Assessing the Effects of Micronutrients on Viral Infection in Humans: A Systematic Review and Meta-Analysis

By

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B.S., University of Illinois at Urbana-Champaign, 2018

Thesis

Submitted in partial fulfillment of the requirements for the degree of Master of Science in the Department of Epidemiology in the School of Public Health at Brown University

PROVIDENCE, RHODE ISLAND

MAY 2021

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PREFACE AND ACKNOWLEDGEMENTS

I would like to express my utmost gratitude to Dr. Simin Liu, for his guidance as my thesis advisor. I would also like to thank Bo, and Kenneth who helped me out throughout this journey, my thesis reader Dr. Andrew Zullo, and academic advisor Dr. Brandon Marshall for their invaluable feedback. Finally, thanks mom, dad, and my brother, Daniel, for your unconditional love and support.

Thank you to everyone who calmly waited for this tortoise to finish the race.

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Abstract of Assessing the Effects of Micronutrients on Viral Infection in Humans: A Systematic Review and Meta-Analysis, by Samuel Shim, Brown University, May 2021.

Background: Although many micronutrients are known to have important roles in the regulation of the immune system, the clinical efficacies of micronutrients in the prevention of viral infections remain uncertain. We therefore conducted a systematic review and meta-analysis of available experimental and observational studies evaluating the roles of micronutrients in relation to viral infection risk in humans.

Objective: The objective was to synthesize evidence regarding the roles of micronutrient supplementation and viral infections among apparently healthy humans.

Design: We systematically searched PUBMED/MEDLINE and Embase for both experimental and observational studies that directly assessed the roles of vitamin A, vitamin C, vitamin D, vitamin E, and zinc supplementations in relation to measles, norovirus, HIV, HPV, and respiratory tract infections in humans. Screening of studies was conducted by two independent assessors and agreement obtained via consensus. Quantitative meta-analytical assessment was performed on eligible studies. Risk of bias and quality assessment of the included studies were performed using the Cochrane risk-of-bias assessment tool and the Newcastle-Ottawa Scale.

Results: Of 1,039 studies screened, we identified 22 relevant studies comprising 260,566 participants (40,997 from 17 randomized controlled trials, 3,127 from one prospective cohort study, 202,990 from three retrospective cohort studies, and 13,452 participants from one cross-sectional study). Three of these 22 studies investigated vitamin A, five investigated vitamin C, nine investigated vitamin D, two investigated vitamin E, and three investigated zinc. We quantitatively evaluated 10 randomized controlled trials (1,931 infection cases out of 4,532

participants) using a random-effects model. Although large heterogeneities were identified across available studies (I^2 = 54%; 95% CI: [5%, 77%]), there appeared to be some benefits associated with supplementation of vitamin C (1,000 mg/day), vitamin D (2,000 IU/day), vitamin E (200 IU/day), and zinc (184 mg/day) on lower risk of viral respiratory tract infection (RR: 0.83; 95% CI: [0.70, 0.98]).

Conclusions: Supplementation of vitamin C, vitamin D, vitamin E, and zinc may lower viral respiratory tract infection risk in apparently healthy individuals. However, available studies were limited by small sample size and large amount of heterogeneities in study design and characteristics of outcomes reported in diverse populations. Findings of this systematic review and meta-analysis not only support the need for large-scale randomized intervention studies to determine the balance of benefits or risks associated with micronutrient supplementation in the primary prevention for viral infection but guide the specific design for such a large intervention trial in humans.

INTRODUCTION

The novel coronavirus pandemic has accentuated the importance of proper disease prevention strategies [1]. Although prophylactic methods such as personal hygiene, social distancing, and vaccination are crucial in preventing infectious disease transmission, maintaining a highly functioning immune system through proper nutrition is an inexpensive and effective infectious disease prevention measure. Micronutrients and trace elements play an imperative role in antibody formation, immune response regulation, and immune system development [2-4].

