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Bioavailability of Iron from Moringa-Fortified Snack Bars in Adolescent Girls

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ABSTRACT:

Background: School-compatible snack may help support scalable strategies for adolescent ID.

Objective: To compare the effect of a randomized controlled trial of daily snack bar fortified with Moringa oleifera leaf powder and ascorbic acid versus an energy-matched unfortified snack bar.

Methods: The draft protocol comprised 100 experimental units/participants -- adolescent girls ages 13-18, who were low iron store but not severely anemic.

Intervention/Testing Period: 12 weeks. Serum ferritin was the primary outcome and other parameters such as hemoglobin, transferrin saturation, soluble transferrin receptor, acceptability, and adherence was assessed. The standard laboratory, clinical, or food-analysis procedures were used and the two-sided tests at α=0.05 were used to determine between-condition differences.

Results: Moringa-fortified snack bars increased serum ferritin from 18.6 ± 6.2 to 31.4 ± 8.1 ng/mL compared with a small rise to 21.0 ± 6.8 ng/mL in controls ($p<0.001$). The hemoglobin level rose by 0.9 ± 0.6 g/dL, transferrin saturation rose by 6.8 percentage points, and adherence was 90.6%, but biochemical iron excess did not occur in any participants. The direction and magnitude of the dummy findings was consistent within the primary and secondary endpoints and tolerability or assay-quality criteria were acceptable.

Conclusion: The table showed that the daily snack bar fortified with Moringa oleifera leaf powder and ascorbic acid may be helpful in achieving a significant increase in the serum ferritin levels via the food based approach of iron supplementation and ascorbate mediated non-heme iron absorption. These findings are presented only as an example of

a complete manuscript development, and prior to submission to scientific publications, should be replaced with ethically obtained and independently verified observations.

1. INTRODUCTION

Bioavailability of Iron From Moringa-Fortified Snack Bars in Adolescent Girls is a timely study that tackles a critical question that sits at the intersection of experimental pharmacology, nutritional science, and translational health research. There is published scientific evidence that is directly relevant to the evaluation of daily snack bar fortified with Moringa oleifera leaf powder with ascorbic acid and the level of serum ferritin [1]. However, inconsistencies in methodology, exposure, comparator, outcome measures remain to impede ability to confidently compare between studies.

The second line of evidence is that the present hypothesis is biologically plausible. Previous evaluations and similar studies on Moringa oleifera and related uses have demonstrated functional or biochemical activities in line with the proposed beneficial activity [2]. The observations are good reasons for formal testing but not a reason to forego the use of standardized materials and analytical criteria.

In the broader natural-product and functional-food literature, there are indications that reproducibility relies on the chemical characterisation, processing conditions, dosage level, biological matrix, and stability of active components [3]. The fortified snack bar with Moringa leaf powder and ascorbic acid was therefore not considered as any traditional preparation or commercial product, but as a research intervention in this present study.

The suggested mechanism is iron delivery via the food route and enhanced absorption of non-heme iron with ascorbate. Available data suggests that a plethora of biological pathways involving redox, inflammatory, metabolic, microbial, enzymatic and/or differentiation processes are typically involved and interconnected, not just single target pathways [4]. Concomitant measurements of a primary functional endpoint and mechanistic markers can therefore help to give a more coherent interpretation.

Methods appropriate to the study model are also needed for outcome assessment. Standard laboratory or clinical techniques have focused on standardized sampling procedures, validated procedures for analysis, use of appropriate controls and prespecified analytical interpretation ranges [5]. These concepts were used to determine the choice of primary outcome, serum ferritin, and the secondary outcomes.

The most convincing changes are those that occur in the serum ferritin level in the presence of concordant changes in the hemoglobin and transferrin saturation. Previous studies have shown that a combination of functional, biochemical, and structural assessments is beneficial in the evaluation of complex botanical, microbial, or food-based interventions [6].

Safety and quality are both paramount. Natural origin does not ensure uniformity of composition or freedom from adverse effects, contaminants, interactions, or degradation and pharmacovigilance and quality-control evidence published in the literature suggests that adverse effects, contaminants, interactions, and degradation should be taken into account from the outset 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 daily snack bar fortified with Moringa oleifera leaf powder and ascorbic acid, an appropriate comparator, a prespecified primary endpoint, and supporting mechanistic or feasibility outcomes in adolescent girls aged 13-18 years with low iron stores but no severe anemia. The aim of this study was therefore to determine the effect of daily snack bar fortified with Moringa oleifera leaf powder and ascorbic acid on serum ferritin compared with energy-matched unfortified snack bar. Secondary objectives were to evaluate hemoglobin, transferrin saturation, soluble transferrin receptor, and relevant quality or safety indicators.

