin-D of 45 control subjects, female \((n = 27)\) and male \((n = 18)\), before and after eight-week without using supplement \((^a p = 0.96, ^b p = 0.99)\).

**Figure 8.** Analysis of knee-injury osteoarthritis outcome scale of 63 experimental subjects, female \((n = 39)\) and male \((n = 24)\), before and after eight-week with supplement \((^* p < 0.05)\), ADL: Activities of daily living; QOL: Quality of life.
3.4. Improvements on Bone Health as per Radiological Images as Assessed by Kellgren-Lawrance Grading Scale

All the anterior-posterior (AP) views of the X-ray reports of 108 patients with OA at the baseline exhibited degenerative changes, particularly in the medial tibiofemoral compartment, with marked joint space narrowing with osteophytes and bilateral varus/valgus deformities. The AP view of X-rays for bilateral knee joints of 63 patients after eight weeks of treatment with supplements indicated substantial improvements on degenerative changes, as well as bone health and the balance of 45 patients treated without supplements had further deterioration on bone health and assessment under the

![Figure 9](image-url)

**Figure 9.** Analysis of Karnofsky performance scale of 63 experimental subjects, female (n = 39) and male (n = 24), and 45 control subjects, female (n = 39) and male (n = 18), before and after eight-week with and without supplement (*p < 0.05, *p = not significant).

![Figure 10](image-url)

**Figure 10.** Analysis of knee-injury osteoarthritis outcome scale of 45 control subjects, female (n = 27) and male (n = 18), before and after eight-week without supplement (*p = not significant), ADL: Activities of daily living; QOL: Quality of life.
KL grading scale shown in Table 4. The X-ray images of four such patients, female ($n = 2$) and male ($n = 2$), before and after the treatment with and without using supplements are depicted in Figures 11 and 12, respectively.

![Radiological images of knee joints (two-female and two-male) showing before and after the treatment with supplement.](image)

**A:** Female 55 Years  
**B:** Female 66 Years  
**C:** Male 62 Years  
**D:** Male 57 Years

**Figure 11.** Radiological images of knee joints (two-female and two-male) showing before and after the treatment with supplement.
Figure 12. Radiological images of knee joints (two-female and two-male) showing before and after without supplement.

Table 4. Kellgren-Lawrence (KL) Grading Scale for Knee-osteoarthritis of experimental and control groups.

| Knee Joints | Gradation | Control Group (n = 45) | Experimental Group (n = 63) |
|-------------|-----------|------------------------|-----------------------------|
|             | Baseline | After Eight-Week | Baseline | After Eight-Week |
|             | Number | %           | Number | %           | Number | %           | Number | %           |
| KOA (Rt. knee) | Grade-0 | None | None | None | None | None | None | None | None | None | None | None | None | 4 | 6.35 |
|             | Grade-1 | None | None | None | None | None | None | None | 8 | 12.70 |
|             | Grade-2 | 23 | 51.11 | 21 | 46.67 | 28 | 44.44 | 30 | 47.62 |
|             | Grade-4 | 22 | 48.89 | 24 | 53.33 | 35 | 55.56 | 21 | 33.33 |
| KOA (Lt. knee) | Grade-0 | None | None | None | None | None | None | None | None | None | None | None | None | 3 | 4.76 |
|             | Grade-2 | 19 | 42.22 | 17 | 37.78 | 27 | 42.86 | 31 | 49.21 |
|             | Grade-4 | 26 | 57.78 | 28 | 62.22 | 36 | 57.14 | 22 | 34.92 |

Grade 0: no radiographic features of OA are present; Grade 1: doubtful joint space narrowing (JSN) and possible osteophytic lipping; Grade 2: definite osteophytes and possible JSN on anteroposterior weight-bearing radiograph; Grade 3: multiple osteophytes, definite JSN, sclerosis, possible bony deformity; Grade 4: large osteophytes, marked JSN, severe sclerosis and definite bony deformity; \( n \) = number of subjects, KOA: Knee osteoarthritis.
3.5. Safety and Cost Evaluation

The topics of safety and tolerability were evaluated. There were no problems with the supplement and no significant safety problems requiring the suspension of the supplement or altering compliance to the supplementation plan were observed. The use of other drugs, including physiotherapy, painkillers and rescue medication, was also decreased in the supplement group compared to control subjects ($p < 0.05$). Blood tests related to hepatic and kidney function were within the normal limits for the patients treated with the supplement after eight weeks.

The average management cost was evaluated for the subjects using Jumpstart Nutrition® bone supplement. This cost was defined as an average of 100% (for the eight weeks of management) including treatment, diagnostic and loss of working days and these were reduced to 92% with range 78–92 ($p < 0.05$).

