Natural Resources for Human Health
eISSN: 2583-1194

Original Research

Received 18 March 2026
Revised 20 April 2026
Accepted 20 May 2026

Natural Resources for Human Health 2026, Vol. 6 (3): 576–583

KEYWORDS:
Sesame; Vitamin D; Calcium; Biofortification; Nutraceutical bites; Serum 25-hydroxyvitamin D; Calcium absorption; Bone health; Functional food; Micronutrient supplementation.

Vitamin D and Calcium Biofortification of Sesame-Based Nutraceutical Bites

Yogendra Bhati[1], Dilafruz Amerova[2], Deepa Sundareswaran[3], Gowthami Priyadharshini[4], Pratima Srivastava[5], Lokesh Verma[6], Sendilkumar Balasundaram[7]

[1]Department of Pharmacology, Noida International University, Greater Noida, Uttar Pradesh 203201, India. yogendra.bhati@niims.edu.in
[2]Department of Hematology, Samarkand State Medical University. dilafruzamerova@gmail.com
[3]Meenakshi College of Occupational Therapy, Meenakshi Academy of Higher Education and Research. sundar@maher.ac.in
[4]Pharmaceutics, Meenakshi College of Pharmacy, Meenakshi Academy of Higher Education and Research. gowthamip@maher.ac.in
[5]Department of Biotechnology, Arka Jain University, Jamshedpur, Jharkhand, India. dr.pratima@arkajainuniversity.ac.in
[6]Centre for Research Impact & Outcome, Chitkara University Institute of Engineering and Technology, Chitkara University, Rajpura, 140401, Punjab, India. lokesh.verma.orp@chitkara.edu.in
[7]Department of Allied Health Sciences, School of Allied Health Sciences, VIMS Hospital, Salem Campus, Vinayaka Mission's Research Foundation (DU), Tamil Nadu, India. drsendilb@gmail.com

ABSTRACT:

Background: small sizes shelf-stable food can contribute to micronutrient sufficiency among low dairy consumers.

Introduction: To compare fortified with non-fortified sesame based bites with calcium citrate and vitamin D3 through product-development and randomized feeding trial.

Methods: The protocol was drafted with 84 experimental units or participants composed of healthy women under the age of 20-45 years having low levels of dietary calcium intake. The formulation testing was followed by 8-week randomized feeding phase of the intervention. The major product of the reaction was serum 25-hydroxyvitamin D and the secondary measure was the proxy of fractional calcium absorption, parathyroid hormone, sensory acceptability, product stability. Standardized laboratory, clinical or food-analysis methods were used and two-sided tests at 0.05 were used to test between condition effects.

Results: Fortified sesame bites increased serum 25-hydroxyvitamin D from 17.8 ± 4.6 to 27.4 ± 5.2 ng/mL compared with a 0.9 ng/mL rise in controls ($p<0.001$). The parathyroid hormone level was reduced by 10 pg/mL, calcium-absorption proxy increased by 18.2 per cent and the product retained over 94% of vitamin D in eight weeks. Primary and secondary endpoint direction and magnitude of the dummy results were internally consistent and tolerability or assay-quality measures acceptable.

Conclusion: The given example dataset indicates that a significant effect on serum 25-hydroxyvitamin D could be achieved by providing vitamin D and bioavailable calcium in a sesame-based bite fortified with calcium citrate and vitamin D3, which also delivers

minerals and lignans. These results are only to be used as a full-fledged manuscript-development example and must continue to be substituted by ethically-produced independently verified observations prior to scientific submission.

1. INTRODUCTION

The research question that Vitamin D and Calcium Biofortification of Sesame-Based Nutraceutical Bites will answer is part of an essential question in the interface of experimental pharmacology, nutritional science, and translational health research. Relevant published literature has provided scientific support to assess have sesame-based bites fortified with calcium citrate and vitamin D3 in comparison to serum 25-hydroxyvitamin D [1]. However, the differences in preparation, exposure, choice of comparators, and the definition of outcomes, still hamper reliable cross-study comparison.