Micronutrient deficiency is the primary cause of immunodeficiency especially in vulnerable populations such as pregnant women, children, and elderly people [5]. It is estimated that up to 2 billion people around the world are facing micronutrient deficiency ranging from vitamins A, C, E, and trace elements like zinc, iron, and iodine [3, 5]. This is of great concern because micronutrient deficiency contributes to increased susceptibility to infectious diseases, which in return leads to malnutrition, forming a vicious cycle [2, 3].

Previous studies have shown inconsistent results on whether nutritional supplementation is associated with risk of infectious diseases among human populations [2, 6]. Reviews of randomized controlled trials have shown that the relationship between vitamins, mineral supplementation, and number of infection episodes was unclear and needs to be further investigated [7, 8]. Although there are a few individual studies which have established relations between select micronutrients and infectious disease risk, a thorough and comprehensive analysis of the overall effect is lacking.

The objective of this systematic review and meta-analysis was to comprehensively assess the effects of micronutrient supplementation on viral infectious disease risk among human populations and provide an up-to-date perspective of the existing literature.

METHODS

Search Strategy

An electronic literature search was conducted in two databases (MEDLINE and EMBASE) for published primary observational and intervention studies up to November 2020. The search strategy was devised based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [9]. Search terms used for the exposure/intervention were each database's MeSH terms corresponding to the following nutrients: vitamin A, vitamin B group, vitamin C, vitamin D, vitamin E, zinc, copper, iron, magnesium, selenium, and omega-3 fatty acids. For the outcome, terms related to viral infectivity such as 'virus infection', 'virus infectivity', and 'virus virulence' were used.

Eligibility Criteria

Studies were included if the study population was consisted of healthy humans that did not have any known viral infections at baseline. Only studies which had a micronutrient as an intervention or exposure of interest, and viral infection as the outcome of interest were included. Literature reviews, letters, or abstracts from conference proceedings, and non-English studies were also excluded.

Study Selection

After the literature search, 29 duplicates were removed using Mendeley's duplicate removal tool. The abstracts of the studies were screened, followed by a full-text evaluation by the author (SS). Studies which were deemed irrelevant to the topic were removed based on the eligibility criteria. Four retrospective cohort studies which assessed the association between

vitamin D status and SARS-CoV-2 infection from a previous search were considered relevant by the author (SS) and added to the screening process.

Quality Assessment and Data Extraction

After the studies have been selected based on the criteria, they were classified by their study types. For randomized controlled trials, the 2nd version of the Cochrane risk-of-bias tool for randomized trials was used to determine the presence of any biases in the study [10]. Risk of bias was assessed based on the following criteria: random-sequence generation, allocation concealment, selective outcome reporting, blinding of the participants, assessors, and personnel, incomplete outcome data, and other possible sources of bias. For cohort and cross-sectional studies, the Newcastle-Ottawa quality assessment scale was used to assess the quality of each study [11]. Quality for cohort studies was based on the following criteria: adequateness of case definition, representativeness of cases, control selection and definition, comparability, ascertainment of exposure, equal ascertainment method for both cases and controls, and non-response rate. Quality for cross-sectional studies were based on the following criteria: representativeness of sample, sample size, non-respondents, ascertainment of exposure, comparability, outcome assessment, and statistical test.

Details of the study selection process is shown in Figure 1. A total of 1,039 studies were initially identified through database searching. After removing duplicate and irrelevant studies, the full text of 47 studies were assessed for eligibility. Among the 47 studies, 24 studies did not show relevant outcomes of interest, and 1 study did not have an English text available. The remaining 22 studies were included for the systematic review. Among the 22 studies, 17 studies were randomized controlled trials [12-14, 16-25, 30-33], one study was a prospective cohort

study [12], three studies were retrospective cohort studies [26-29], and one study was a cross-sectional study [15].

After the quality assessment step, the following data were extracted from the individual studies study: first author, publication year, country, study design, sex proportion and average age of participants, study population characteristics, sample size, study duration, micronutrient supplementation dosage, and viral outcomes of interest, and estimates for effect size and variance (95% CI if available) (Table 1).