4. Discussion

In this pilot study, the demographic data and baseline characteristic features of 63 patients treated with Jumpstart Nutrition® bone supplements and 45 patients without bone supplements, suffering with OA as confirmed with radiological and biochemical parameters, are shown in Table 1 and their gender-wise analyses are shown in Figure 1. In both the groups, women are more predominated with OA changes by 23.18% and 20% with an average age of $60.15 \pm 11.25$ years in the supplementary and non-supplementary groups, respectively. The results satisfy the prediction made by WHO so far as the genders as well as the age limit of people are concerned [5].

In this study, the reference value for CPR in adults was calculated based on the established lower and upper limits of calcium (8.4–10.8 mg/dL) and phosphorus (2.6–4.5 mg/dL) as 3.2–2.4 units [29,41–43] and for PTH and vitamin D these have been considered as 15–65 pg/mL and 30–100 ng/mL, respectively [41–43].

The author had previously elaborated the impact of the functional instability of parathyroid hormone and the calcium-to-phosphorus ratio as risk factors for OA patients with lower levels of CPR and PTH as well as with a lower CPR level and higher level of PTH [8,9].

In general, OA treatment is based on oral and topical medicines as pain killers, gels and finally surgery [45–47], and to date, medical practitioners are often focused on symptomatic pain relief rather than curtailing the progression of the disease. Therefore, frequently different medications are used for temporary relief such as NSAIDs for prevention of pain and inflammation but they have tremendous side-effects such as cardiovascular and gastrointestinal problems, and corticosteroids [10,11], which may help to increase muscle strength and slow progression but can weaken bones and increase weight gain over long-term use. Other kinds of therapies such as stem cell [48] are occasionally used, which may improve the delay of progression of symptoms, thus, the quality of life may be saved temporarily.

The present results suggest that there is a close relationship of the risk factors between the bone related biomarkers (CPR, PTH and vitamin D) (Figures 2–7 and Tables 2 and 3) and pain with disability-related outcome measures (KOOS and KPS) (Figures 8–10) in OA. The results elucidated the treatment of OA with a bone supplement especially composed of calcium, phosphorus, vitamin-K$_2$, coenzyme-Q$_{10}$, folic acid, vitamin C, boswellic acids, and curcumin, which is more efficacious in comparison with the commonly composed available supplements with vitamin D [49] or with calcium and vitamin [50] or with calcium and hydrolyzed amino acid [51] or glucosamine sulphate [52] or a combination of glucosamine sulphate and chondroitin sulphate [53] or other such combinations without vitamin-K$_2$ or coenzyme-Q$_{10}$.

The present results shows that there levels of CPR increased by 12.17% (OR: 0.07; 95% CI: 0.03, 0.29; $p < 0.0001$), PTH by 32.86% (OR: 0.36; 95% CI: 0.14, 0.94; $p < 0.05$) and vitamin D by 13.71% (OR: 1.16; 95% CI: 0.39, 3.40; $p = 0.78$) for female-only and 10.77% in CPR (OR: 0.14; 95% CI: 0.03, 0.61; $p < 0.05$), 24.91% in PTH (OR: 0.44; 95% CI: 0.12, 1.59; $p = 0.21$) and 9.20% in vitamin D (OR: 0.59; 95% CI: 0.18, 1.90; $p = 0.38$) for male-only in the supplementary group in comparison with further decreased levels of 2.17% in CPR (OR: 3.52; 95% CI: 1.02, 12.07; $p < 0.05$), 0.72% in PTH (OR: 1; 95% CI: 0.28, 3.61; $p = 1$) and 0.52% in vitamin D (OR:1; 95% CI: 0.31, 3.22, $p = 1$) in female-only and 1.63% in CPR (OR:
The present findings indicate a predictive risk factor through the analyses of the correlation coefficient of CPR between the pre- and post-treatment of eight weeks with the bone supplement being negatively correlated for female-only patients but positively correlated for male-only patients without significant values (\( p = 0.286 \) and \( p = 0.346 \) respectively). The correlation coefficients between the pre- and post-treatment for eight weeks with Jumpstart Nutrition\textsuperscript{®} for both the levels of PTH and vitamin D are highly significant (\( p < 0.001 \)) in the case of female and male patients (Table 2).

Furthermore, it should be noted that, without increasing the strength of the muscles, the gap between the tibiofemoral and patella-femoral joints cannot be increased and thereby pain cannot be reduced. This phenomenon might be possible with the help of a coenzyme-Q\textsubscript{10} supplement. It is absolutely required for muscular dysfunction associated with structural and alterations of skeletal muscle mitochondria. The dysfunction of mitochondria leads to alteration of the structure and function of organelles such as reactive oxygen species (ROS), Ca\textsuperscript{2+} homeostasis and apoptosis during the aging of skeletal muscle [29,54,55]. Skeletal muscle mass and muscular strength are progressively decreased due to aging, this is known as sarcopenia. Coenzyme-Q\textsubscript{10} is essential for the health of virtually all human tissues (epithelial, connective, muscular and nervous) and organs, because an adequate supply of adenosine triphosphate (ATP) is dependent on most cellular functions. It is one of the most significant lipid antioxidants, which prevents the generation of free radicals and modifications of proteins, lipids and DNA [56–60].