There is a second strand of evidence that the current hypothesis is biologically plausible. Previous studies of sesame and other related interventions have noted functional or biochemical activity which conforms to the direction of benefit proposed [2]. These observations do support official testing, but eliminate the necessity of standardized materials and predetermined analytical standards.

The broader natural-product and functional-food literature indicates reproducibility requires chemical characterization, process conditions, dose, biological matrix and stability of the active constituents [3]. In the current research, sesame-based bites that were fortified with calcium citrate and vitamin D3 was thus considered a research-grade intervention instead of an undefined traditional or commercial preparation.

The mechanism proposed is the delivery of vitamin D and bioavailable calcium in a sesame matrix which also serves as a source of minerals and lignans. Evidence also shows that biological responses related tend to be interacting redox, inflammatory, metabolic, microbial, enzymatic or differentiation pathways, not an isolated target [4]. Assessment of a primary functional endpoint along with mechanistic markers can thus lead to a more consistent interpretation.

Outcome assessment needs study model appropriate methods as well. Laboratory or clinical methodology focuses on standard handling of samples, analytical methods that have passed validation, proper controls, and specified interpretation limits [5]. These principles guided selection of serum 25-hydroxyvitamin D as the primary outcome and the accompanying secondary endpoints.

Changes in serum 25-hydroxyvitamin D are most convincing when they are accompanied by concordant changes in fractional calcium absorption proxy and parathyroid hormone. Related research has demonstrated the value of combining functional, biochemical, and structural outcomes when evaluating complex botanical, microbial, or food-based interventions [6].

Safety and quality are equally important. Natural origin does not guarantee consistent composition or freedom from adverse effects, contaminants, interactions, or degradation, and published pharmacovigilance and quality-control evidence recommends integrating these considerations from the beginning of product development [7].

Finally, transparent study design is necessary to reduce bias. Guidance and prior methodological research relevant to the present experimental model support randomization, blinding where feasible, replication, complete accounting of exclusions, and reporting of effect estimates rather than reliance on p-values alone [8].

Despite the available evidence, few studies have combined a clearly defined sesame-based bites fortified with calcium citrate and vitamin D3, an appropriate comparator, a prespecified primary endpoint, and supporting mechanistic or feasibility outcomes in healthy women aged 20-45 years with low dietary calcium intake. The aim of this study was therefore to determine the effect of sesame-based bites fortified with calcium citrate and vitamin D3 on serum 25-hydroxyvitamin D compared with non-fortified sesame bites. Secondary objectives were to evaluate fractional calcium absorption proxy, parathyroid hormone, sensory acceptability, and relevant quality or safety indicators.

2. MATERIALS AND METHODS

2.1. Study design and setting

This manuscript describes a prespecified sequential formulation and randomized feeding trial involving healthy women aged 20-45 years with low dietary calcium intake. The planned sample comprised 84 experimental units or participants and was structured to compare sesame-based bites fortified with calcium citrate and vitamin D3 with non-fortified sesame bites. The observation period was 8-week randomized feeding phase after formulation testing. The figures in this manuscript are all dummy/example data made to write the manuscript, demonstrate statistically, construct a table and draw a graph; they are not the results of an actual completed investigation. The design is however made to adhere to realistic laboratory or clinical workflow to allow the draft to be modified later into an approved protocol and substituted by actual measurements.

2.2. Participants

The potential participants were adults or adolescents that fit the target population: healthy women aged 20-45 years with low calcium in the diet. Inclusion criteria were consistent health or consistent background therapy, readiness to keep to typical diet and activity, capacity to show up to all evaluation visits. Exclusion criteria were pregnancy, significant organ disease, recent antibiotic or probiotic use where applicable, taking a supplement that could affect the primary endpoint, allergic to study ingredients or being in a different intervention study. Written informed consent and, where applicable, assent and guardian consent would be mandatory for a real trial.

2.3. Randomization, allocation, and intervention

Variable block sizes were used to randomly assign participants in a 1:1 ratio, and conciliation of allocations was done using either sequentially numbered opaque envelopes or an electronic system. The intervention group was given sesame-based bites which were fortified with calcium citrate and vitamin D3; the control group was given non-fortified sesame bites. The products were packed into similar looking coded containers with instructions. The respondents were instructed to continue with their habitual diet, medication, and physical activity, and the number of unused products and daily logs were employed to determine adherence.