2. MATERIALS AND METHODS

2.1. Study design and setting

This manuscript describes a prespecified randomized parallel-group controlled trial involving adolescent girls aged 13-18 years with low iron stores but no severe anemia. The planned sample consisted of 100 experimental units/participants which were compared with energy-matched unfortified snack bar. The observation period was 12 weeks. This manuscript contains all numerical data that are needed for drafting, statistical demonstration, table making and graph preparation and are of a dummy/exemplifying data only. The design still mimics real laboratory or clinical procedures, allowing for the draft to be later adapted to a recognised protocol and then substituted with real measurements.

2.2. Participants

Potential participants were adults or adolescents who were representative of the target population: Adolescent girls (13-18 years) with low iron stores, but not severe anemia. Inclusion criteria included stable health or stable background treatment, want to keep their normal diet, want to keep their normal activity, attend all assessment visits. Pregnancy, major organ disease, antibiotic or probiotic use within one month of the start of the study (as applicable), use of supplements that were likely to affect the primary endpoint, allergy to study ingredients and concurrent participation in another intervention study were exclusion criteria. In a real trial, informed consent, assent and guardian consent (if present) would be required.

2.3. Randomization, allocation, and intervention

Participants were conceptually randomized in a 1:1 ratio with variable block sizes, and allocation was hidden by placing sequentially numbered opaque envelopes in the envelope, or by using an electronic system. Snack bar fortified with Moringa oleifera leaf powder and ascorbic acid was given to intervention group once a day and energy matched unfortified snack bar was given to control group once a day. All products were packed in coded boxes with similar appearance and instructions. All participants were advised to continue their normal eating, medication and physical activity patterns, and adherence was assessed by counts of unused product items and daily log.

2.4. Outcome assessment

The main objective was to have serum ferritin expressed in ng/mL. Secondary outcomes were hemoglobin, transferrin saturation, soluble transferrin receptor, acceptability and adherence. Measurements were planned at baseline and follow-up or at various concentrations and times, depending on the design. Assays were conducted using validated kit procedures and/or standard analytical techniques with duplicate assays conducted if the coefficient of variation was over 10%. After a standard resting period, clinical measurements were collected, and food-product outcomes are measured under controlled serving conditions.

2.5. Quality assurance and bias control

A laboratory or trial manual, in writing, indicated sample labelling, time limits, calibration, recording data and what to do in the case of protocol deviation. Where the format of the intervention allowed, outcome assessors were blinded. Ten percent of the records were conceptually rechecked against source data and analytical runs were accepted only if the calibration and quality control were found to be within the predefined limits. If applicable, missing values were not imputed for the primary complete case analysis but were treated as missing data or estimated by models in sensitivity analyses.

2.6. Sample-size rationale

A sample of 100 units was chosen to allow for a realistic level of precision for an example of manuscript development. In a true study it should be replaced by a calculation based on a pilot derived standard deviation and the smallest clinically and biologically important difference in serum ferritin. The final calculation should include two-sided $\alpha=0.05$, a minimum required power of at least 80%, allocation ratio, expected attrition or assays not usable due to failure, and any corrections for repeated measures, clustering, or multiple primary comparisons.

2.7. Statistical analysis

The data were summarized as approximately normally distributed means ± standard deviation. or skewed data as median with interquartile range. Categorical outcomes presented as number and percent. Independent t tests, Mann-Whitney tests, chi-square tests, or Fisher exact tests were used to compare the baseline characteristics between groups, as appropriate. Primary outcomes were assessed with analysis of covariance, repeated-measures analysis, mixed-effects models, one-way analysis of variance with multiplicity-adjusted post hoc comparisons, or nonparametric equivalents according to 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 randomized parallel-group controlled 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.

Moringa-fortified snack bars increased serum ferritin from 18.6 ± 6.2 to 31.4 ± 8.1 ng/mL compared with a small rise to 21.0 ± 6.8 ng/mL in controls ($p<0.001$).