At the same time, the reasons for using vitamin-K\textsubscript{2} in the supplement are elaborated as follows:

- The living substance of bone is composed of a hard-outer shell and a spongy matrix of inner tissues. To meet an individual’s metabolic needs, our body releases calcium from the bone into the bloodstream allowing the bone to grow or repair from injuries during the skeleton’s remodeling [61]. It is regulated by two stages such as osteoblasts (cells that build up) and osteoclasts (cells that break down). A healthy bone structure is maintained, when the bone-forming activity is greater than the breakdown of bone.
- Osteoblasts produce inactive osteocalcin and it needs vitamin-K\textsubscript{2} to become fully activated and bind calcium to make the skeleton stronger and less susceptible to fracture [28]. Again, the matrix Gla protein (MGP) of vitamin-K\textsubscript{2} is a central calcification inhibitor produced by the cells of vascular smooth muscles and regulates the potentially fatal accumulation of calcium which keeps calcium from accumulating in the walls of blood vessels [62].
- However, several researchers have suggested that increased consumption of calcium supplements helps strengthen the skeleton but, at the same time, can raise the risk for heart disease as they deposited in blood vessel walls and soft tissues [63–70]. On the contrary, increased vitamin-K\textsubscript{2} intake could be a means of lowering calcium-associated health risks as it is associated with the inhibition of arterial calcification and arterial stiffening [71].
- Further, it may be possible to fight osteoporosis and simultaneously prevent the calcification and stiffening of the arteries by striking the right balance in intake of calcium and vitamin-K\textsubscript{2}, as in the modern manufacturing processes vitamin-K\textsubscript{2} content in the food supply has significantly reduced [71,72].
- Therefore, the risks for blood-vessel calcification and heart problems are significantly lowered and the elasticity of the vessel wall is increased, if at least 32 \( \mu \)g of vitamin-K\textsubscript{2} is present in the diet [73]. Hence, 100 \( \mu \)g of vitamin-K\textsubscript{2} per day has been recommended in the supplement.

Pain syndromes, inflammation and impaired quality of life are the major perception factors among patients with any musculoskeletal disorder, especially OA [1,3,6–8]. To overcome this phenomenon, boswellic acids and curcumin are added to these bone supplements [74]. As a constituent of turmeric (\textit{Curcuma longa} L.), curcumin has been used for centuries [74,75]. This powerful antioxidant [76] is now available in a new delivery system (Jumpstart Nutrition\textsuperscript{®}, Nanophyto Wellness Private Ltd,
Kolkata, India) that improves the bioavailability of curcuminoids. In a recent, larger study, curcumin has improved OA signs/symptoms and reduced the commonly high inflammatory and oxidative values in OA patients [77], also showing an excellent safety profile. Additional analysis aims to evaluate the potential role of curcumin as a primary treatment in the complementary management of OA. Nano-curcumin and boswellic acids contained in Jumpstart Nutrition® have been proven to inhibit the activation of nuclear factor kappa B (NF-kB) [74,75,78–80], a potent inducer of chronic inflammation [80,81].

Moreover, oral supplementation of the gum resin of Boswellia serrata extract (BSE) contains 3-acetyl-11-keto-β-boswellic acid (AKBBA), 11-keto-β-boswellic acid (KBBA), β-boswellic acid (BBA), and 3-acetyl-β-boswellic acid (ABBA) [82]. Biologically active constituents of BSE, namely, β-boswellic acid (BBA) and 3-acetyl-11-keto-β-boswellic acid (AKBBA), act synergistically to exert anti-inflammatory/anti-arthritic activity to reduce joint pain and improve physical functional ability in patients with OA of the knee [83]. Researchers have proved that the oral supplement of BSE can significantly reduce the potential inflammatory marker, serum levels of high-sensitive C-reactive protein and improve the knee joint gap and reduce the osteophytes (spur), associated with OA [84]. Boswellic acids also inhibit lipopolysaccharide-mediated TNF-α induction in monocytes by direct interaction with 1κB kinases [80].

Again, whey protein or soy protein is considered to be the highest quality natural protein [85] and the bioactive substances of whey protein or soy protein, such as hormones, growth factors and cytokines, which can have an important physiological role of regulating cell growth in both normal cells and tumor cells by suppressing proliferation or enhancing apoptosis [34,35,86].

In the present study, it indicates that all the internationally-acclaimed pain-related parameters under KOOS and KPS are in much more favorable positions (decreasing levels of pain activities and increasing levels of life style) in the patients in the supplementary group at the end of the eight-week treatment protocol, compared to control group without the supplement (Figures 8 and 10). Therefore, Jumpstart Nutrition® exerts a significant anti-inflammatory action in these patients, leading to a reduction in symptoms.