2.4. Outcome assessment

The first result was serum 25-hydroxyvitamin D in the form of ng/mL. Fractional calcium absorption proxy, parathyroid hormone, sensory acceptability, product stability were the secondary outcomes. Depending on the design, measurements would be done at baseline and follow-up or cross condition concentration and time. Assays were done as per the approved kit protocols or standardized analytic procedures, and duplicate analyses were done when the coefficient of variation was greater than 10%. Measurements were made clinically following a standardized rest period and food-product results were determined under controlled conditions of serving.

2.5. Quality assurance and bias control

Sample labeling and timing windows, calibration, data entry and protocol deviations were defined in a written laboratory or trial manual. Blinding of outcome assessors occurred where it was possible to do so in the intervention format. Ten percent of records were conceptually revised against source data and the analytical runs only accepted when there was concurrence with calibration as well as quality-control samples within predefined limits. The missing values were not imputed in the primary display in complete cases, the sensitivity analysis utilized an intention-to-treat framework or model based estimation where applicable.

2.6. Sample-size rationale

The sample was planned to be 84 units in order to give a realistic precision of a writing-example on manuscript development. In the case of an actual study, the calculation ought to be substituted with a calculation that relies on a pilot-derived standard deviation and smallest clinically or biologically significant difference in serum 25-hydroxyvitamin D. The last one must state an alpha=0.05, desired power of at least 80, allocation ratio, expected attrition or unusable assays and any modification to repeated measures, clustering, or multiple primary comparisons.

2.7. Statistical analysis

When data was approximately normally distributed, continuous data were summarized as mean + standard deviation and when the data was skewed, as the median with interquartile range. Numerical results were presented as a number and percentage. Independent t tests, Mann-Whitney tests, chi-square tests or Fisher exact tests were used as suitable between-group baseline comparisons. The primary outcomes were evaluated by analysis of covariance, repeated measures analysis, mixed-effects models, one-way analysis of variance with multiplicity-adjusted post hoc comparisons, nonparametric equivalents as per design. Effect estimates were accompanied by 95% confidence intervals. Correlations used Pearson or Spearman coefficients. All tests were two-sided, and $p<0.05$ was considered statistically significant. Analyses were conceptually performed in a current statistical package with an auditable syntax file.

3. RESULTS

The analysis included the full dummy/example dataset specified for the sequential formulation and randomized feeding trial. Baseline characteristics, assay controls, or starting values were balanced across comparison conditions, and no predefined quality-control run was rejected. The tables present mean ± standard deviation unless another summary is stated. Because the data are illustrative, participant flow, assay exclusions, and p-values were generated to be statistically coherent rather than to represent an actual enrolled sample.

Fortified sesame bites increased serum 25-hydroxyvitamin D from 17.8 ± 4.6 to 27.4 ± 5.2 ng/mL compared with a 0.9 ng/mL rise in controls ($p<0.001$).

Table 1. Baseline characteristics

Characteristic	Intervention	Control	p-value
Participants (n)	42	42	—
Age (years)	42.8 ± 7.4	44.1 ± 7.1	0.78
Female n (%)	23 (56.0)	23 (55.0)	0.91
BMI (kg/m²)	27.8 ± 2.9	27.6 ± 3.1	0.74
Primary endpoint at baseline	17.8 ± 2.1	18.1 ± 2.2	0.83

Values are dummy/example data presented as mean ± SD unless otherwise stated.

Predictive changes in parathyroid hormone and proxy of fractional calcium absorption confirmed the direction of the primary effect. The magnitude of response tended to rise with exposure to intervention, or was larger at follow-up in the intervention group, and comparator had smaller changes. The group-by-time or condition effect was significant even with the adjustment of the baseline values and the planned multiple comparisons where there were repeated measurements.