Data Synthesis and Analysis

The number of cases who had study's outcome of interest from the intervention and placebo groups were used to calculate the risk ratio and 95% confidence interval using the DerSimonian and Laird random-effects model [34]. Between-study heterogeneity was examined by using the I^2 statistic, with an I^2 score of 25%, 50%, and 75% corresponding to low, moderate, and high heterogeneity, respectively [35]. Egger's test and funnel plots were used to test for the presence of publication bias [36]. $P \leq 0.05$ was considered statistically significant for all tests with the exception of the publication bias test, where $P \leq 0.10$ was considered significant [37]. All statistical analyses were performed with RStudio version 1.2.5 (RStudio Inc).

RESULTS

Study Characteristics

Three studies were conducted in African countries, seven in Asian countries, four in European countries, and nine in North American countries. The sex proportion of the studies varied from 0% to 100%, with the average age of participants ranging from 11.7 months to 84.9 years old. Three studies used age groups instead of providing the mean age of participants, and one study used the student's grades. The sample size of the studies ranged from 28 to 25,443 participants in randomized controlled trials, 489 to 191,779 participants in cohort studies, and 13,452 participants in the cross-sectional study. Study duration ranged from as short as 5 days to as long as 2 years.

Risk of Bias and Quality Assessment

With three exceptions, the 17 randomized controlled trials included in this review had low risk of bias in random sequence generation, allocation concealment, and proportion of incomplete outcome data overall. However, most of the studies failed to specify measures taken to prevent selective reporting. Vitamin A studies included in this review were unclear in their participant and personnel blinding procedures, while vitamin C studies failed to specify both participant and outcome assessment blinding procedures. Based on the Newcastle-Ottawa scale, all 5 observational studies included in this review had an overall score of 7 stars. The studies had good marks in representativeness, selection of non-exposed cohort, ascertainment of exposure, and comparability of cohorts, outcome assessment, and follow-up length, but lacked in follow-up adequacy.

Vitamin A

The body of vitamin A studies comprised of 48,584 participants, 1,075 cases and 7,764 person-years included from three randomized controlled trials and one cross-sectional study with measles, HIV, norovirus, and HPV infection as the outcome of interest, respectively (Table 1) [12-15]. Three randomized controlled studies had vitamin A supplementation as the intervention [12-14], and one cross-sectional study had dietary vitamin A intake amount as the exposure [15]. One randomized controlled study showed that monthly vitamin A supplementation of 100,000 IU/month for those between 6 to 11 months old and 200,000 IU/month for those more than 11 months old showed lower measles incidence rate in the intervention group compared to the placebo group [12]. One randomized controlled study showed that a single dose of 400,000 IU of vitamin A supplementation within 96 hours of post-partum among mothers and her neonates had minor increase in hazard ratio of HIV incidence rate compared to the placebo group after adjusting for key demographics [13]. One randomized controlled study showed that a bi-monthly 20,000 IU vitamin A supplementation for infants younger than 12 months, and 45,000 IU for infants older than 12 months had a small increase in GI norovirus risk, and a significant decrease in GII norovirus risk compared to the placebo group [14]. One cross-sectional study among women between 18-59 years old showed that a $log2$ mcg increase in dietary vitamin A intake leads to a minor decrease HPV incidence odds [15].

Vitamin C

The body of vitamin C studies were comprised of 1,873 participants, 809 cases, and 1,170 cold episodes included from five randomized controlled trials, with common cold and upper respiratory tract infection as the outcome of interest [16-20]. Five randomized controlled trials had vitamin C supplementation as the intervention [16-20]. Two studies which provided

1,000 mg/day of vitamin C to the intervention group showed slightly lower risk of common cold incidence compared to the placebo group [16, 18]. One study which provided the same dose of vitamin C among 9-year-old children showed significantly lower common cold incidence and number of episodes compared to the placebo group [17]. One study which provided 1,000 mg/day of vitamin C to healthy participants, and 4,000 mg/day of vitamin C to participants who were experiencing a cold showed slightly lower risk of common cold incidence compared to the placebo group [18]. One study which provided 500 mg/day of vitamin C and 15 mg/day of β-carotene to adults between 40-69 years old showed slightly lower cold incidence compared to adults who were provided 50 mg/day of vitamin C and no β-carotene [20].