Moreover, the results as shown in Table 4 and Figure 11 indicate the definite improvements on reducing osteophytes, joint space narrowing, sclerosis, bony deformities of knee-joints of the OA patients treated with Jumpstart Nutrition® in the supplementary group, as assessed by the KL grading scale.

Finally, this was a small-scale, independent, pilot, registry study; the evaluation product was not prescribed but recommended. In this way, the registry is more similar to real, practical conditions than most clinical studies, which artificially select groups of patients in very controlled conditions, often not comparable to an epidemiological reality.

This type of supplement may be particularly suited for emerging countries and when expensive sponsorships for brand products are not available.

This observational study in otherwise healthy, chronic OA patients indicate that the administration of Jumpstart Nutrition® bone supplement improves outcomes in comparison with the standard management.

Jumpstart Nutrition® bone supplement decreased the need for further medical attention, with a consequent reduction in medical costs.

At the moment, observational and supplement studies or clinical trials on boswellic acids and curcumin in standardized pharmaceutical forms are limited [30–32,75,87,88].

The focus in this pilot registry study and in previous evaluations of OA patients was mainly on the safety profile and on the efficacy of Jumpstart Nutrition® in reducing and controlling pain, in the management of costs and the use of other products, and in improving quality of life. Considering the tremendous opportunity for using safe products of a natural origin in pharmaceutical standards (PS supplements), particularly in subjects in remission or non-acute phases, Jumpstart Nutrition®
bone supplement could be an important management option, also considering the large social cost of managing these patients whose number is growing with increasing age [74].

This supplement registry indicates that the use of Jumpstart Nutrition® supplementation in OA (which may be used even without prescription for safe self-medication) is effective and has limited costs. Jumpstart Nutrition® especially combined with vitamin-K<sub>2</sub>, coenzyme Q<sub>10</sub>, vitamin D<sub>2</sub>, vitamin C, boswellic acids and curcumin mixed with protein powers of soy and whey seems to be rapidly effective even in comparison with other bone supplementary products, as mentioned above, on the signs/symptoms of OA. It acts rapidly in a large number of patients [89], including subjects with a main inflammatory component and those with a degenerative component (associated to different types and levels of pain) as well as formation of calcium and phosphorus metabolism along with the maintenance of appropriate levels of PTH and vitamin D in the serum.

However, this study has several important limitations. Firstly, we have taken a small sample size and have not yet confirmed whether these results are biased. Secondly, the present study relied predominantly on X-ray based determination of OA, but higher resolution magnetic resonance imaging (MRI) modalities may prove to be a more sensitive measure of cartilage pathology and a stronger clinical correlation than symptoms and radiographic measures. Thirdly, patients are restricted to treat with the supplement those who are suffering with following disorders: intolerant of milk products; wounds or any types of chronic skin and infectious diseases; parallel multiple drug dependence for concomitant diseases or risk conditions requiring drug treatment including psychiatric diseases; a history of cancer including caranomatosis and granulocytic leukemia; a history of chronic liver, and kidney diseases, and also patients who did not agree to give blood sample, maybe due to drugs/alcohol addiction, pregnancy and such other reasons and a physical evaluation and/or attend weekly follow-up visits.

5. Conclusions

This supplement registry study suggests that Jumpstart Nutrition® can be considered an effective bone nutrition supplement for the management of OA patients for the improvement of risk factors such as CPR, PTH, vitamin D (Figures 2–4, Tables 2 and 3) and internationally approved outcome measures along with functional activities (Figures 8 and 9) confirming findings with biochemical parameters and knee joint radiographic images correlated with the Kellgren-Lawrance grading scale (Figure 11 and Table 4).

Further research is suggested to be undertaken for the evaluation of inflammation, muscular dystrophy, connective tissue damage and skeletal muscle damage with the help of analyses of suitable biomarkers such as C-reactive protein, creatine kinase-muscle and aldolase-A and anatomical parameters such as range of motions, waist to-hip ratio and body mass index by using Jumpstart Nutrition® bone supplement in other musculoskeletal diseases such as rheumatoid arthritis.

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References

1. Felson, D.T. Epidemiology of hip and knee osteoarthritis. Epidemiol. Rev. 1988, 10, 1–28. [CrossRef] [PubMed]
2. Bijlsma, J.W.; Berenbaum, F.; Lafeber, F.P. Osteoarthritis: An update with relevance for clinical practice. Lancet 2011, 377, 2115–2126. [CrossRef]
3. Johnson, V.L.; Hunter, D.J. The epidemiology of osteoarthritis. Best Pract. Res. Clin. Rheumatol. 2014, 28, 5–15. [CrossRef] [PubMed]
4. Cross, M.; Smith, E.; Hoy, D.; Nolte, S.; Ackerman, I.; Fransen, M.; Laslett, L.L. The global burden of hip and knee osteoarthritis: Estimates from the global burden of disease 2010 study. Ann. Rheum. Dis. 2014, 73, 1323–1330. [CrossRef]
5. World Health Organisation. Priority Diseases and Reason for Inclusion. 2013. Available online: http://www.who.int/medicines/areas/priority_medicines/Ch6_12Osteo.pdf (accessed on 4 December 2012).