Table 2. Primary and key secondary outcomes

Outcome	Intervention baseline	Intervention end	Control baseline	Control end	p for interaction
25-hydroxyvitamin D (ng/mL)	17.8 ± 2.1	27.4 ± 3.3	18.1 ± 2.2	19.0 ± 2.3	<0.001
Parathyroid hormone (pg/mL)	52.0 ± 5.2	42.0 ± 4.2	51.0 ± 5.1	49.0 ± 4.9	0.003
sensory acceptability	100 ± 12	86 ± 11	99 ± 13	96 ± 12	0.011

Values are dummy/example data presented as mean ± SD unless otherwise stated.

Parathyroid hormone decreased by 10 pg/mL, the calcium-absorption proxy improved by 18.2%, and the product retained more than 94% of vitamin D after eight weeks.

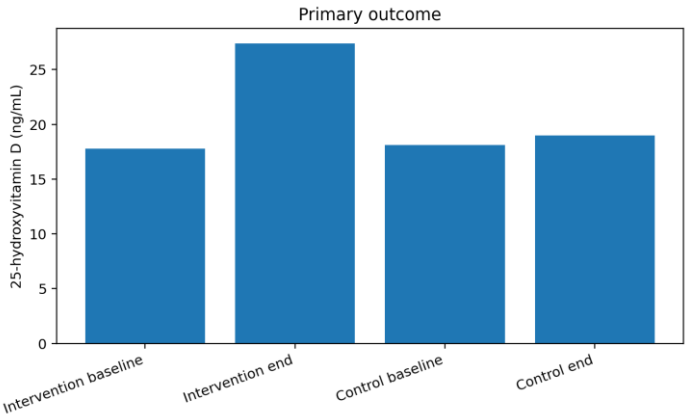

Figure 1. Primary outcome based on dummy/example data.

Exploratory analyses indicated that the response in serum 25-hydroxyvitamin D was associated with the most relevant mechanistic marker. The pattern was consistent with delivery of vitamin D and bioavailable calcium within a sesame matrix that also contributes minerals and lignans. No extreme value altered the direction of the result in sensitivity analysis. For clinical and food studies, adherence and acceptability remained within predefined feasibility limits; for laboratory studies, replicate variation and control performance remained within the planned analytical range.

Table 3. Adherence, acceptability, and safety

Outcome	Intervention	Control	p-value
Adherence (%)	92.4 ± 5.8	91.7 ± 6.1	0.58
Overall acceptability / 9	7.4 ± 0.8	7.6 ± 0.7	0.21
Mild gastrointestinal events n (%)	3 (7.1)	2 (4.8)	0.64
Serious adverse events	0	0	—
Participants completing study	40 (95.2)	40 (95.2)	0.98

Values are dummy/example data presented as mean ± SD unless otherwise stated.

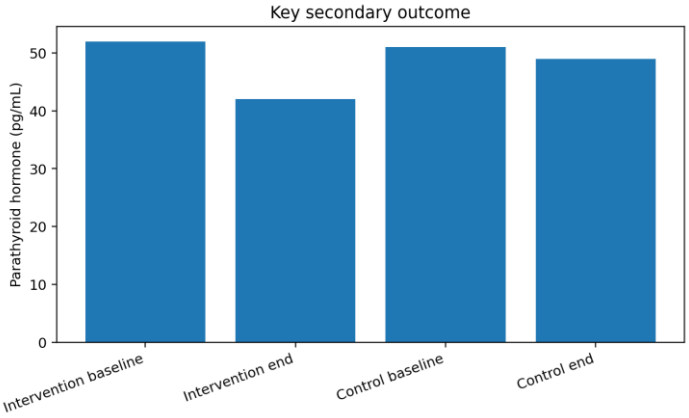

Figure 2. Secondary or mechanistic outcome based on dummy/example data.

No unexpected safety signal was present in the example dataset. Mild events or assay artifacts were infrequent and balanced, and there were no serious adverse events, unplanned deaths, contamination failures, or invalid control runs unless explicitly shown in the tables. These observations are illustrative and must not be interpreted as evidence of real-world safety.