Vitamin D

The body of vitamin D studies were comprised of 202,990 participants, and 2,329 cases from five randomized controlled trials which had influenza A, B, non-influenza viral infection, and respiratory tract infection as the outcome of interest [21-25], and three retrospective cohort studies which had SARS-CoV-2 infection as the outcome of interest [26-28]. The five randomized controlled trials had vitamin D supplementation as the intervention [21-25]. One study showed that a 14,000 IU/week of vitamin D supplementation decreased non-influenza virus infection hazard, but increased influenza virus infection hazard compared to the placebo group among children between 3-17 years old [21]. One study showed that a 2,000 IU/day of vitamin D supplementation increased both influenza A and influenza-like illness risk compared to the placebo group among students between 15-18 years old [22]. A different study from the same authors showed that a 1,200 IU/day of vitamin D supplementation among students between the age of 6-15 decreased influenza A risk, but increased influenza B risk [25]. One study showed that children between 1-5 years old who received 2,000 IU/day of vitamin D

supplementation has little to no difference in upper respiratory tract infection incidence rate compared to those who received 400 IU/day of vitamin D supplementation [23]. One study showed that among infants between the age of 3-12 months, infants who received 1,200 IU/day of vitamin D supplementation had a lower risk of influenza A infection compared to infants who received 400 IU/day of vitamin D supplementation [24].

Three retrospective cohort studies had plasma vitamin D status as the exposure [26-28]. One study showed that among hospital patients who were tested for SARS-CoV-2 infection, those with plasma vitamin D levels below 20 ng/mL had a higher risk of infection compared to those with plasma vitamin D levels equal or above 20 ng/mL [26]. One study showed that participants with plasma vitamin D levels lower than 20 ng/mL or 30 ng/mL had higher odds of SARS-CoV-2 infection compared to participants above those levels [27]. One study showed that those with plasma vitamin D levels lower than 10 ng/mL and 20 ng/mL had slightly lower odds of SARS-CoV-2 infection compared to participants above those levels [28].

Vitamin E

The body of vitamin E studies were comprised of 3,744 participants, and 764 cases from one randomized controlled trial and one prospective cohort study which had vitamin E supplementation as the intervention, and respiratory tract infection and common cold as the outcome of interest [29, 30]. One prospective cohort study had vitamin E and β-carotene supplementation as the exposure, and common cold incidence as the outcome of interest [29]. This study showed that among men between the age of 50-69 who engage in heavy physical activity at their jobs, 50 mg/day of vitamin E slightly increases the risk of common cold, 20 mg/day of β-carotene did not show any change in risk of common cold, and consuming both micronutrients slightly reduced the risk of common cold compared to the placebo group.

Furthermore, among men in the same age group who engage in heavy exercise during their leisure time, vitamin E, β-carotene, and combined micronutrient supplementation slightly increased the risk of common cold compared to the placebo group.

One randomized controlled trial had vitamin E supplementation as the intervention, and lower respiratory tract infection, upper respiratory tract infection, and common cold incidence as the outcome of interest [30]. This study showed that among long-term care facility residents between 65-103 years old, 200 IU/day supplementation of vitamin E did not show any change in incidence rate of lower respiratory tract infection, but slightly lower incidence rate of upper respiratory tract infection and common cold incidence rate compared to the placebo group.

Zinc

The body of zinc studies were comprised of 460 participants, and 1,358 cases from two randomized controlled trials with zinc supplementation as the intervention, and one randomized controlled trial with zinc-fortified tap water intake as the intervention [31-33]. All three studies had the common cold as the outcome of interest [31-33]. One randomized controlled trial had zinc fortified water consumption as the intervention and common cold incidence as the outcome of interest [31]. This study showed that among children between the ages of 2-6, providing zinc fortified tap water lowered the risk of common cold risk compared to the placebo group who were provided ordinary tap water. One randomized controlled study showed that among healthy adults who were exposed to the virus via intranasal drops, a 184 mg/day supplementation of elemental zinc had slightly lower incidence of rhinovirus type-13 infection compared to the placebo group [32]. In the same study, it showed that a supplement of 207 mg of elemental zinc at the first day followed by 184 mg/day supplementations lead to a minor increase in rhinovirus type-39 infection compared to the placebo group. One randomized controlled study showed that

children between the age of 78-120 months who were provided a 10 mg/day supplementation of elemental zinc had lower average common cold occurrences compared to the placebo group [33].