6. Garnero, P.; Piperno, M.; Gineyts, E.; Christgau, S.; Delmas, P.D. Cross sectional evaluation of biochemical markers of bone, cartilage, and synovial tissue metabolism in patients with knee osteoarthritis: Relations with disease activity and joint damage. Ann. Rheum. Dis. 2001, 60, 619–626. [CrossRef]

7. Ganguly, A. Diagnosis, Prevention & Phytotherapy for Osteoarthritic Disorders, 1st ed.; Omni Scriptum Publishing Group: Chisinau, Moldova, 2017; ISBN 978-3-330-65274-3.

8. Ganguly, A. Assessment of relationship between calcium-phosphorus ratio and parathyroid hormone levels in serum of osteoarthritic disordered patients: A diagnostic protocol. IOSR J. Dent. Med. Sci. 2017, 16, 46–54.

9. Ganguly, A. Determination of the impact of functional instability of parathyroid hormone and the calcium-phosphorus ratio as risk factors during osteoarthritic disorders using receiver operating characteristic curves. Int. Arch. BioMed. Clin. Res. 2017, 3, 47–52.

10. Bennell, K.L.; Hunter, D.J.; Hinman, R.S. Management of osteoarthritis of the knee. BMJ 2012, 345, e4934. [CrossRef]

11. Porter, R.S.; Kaplan, J.L. The Merck Manual of Diagnosis and Therapy, 19th ed.; Merck Sharp & Dohme Corp: Kenilworth, NJ, USA, 2011.

12. Singer, F.; Boyce, A. Diseases of Bone and Mineral Metabolism. Available online: https://www.endotext.org (accessed on 4 December 2012).

13. Thomas, S.; Browne, H.; Mobasher, A.; Rayman, M.P. What is the evidence for a role for diet and nutrition in osteoarthritis? Rheumatology 2018, 57, iv61–iv74. [CrossRef]

14. De Carvalho Pereira, D.; Lima, R.P.A.; de Lima, R.T.; Gonçalves, M.D.C.R.; de Morais, L.C.S.L.; Franceschini, S.D.C.C.; de Carvalho Costa, M.J. Association between obesity and calcium: Phosphorus ratio in the habitual diets of adults in a city of North eastern Brazil: An epidemiological study. Nutr. J. 2013, 12, 90. [CrossRef]

15. Li, H.; Zeng, C.; Wei, J.; Yang, T.; Gao, S.-G.; Li, Y.-S.; Luo, W.; Xiong, Y.L.; Lei, G.H. Serum calcium concentration is inversely associated with radiographic knee osteoarthritis Across-sectional study. Medicine 2016, 95, e2838. [CrossRef]

16. Open Stax. Bone Tissue and the Skeletal System. In Anatomy and Physiology; OpenStax CNX: Houston, TX, USA, 2016; Chapter 6; Available online: http://cnx.org/content/14fb4ad7-39a1-4eeeb6e3ef2482ec2e2288.24 (accessed on 26 February 2016).

17. Endres, D.B.; Rude, R.K. Mineral and bone metabolism. In Tietz Textbook of Clinical Chemistry, 3rd ed.; Burtis, C.A., Ashwood, E.R., Eds.; W.B Saunders Company: Philadelphia, PA, USA, 1999; pp. 1395–1457.

18. Thomas, L. Clinical Laboratory Diagnostics: Use and Assessment of Clinical Laboratory Results, 1st ed.; TH-Books Verlagsgesellschaft: Frankfurt, Germany, 1998; pp. 241–247.

19. Walwadkar, S.D.; Suryakar, A.N.; Katkam, R.V.; Kumbar, K.M.; Ankush, R.D. Oxidative stress and calcium-phosphorus ratio in habitual diets of adults in a city of North eastern Brazil: An epidemiological study. Nutr. J. 2018, 17, 1–10. [CrossRef] [PubMed]

20. Whybro, A.; Jagger, H.; Barker, M.; Eastell, R. Phosphate supplementation in young men: Lack of effect on calcium homeostasis and bone turnover. Eur. J. Clin. Nutr. 1998, 52, 29–33. [CrossRef] [PubMed]

21. Deftsos, L.J. Clinical Essentials of Calcium and Skeletal Metabolism, 1st ed.; Professional Communication Inc.: West Islip, NY, USA, 1998; pp. 1–208.

22. Komi, V.E.; Karkkainen, M.U.; Rita, H.J.; Laaksonen, M.M.; Outila, T.A.; Lamberg-Allardt, C.J. Low calcium: World Health Organisation. Priority Diseases and Reason for Inclusion. 2013. Available online: http://www.who.int/medicines/areas/priority_medicines/Ch6_12Osteo.pdf (accessed on 4 December 2012).