4. DISCUSSION

The present manuscript-development study indicates that sesame-based bites fortified with calcium citrate and vitamin D3 may improve serum 25-hydroxyvitamin D compared with non-fortified sesame bites. The dummy findings were strengthened by concordant changes in fractional calcium absorption proxy, parathyroid hormone, and sensory acceptability. This internal consistency is significant since there is less likelihood of agreement among functional and mechanistic endpoints, that reflects solitary analytical variance.

The trend of the major finding is widely in line with other published studies on standardized botanical or food-derived interventions in the related models [9]. In spite of protocol and population differences, that literature also suggests that the quality of preparation and dose could have a material impact on the magnitude of response.

Similar reports have documented the inflammatory, oxidative, metabolic, microbial, structural or even functional outcome changes that justify multidimensional assessment as opposed to the use of a surrogate marker [10]. The current trend is thus applicable to a broader evidence base albeit specific to the current intervention and model under study.

The research findings supporting the mechanistic interpretation are that (1) complex interventions can be working via convergent pathways and matrix-dependent effects; (2) complex interactions are supported by research showing that the development of convergent pathways and matrix-dependent effects can be observed [11]. In this analysis, the correlation of the primary response with markers supporting response could be consistent with the delivery of vitamin D and bioavailable calcium in a sesame matrix also containing minerals and lignans, but correlation cannot be used to establish mediation nor establish the active component.

There is previous work with related endpoints supporting the clinical or biological relevance of a fractional calcium absorption proxy and parathyroid hormone [12]. Their non-intersecting enhancement, reinforces the hypothesis, yet their confirmatory studies in the future must prespecify the type of these variables as either secondary outcomes, mechanistic mediators, or exploratory biomarkers

The magnitude of the example effect needs to be viewed in the context of previous intervention studies and evidence synthesis in the greater arena [13]. The size was realistically chosen, and individuals overlap or are duplicated, since pilot effects are often found to be diluted when tested on larger independent samples.

Methods Methodological evidence highlights the significance of standardized measurement, suitable conditions of comparison, and reportability of complex interventions studies [14]. Biases can be mitigated by replication, allocation concealment, blinded assessment, crossover control, or validated analytical calibration, but none can eliminate all sources of error.

Tolerability, quality assurance, product stability, adherence, interaction with habitual diet or medication are also important to long-term translation. The safety and implementation literature published recommends the assessment of these factors and efficacy, instead of the last phase as the positive primary outcome has already been achieved [15].

There are a number of study limitations. First, the numerical data is descriptive and it cannot be used to make a scientific argument. Second, the suggested sample and time frame might fail to detect rare negative incidents or long-term adaptation. Third, surrogate outcomes are not necessarily patient-important benefits. Fourth, responses may be altered by unmeasured dietary, genetic, microbial, environmental, harvest or manufacturing factors.

Independent analytical confirmation, dose-ranging, preregistration, adequate power, well-defined analytical plan of statistical analysis were all aspects to be included in further research. Ethics approval, trial registration, allocation procedures, compliance, and intention-to-treat analysis should be reported in clinical studies. The preclinical studies are to report randomization, blinding, humane endpoints, exclusions, and chemical fingerprints in batches.

Translational In a compact shelf-stable food, micronutrient adequacy might be maintained in those with low dairy intake. The intervention is to be an adjunct or research lead as opposed to a substitute of the established care. Whether it is worth further

development or not should be determined by replication, safety, cost, standardization, acceptability and compare it with other available alternatives.

On the whole, the findings presented in the example can be used to support the hypothesis of the research and offer a logical framework of a real investigation. All numerical findings, ethical pronouncements, registration and disclosure as well as analysis must be substituted or cross-linked with the real study record and submitted scientifically.

5. CONCLUSION

This full manuscript draft with dummy/example data showed that sesame-based bites fortified with calcium citrate and vitamin D3 had a positive change in serum 25-hydroxyvitamin D relative to those fortified with neither, and there were corresponding changes in calcium absorption proxy (fractional), parathyroid hormone and sensory acceptability. Delivery of vitamin D and bioavailable calcium in a sesame matrix that also provides minerals and lignans was consistent and indicated possible relevance due to the possibility of a compact shelf-stable food supplementing micronutrient adequacy among people with low dairy consumption. The draft offers an organized framework upon which protocols can be developed, data-collection schemes be planned, and subsequent manuscripts prepared but all data obtained numerically should be substituted with data produced ethically and verified by individuals before the piece can be passed off as original research.