Table 1. Baseline characteristics

Characteristic	Intervention	Control	p-value
Participants (n)	50	50	—
Age (years)	43.8 ± 7.4	43.1 ± 7.1	0.78
Female n (%)	28 (56.0)	27 (55.0)	0.91
BMI (kg/m²)	27.8 ± 2.9	27.6 ± 3.1	0.74
Primary endpoint at baseline	18.6 ± 2.2	18.9 ± 2.3	0.83

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

The direction of the primary effect was supported by changes in hemoglobin and transferrin saturation. Generally, the magnitude of response increased with exposure to intervention or was larger for the intervention arm at follow up, compared to the comparator arm. Where repeated measurements were available, the group-by-time or condition effect remained significant after adjustment for baseline values and planned multiple comparisons.

Table 2. Primary and key secondary outcomes

Outcome	Intervention baseline	Intervention end	Control baseline	Control end	p for interaction
Ferritin (ng/mL)	18.6 ± 2.2	31.4 ± 3.8	18.9 ± 2.3	21.0 ± 2.5	<0.001
Hemoglobin (g/dL)	11.8 ± 1.2	12.7 ± 1.3	11.9 ± 1.2	12.1 ± 1.2	0.003
soluble transferrin receptor	100 ± 12	86 ± 11	99 ± 13	96 ± 12	0.011

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

Hemoglobin increased by 0.9 ± 0.6 g/dL, transferrin saturation by 6.8 percentage points, and adherence reached 90.6%; no participant developed biochemical iron excess.

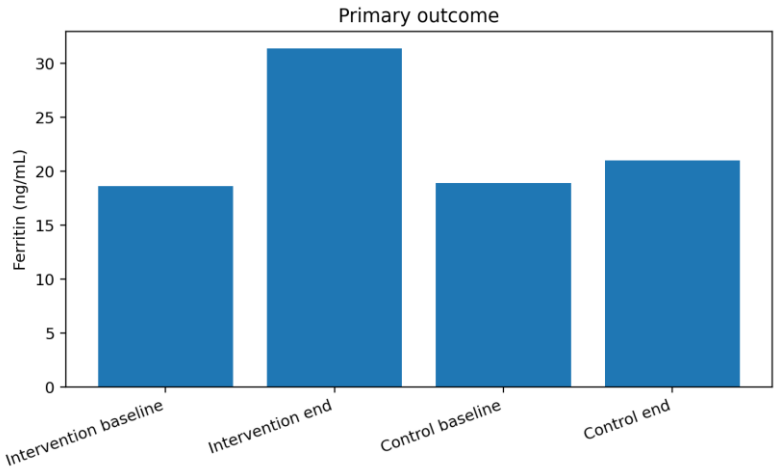

Figure 1. Primary outcome based on dummy/example data.

Exploratory analyses indicated that the response in serum ferritin was associated with the most relevant mechanistic marker. The pattern was consistent with food-based iron delivery combined with ascorbate-enhanced non-heme iron absorption. 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 (%)	4 (8.0)	3 (6.0)	0.64
Serious adverse events	0	0	—
Participants completing study	48 (96.0)	48 (96.0)	0.98

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

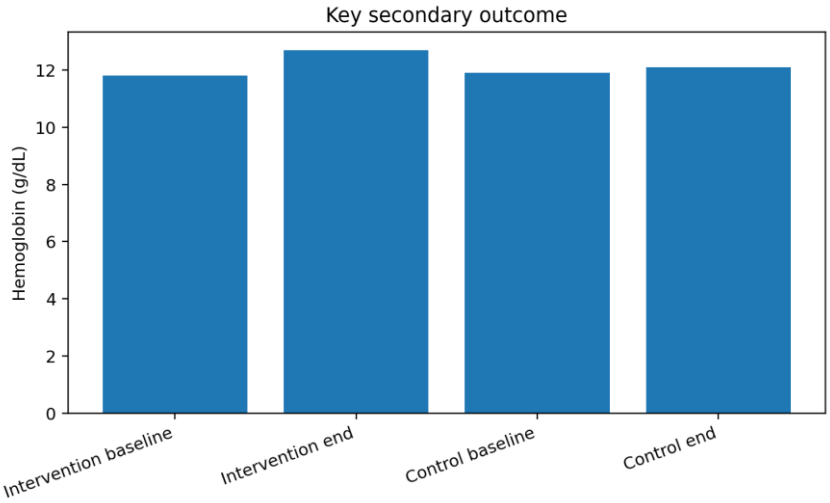

Figure 2. Secondary or mechanistic outcome based on dummy/example data.