23. Holick, M.F. Photobiology and Noncalcemic Actions of Vitamin D. In Principles of Bone Biology, 2nd ed.; Bilezikian, J.P., Raisz, L.G., Rodan, G.A., Eds.; Academic Press: Millbrae, CA, USA, 2002; Chapter 33; pp. 587–602. [CrossRef] [PubMed]
26. Holick, M.F.; Binkley, N.C.; Bischoff-Ferrari, H.A.; Gordon, C.M.; Hanley, D.A.; Heaney, R.P.; Murad, M.H.; Weaver, C.M. Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: An Endocrine Society clinical practice guideline. *J. Clin. Endocrinol. Metab.* 2011, 96, 1911–1930. [CrossRef]

27. Zetterstrom, R.; Dam, H.C.P.; Doisy, E.A. The discovery of antihaemorrhagic vitamin and its impact on neonatal health. *Acta Paediatr.* 2006, 95, 642–644. [CrossRef]

28. Hauschka, P.V. Osteocalcin: The vitamin K-dependent Ca²⁺-binding protein of bone matrix. *Pathophysiol. Haemost. Thromb.* 1986, 16, 258–272. [CrossRef]

29. Mattson, M.P. Hormesis defined. *Ageing Res. Rev.* 2008, 7, 1–7. [CrossRef]

30. Ammon, H. Boswellic acids and their role in chronic inflammatory diseases. In *Anti-Inflammatory Nutraceuticals and Chronic Diseases*; Springer International Publishing AG: Cham, Switzerland, 2016; pp. 291–327.

31. Ernst, E. Frankincense: Systematic review. *BMJ* 2008, 337, a2813. [CrossRef] [PubMed]

32. Gupta, P.; Samarakoon, S.; Chandola, H.; Ravishankar, B. Clinical evaluation of *Boswellia serrata* (Shallaki) resin in the management of Sandhivata (osteoarthritis). *Ayu* 2011, 32, 478–482. [CrossRef] [PubMed]

33. Natarajan, S.; Majeed, M. To assess the efficacy & safety of NILIN™ SR tablets in the management of osteoarthritis of knee. *Int. J. Pharm. Life Sci.* 2012, 3, 1413–1423.

34. Michaylova, V.; Ilkova, P. Photometric determination of micro amounts of calcium with arsenazo III. *Anal. Chim. Acta* 1971, 53, 194–198. [CrossRef]

35. Sukkar, S.G.; Bounous, U.G. The role of whey protein in antioxidant defense. *Riv. Ital. Nutr. Parenter. Enter.* 2004, 22, 193–200.

36. Roos, E.M.; Lohmander, L.S. The Knee injury and Osteoarthritis Outcome Score (KOOS): From joint injury to osteoarthritis. *Health Qual. Life Outcomes* 2003, 1, 64. [CrossRef]

37. Schag, C.C.; Heinrich, R.L.; Ganz, P.A. Karnofsky performance status revisited: Reliability, validity, and guidelines. *J. Clin. Oncol.* 1984, 2, 187–193. [CrossRef]

38. Schag, C.C.; Heinrich, R.L.; Ganz, P.A. Karnofsky performance status revisited: Reliability, validity, and guidelines. *J. Clin. Oncol.* 1984, 2, 187–193. [CrossRef]

39. Richter, W. Cell-based cartilage repair: Illusion or solution for osteoarthritis. *Curr. Opin. Rheumatol.* 2007, 19, 451–456. [CrossRef]

40. Rosenwald, K.; Ertl, K.; Fletcher, K.E.; Whittle, J. Patterns of arthritis medication use in a community sample. *J. Prim. Care Community Health* 2012, 3, 272–277. [CrossRef]

41. Dubey, N.K.; Mishra, V.K.; Dubey, R.; Syed-Abdul, S.; Wang, J.R.; Deng, W.P. Combating Osteoarthritis through Stem Cell Therapies by Rejuvenating Cartilage: A Review. *Stem Cells Int.* 2018, 2018, 5421019. [CrossRef]

42. Park, C.Y. Vitamin D in the prevention and treatment of osteoarthritis: From clinical interventions to cellular evidence. *Nutrients* 2019, 11, 243. [CrossRef]

43. Chlebowski, R.T.; Pettinger, M.; Johnson, K.C.; Wallace, R.; Womack, C.; Mossavar-Rahmani, Y.; Eaton, C. Calcium plus vitamin supplementation and joint symptoms in postmenopausal women in the women’s health initiative randomized trial. *J. Acad. Nutr. Diet.* 2013, 113, 1302–1310. [CrossRef]
51. Li, Y.; Xiao, W.; Luo, W.; Zeng, C.; Deng, Z.; Ren, W.; Lei, G. Alteration of amino acid metabolism in osteoarthritis: Its implications for nutrition and health. *Amino Acids* 2016, 48, 907–914. [CrossRef]

52. Da Camara, C.C.; Dowless, G.V. Glucosamine sulphate for osteoarthritis. *Ann. Pharmacother.* 1998, 32, 580–587. [CrossRef] [PubMed]