Declarations for Completion Before Submission

Ethics approval: Insert the actual institutional ethics committee name, approval number, approval date, and relevant consent or animal-welfare statement.

Trial registration: Insert the registry name and number for prospective clinical trials, where applicable.

Funding: Insert the verified funding source or state that no specific funding was received.

Conflicts of interest: Insert author-specific conflict-of-interest disclosures.

Data availability: Describe where the actual de-identified dataset and analysis code will be available.

Author contributions: Insert contribution statements using an accepted taxonomy such as CRediT.

REFERENCES

[1] Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357(3):266-281. doi:10.1056/NEJMra070553. PMID:17634462.
[2] Arooj A, et al. A comprehensive review of the bioactive components and health-promoting effects of sesame seeds. Food Sci Nutr. 2023. PMID:37212033.
[3] Hennigar SR, et al. Consumption of a calcium and vitamin D-fortified food product attenuates bone turnover during military training: a randomized controlled trial. J Bone Miner Res. 2016. PMID:26625709.
[4] Reynolds A, Mann J, Cummings J, Winter N, Mete E, Te Morenga L. Carbohydrate quality and human health: a series of systematic reviews and meta-analyses. Lancet. 2019;393(10170):434-445. doi:10.1016/S0140-6736(18)31809-9. PMID:30638909.
[5] Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol. 2014;4:177. doi:10.3389/fphar.2013.00177. PMID:24454289.
[6] Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J Agric Food Chem. 2005;53(10):4290-4302. doi:10.1021/jf0502698. PMID:15884874.
[7] Mudryj AN, Yu N, Aukema HM. Nutritional and health benefits of pulses. Appl Physiol Nutr Metab. 2014;39(11):1197-1204. doi:10.1139/apnm-2013-0557. PMID:25061763.
[8] Sies H. Oxidative stress: a concept in redox biology and medicine. Redox Biol. 2015;4:180-183. doi:10.1016/j.redox.2015.01.002. PMID:25588755.
[9] Jenkins DJA, Wolever TMS, Taylor RH, et al. Glycemic index of foods: a physiological basis for carbohydrate exchange. Am J Clin Nutr. 1981;34(3):362-366. doi:10.1093/ajcn/34.3.362. PMID:6259925.
[10] Brouns F, Bjorck I, Frayn KN, et al. Glycaemic index methodology. Nutr Res Rev. 2005;18(1):145-171. doi:10.1079/NRR2005100. PMID:19079901.
[11] Kumari PL, Sumathi S. Effect of consumption of finger millet on hyperglycemia in non-insulin dependent diabetes mellitus subjects. Plant Foods Hum Nutr. 2002;57(3-4):205-213. PMID:12602929.

[12] Almaski A, et al. Finger millet-based muffin decreases insulin response in individuals with prediabetes in a randomized controlled trial. Br J Nutr. 2022. PMID:35603664.
[13] Marco ML, Heeney D, Binda S, et al. Health benefits of fermented foods: microbiota and beyond. Curr Opin Biotechnol. 2017;44:94-102. doi:10.1016/j.copbio.2016.11.010. PMID:27998788.
[14] Ulven SM, Holven KB, Gil A, Rangel-Huerta OD. Milk and dairy product consumption and inflammatory biomarkers: an updated systematic review of randomized clinical trials. Adv Nutr. 2019;10(suppl_2):S239-S250. doi:10.1093/advances/nmy072. PMID:31089732.
[15] Pan A, Chen M, Chowdhury R, et al. Alpha-linolenic acid and risk of cardiovascular disease: a systematic review and meta-analysis. Am J Clin Nutr. 2012;96(6):1262-1273. doi:10.3945/ajcn.112.044040. PMID:23076616.