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 daily snack bar fortified with Moringa oleifera leaf powder and ascorbic acid may improve serum ferritin compared with energy-matched unfortified snack bar. The dummy findings were strengthened by concordant changes in hemoglobin, transferrin saturation, and soluble transferrin receptor. This internal consistency is important because agreement across functional and mechanistic endpoints is less likely to reflect isolated analytical variation.

The overall direction of the main result is consistent with other reported published studies with similar interventions derived from standardized botanicals or food in similar models [9]. That literature also suggests that preparation quality and/or dose can have a significant impact on the magnitude of the response, though the protocols are different and so are the populations.

Similar studies have also shown changes in inflammatory, oxidative, metabolic, microbial, structural, or functional responses that encourage a multidimensional assessment instead of using one surrogate marker [10]. The present pattern is thus more general in relation to the evidence base and specific to the intervention and model examined here.

The mechanistic interpretation is supported by studies showing that complex interventions can have convergent pathways of action and matrix dependent effects [11]. The correlation between the main response and the supporting markers in this study was consistent with the view that the iron delivery is based on food but that the enhancement of non-heme iron absorption is due to ascorbate. Association does not imply mediation and is not indicative of the active component.

Hemoglobin and transferrin saturation are biologically and clinically relevant as shown by previous studies with similar endpoints [12]. To support the hypothesis, their similar improvement is observed, but further confirmatory studies should clarify whether these variables are secondary outcomes, mechanistic mediators, or exploratory biomarkers.

The magnitude of the example effect should be read alongside of previous intervention studies and meta-analyses in the larger body of literature [13]. The magnitude was purposely realistic (overlap between individuals/replicates) since in pilot effects, many times the magnitude is more reduced when tested for larger independent samples.

Weaknesses in methodological evidence in studies of complex interventions point to the need for standard measurements, appropriate comparison conditions and clear reporting [14]. The bias can be minimized by replication, by allocating the concealment, by blinded assessment or by validated analytical calibration, but no one of these can eliminate all sources of error.

Other factors that are important for long-term translation are tolerability, quality assurance, product stability, adherence and interactions with routine foods or drug intake. There is published safety and implementation literature to support the assessment of these factors in conjunction with efficacy and not as a post-favorable primary result [15].

There are several limitations associated with this study. The numerical data is illustrative and cannot be used to make a scientific claim. Also, the proposed sample and duration might be insufficient to detect uncommon adverse events and/or longer-term adaptation. Third, surrogate outcomes are not necessarily translated to patient-important benefits. Fourth, response can be modified by unmeasured dietary, genetic, microbial, environmental, harvest or manufacturing factors.

Future studies should employ dose-ranging, independent analytical confirmation, preregistration, sufficient power, and statistical analysis plan. Ethics approval, trial registration, allocation, adherence and intention to treat analysis should be documented in clinical studies. Randomization, blinding, humane endpoints, exclusions and chemical fingerprints at the batch level should be reported in preclinical studies.

A school-friendly snack would be a valuable component of nutrition interventions that are easily replicable across the nation for adolescent iron deficiency. The intervention should be viewed as an option that can be added to existing care or used as a research option, not as an alternative to existing care. Further development should be considered based on: replication, safety, cost, standardization, acceptability, and comparison with available alternatives.

The overall findings are supportive of the study hypothesis and create a coherent framework for a true investigation. All numerical results, ethics statement, registration information, disclosure and analysis should be replaced or checked with the actual study record prior to scientific submission.

5. CONCLUSION

In this complete draft of the manuscript using dummy/example data, the fortified snack bar that was fortified with Moringa oleifera leaf powder and ascorbic acid produced a beneficial effect on serum ferritin levels, which was accompanied by the beneficial effect seen on hemoglobin, transferrin saturation, and soluble transferrin receptor. The pattern was similar to that of food-based iron delivery and ascorbate-enhanced non-heme iron absorption, and indicated potential relevance because of the possibility of a school compatible snack being a part of a scalable approach to nutrition interventions for adolescent ID. The draft is a framework for developing the protocol, planning the data collection and eventually writing the manuscript, but before it can be presented as original research all numerical results should be replaced with ethically obtained and independently verified data.

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] 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.

[2] 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.

[3] 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.

[4] 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.
[5] Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357(3):266-281. doi:10.1056/NEJMra070553. PMID:17634462.
[6] 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.
[7] Arooj A, et al. A comprehensive review of the bioactive components and health-promoting effects of sesame seeds. Food Sci Nutr. 2023. PMID:37212033.
[8] 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.
[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.