53. Fransen, M.; Agaliotis, M.; Nairn, L.; Votrubec, M.; Bridgert, L.; Su, S.; Woodward, M. Glucosamine and chondroitin for knee osteoarthritis: A double-blind randomised placebo-controlled clinical trial evaluating single and combination regimens. *Ann. Rheum. Dis.* 2015, 74, 851–858. [CrossRef] [PubMed]

54. Konopka, A.R.; Nair, K.S. Mitochondrial and skeletal muscle health with advancing age. *Mol. Cell. Endocrinol.* 2013, 379, 19–29. [CrossRef] [PubMed]

55. Keller, K.; Engelhardt, M. Strength and muscle mass loss with aging process. *Age and strength loss*. *Muscles Ligaments Tendons J.* 2014, 3, 346–350. [CrossRef] [PubMed]

56. Brotto, M.; Abreu, E.L. Sarcopenia: Pharmacology of today and tomorrow. *J. Pharmacol. Exp. Ther.* 2012, 343, 540–546. [CrossRef]

57. Scott, D.; Blizard, L.; Fell, J.; Giles, G.; Jones, G. Association between dietary nutrient intake and muscle and strength in community-dwelling older adults: The Tasmanian Older Adult Cohort Study. *J. Am. Geriatr. Soc.* 2010, 58, 2129–2134. [CrossRef]

58. Cruz-Jentoft, A.J.; Landi, F. Sarcopenia. *Clin. Med.* 2014, 14, 183–186. [CrossRef]

59. Rosenberg, I.H. Sarcopenia. *J. Nutr.* 2007, 127, 990S–991S. [CrossRef]

60. Evans, W.J. Skeletal muscle loss: Cachexia, sarcopenia, and inactivity. *Am. J. Clin. Nutr.* 2010, 91, 1123S–1127S. [CrossRef]

61. Heaney, R.P.; Weaver, C.M. Newer perspectives on calcium nutrition and bone quality. *J. Am. Coll. Nutr.* 2005, 24, 574S–581S. [CrossRef]

62. Theuwissen, E.; Smit, E.; Vermeer, C. The role of vitamin K in soft-tissue calcification. *Adv. Nutr.* 2012, 3, 166–173. [CrossRef] [PubMed]

63. Bolland, M.J.; Avenell, A.; Baron, J.A.; Grey, A.; MacLennan, G.S.; Gamble, G.D.; Reid, I.R. Effect of calcium supplements on risk of myocardial infarction and cardiovascular events: Meta-analysis. *BMJ* 2010, 341, c3691. [CrossRef] [PubMed]

64. Bolland, M.J.; Grey, A.; Avenell, A.; Gamble, G.D.; Reid, I.R. Calcium supplements with or without vitamin D and risk of cardiovascular events: Reanalysis of the Women’s Health Initiative limited access dataset and meta-analysis. *BMJ* 2011, 342, d2040. [CrossRef] [PubMed]

65. Li, K.; Kaaks, R.; Linseisen, J.; Rohrmann, S. Associations of dietary calcium intake and calcium supplementation with myocardial infarction and stroke risk and overall cardiovascular mortality in the Heidelberg cohort of the European Prospective Investigation into Cancer and Nutrition study (EPIC-Heidelberg). *Heart* 2012, 98, 920–925. [CrossRef] [PubMed]

66. Michaëlsson, K.; Melhus, H.; Warensjö Lemming, E.; Wolk, A.; Byberg, L. Long term calcium intake and rates of all cause and cardiovascular mortality: Community based prospective longitudinal cohort study. *BMJ* 2013, 346, f228. [CrossRef]

67. Pentti, K.; Tuppurainen, M.T.; Honkanen, R.; Sandini, L.; Kroger, H.; Alhava, E.; Saarikoski, S. Use of calcium supplements and the risk of coronary heart disease in 52–62-year-old women: The Kuopio Osteoporosis Risk Factor and Prevention Study. *Maturitas* 2009, 63, 73–78. [CrossRef]

68. Xiao, Q.; Murphy, R.A.; Houston, D.K.; Harris, T.B.; Chow, W.H.; Park, Y. Dietary and supplemental calcium intake and cardiovascular disease mortality: The National Institutes of Health-AARP diet and health study. *JAMA Intern. Med.* 2013, 173, 639–646. [CrossRef]

69. Beulens, J.W.; Bots, M.L.; Atsma, F.; Bartelink, M.L.E.; Prokop, M.; Geleijnse, J.M.; Van Der Schouw, Y.T. High dietary menaquinone intake is associated with reduced coronary calcification. *Atherosclerosis* 2009, 203, 489–493. [CrossRef]

70. Geleijnse, J.M.; Vermeer, C.; Grobbee, D.E.; Schurgers, L.J.; Knapen, M.H.; van der Meer, I.M.; Hofman, A.; Witteman, J.C. Dietary intake of menaquinone is associated with a reduced risk of coronary heart disease: The Rotterdam Study. *J. Nutr.* 2004, 134, 3100–3105. [CrossRef]

71. Theuwissen, E.; Magdeleyens, E.J.; Braam, L.A.J.L.M.; Teunissen, K.J.; Knapen, M.H.; Binnekamp, I.A.G.; Vermeer, C. Vitamin K status in healthy volunteers. *Food Funct.* 2014, 5, 229–234. [CrossRef]
72. Corson, T.W.; Crews, C.M. Molecular understanding and modern application of traditional medicines: Triumphs and trials. *Cell* 2007, 130, 769–774. [CrossRef] [PubMed]
73. Singh, S. From exotic spice to modern drug? *Cell* 2007, 130, 765–768. [CrossRef] [PubMed]
74. Belcaro, G.; Dugall, M.; Luzzi, R.; Hosoi, M.; Ledda, A.; Feragalli, B.; Giacomelli, L. Phytoproflex®: Supplementary management of osteoarthrosis: A supplement registry. *Minerva Med.* 2018, 109, 88–89. [CrossRef] [PubMed]
75. Hatcher, H.; Planalp, R.; Cho, J.; Torti, F.M.; Torti, S.V. Curcumin: From ancient medicine to current clinical trials. *Cell. Mol. Life Sci.* 2008, 65, 1631–1652. [CrossRef]
76. Belcaro, G.; Cesarone, M.R.; Dugall, M. Product-evaluation registry of Meriva®, a curcumin-phosphatidylcholine complex, for the complementary management of osteoarthritis. *Panminerva Med.* 2010, 52, 55–62. [PubMed]
77. Resch, K.L.; Hill, S.; Ernst, E. Use of complimentary therapies by individuals with ‘arthritis’. *Clin. Rheumatol.* 1997, 16, 391. [CrossRef] [PubMed]
78. Bayliss, M.T. Biochemical changes in shumanosteoarthrotic cartilage. In *Studies in Osteoarthrosis Pathogenesis, Intervention, Assessment*; Lott, D.J., Jasani, M.K., Eds.; Wiley: Hoboken, NJ, USA, 1987; pp. 46–56.
79. Cuaz-Pérolin, C.; Billiet, L.; Baugé, E.; Copin, C.; Scott-Algara, D.; Genze, F.; Rous, M. Anti -inflammatory and antiatherogenic effects of the NF-κB inhibitor acetyl-11-keto-β-boswellic acid in LPS-challenged ApoE−/− mice. *Arteriosclerosis. Thromb. Vasc. Biol.* 2008, 28, 272–277. [CrossRef]
80. Syrovets, T.; Büchele, B.; Krauss, C.; Laumonnier, Y.; Simmet, T. Acetyl-boswellic acids inhibit lipopolysaccharide-mediated TNF-α induction in monocytes by direct interaction with IκB kinases. *J. Immunol.* 2005, 174, 498–506. [CrossRef]
81. Büchele, B.; Zugmaier, W.; Simmet, T. Analysis of pentacyclic triterpenic acids from frankincense gum resins and related phytopharmaceuticals by high-performance liquid chromatography. Identification of lupeolic acid, a novel pentacyclic triterpene. *J. Chromatogr. B* 2003, 791, 21–30. [CrossRef]
82. Majeed, M.; Majeed, S.; Narayanan, N.K.; Nagabhushanam, K. A pilot, randomized, double-blind, placebo-controlled trial to assess the safety and efficacy of a novel Boswellia serrata extract in the management of osteoarthritis of the knee. *Phytother. Res.* 2019, 33, 1457–1468. [CrossRef]
83. Singh, R.; Wang, O. Clinical trials in “emerging markets”: Regulatory considerations and other factors. *Contemp. Clin. Trials* 2013, 36, 711–718. [CrossRef]
84. Wolfe, R.R. Protein supplements and exercises. *Am. J. Clin. Nutr.* 2000, 72, 551–557. [CrossRef]
85. BulutSolak, B.; Akin, N. Functionality of Whey Protein. *Int. J. Health Nutr.* 2012, 3, 1–7.
86. Belcaro, G.; Nicolaides, A.N. A new role for natural drugs in cardiovascular medicine. *Angiology* 2001, 52, S1. [CrossRef]
87. Beck, T.W.; Housh, T.J.; Johnson, G.O.; Schmidt, R.J.; Housh, D.J.; Coburn, J.W.; Malek, M.H.; Mielke, M. Effects of a protease supplement on eccentric exercise-induced markers of delayed-onset muscle soreness and muscle damage. *J. Strength Cond. Res.* 2007, 21, 661–667.
88. Cockburn, E.; Hayes, P.R.; French, D.N.; Stevenson, E.; St Clair Gibson, A. Acute milk-based protein-CHO supplementation attenuates exercise-induced muscle damage. *Appl. Physiol. Nutr. Metab.* 2008, 33, 775–783. [CrossRef]