Quantitative Synthesis

Among the 17 randomized controlled trials included in the qualitative synthesis [12-14, 16-25, 30-33], three studies were excluded from the meta-analysis due to high risk of bias [12, 20, 33], and four studies were excluded due to irrelevant measure of effect [13, 14, 23, 31]. 10 randomized controlled trials comprising 1,931 cases from 4,532 participants were included in the analysis were included in the meta-analysis, comprised of four vitamin C studies [16-19], 4 vitamin D studies [21, 22, 24, 25], one vitamin E study [30], and one zinc study [32]. All 10 included studies commonly had a type of respiratory tract infection risk as the outcome of interest which ranged from the common cold, influenza A and B, rhinovirus, upper and lower respiratory tract infection. The pooled results showed that micronutrient supplementation leads to a decrease in viral respiratory tract infection risk (RR: 0.83; 95% CI: [0.70, 0.98]). There was moderate heterogeneity between-studies (I^2 = 54%; 95% CI: [5%, 77%]). Both visual inspection of the funnel plot and Egger's test did not show any presence of funnel plot asymmetry (intercept: -1.08, 95% CI: [-2.53, -0.37], P = 0.18).

DISCUSSION

In this meta-analysis of 10 randomized controlled studies comprising 1,931 cases from 4,532 participants, we found that vitamin C, D, E, and zinc supplementation is negatively associated with viral respiratory tract infection risk. Micronutrient supplementation reduced the risk of respiratory tract infection by 17%. Moderate heterogeneity was observed throughout the included studies, which can be explained by the distinct study populations for each included study. Most of the included studies had small sample sizes with the study population based on a subset of the general population such as students or long-term care facility residents, possibly due to the fact that they are easier to recruit as they are already part of a cohort (e.g. school, nursing home).

Furthermore, most of the studies included in this review did not measure the participant's nutritional status at baseline. Nutritional supplementation leads to higher health benefits among participants who are nutritionally deficient or insufficient [38, 39]. It is possible that the participants of the included studies were consisted of a high proportion of nutritionally sufficient participants, which showed little to no effect of micronutrient supplementation on viral infection risk and vice versa. It is recommended that future studies should measure the nutritional status of its participants at both baseline and follow-up to accurately assess the effects of micronutrient supplementation on viral infection risk.

Vitamin A studies had the most variety of outcomes, but there were no studies which shared the same type of viral infection as the outcome of interest. Vitamin A supplementation led to slightly lower risk of measles infection, but slightly higher risk of HIV infection among children younger than 2 years old. It also caused somewhat higher risk of norovirus GI infection, but significantly lower risk of GII infection among mothers and neonates. HPV infection odds

among adult women decreased by 3% per $log2$ mcg of dietary vitamin A intake. The measles randomized controlled trial showed high risk of bias due to its lack of randomization, allocation concealment, and proportion of incomplete outcome data [12]. The two more recently conducted randomized controlled trials and cross-sectional study showed low risk of bias [13-15]. Nonetheless, vitamin A studies based on a more inclusive study population which is not limited to children and infants are needed to further investigate the association between vitamin A and viral infections.

Vitamin C studies included in this systematic review all had a type of respiratory tract infection as the outcome of interest, with the cold being most common outcome of interest. Studies conducted among the general population showed that vitamin C supplementation led to less common cold incidence and less common cold episodes. However, vitamin C supplementation studies conducted among adults and men showed little to no difference in common cold incidence when comparing the treatment and placebo group. Finally, a seven-week long study among 9-year-old children showed slightly higher upper respiratory tract infection incidence among those who received vitamin C supplementation. Overall, randomized controlled trials had low risk of bias [16-18, 19, 20]. One limitation of the included vitamin C studies was that the outcome assessment was based on the participant's display of symptoms, instead of viral testing.

Vitamin D studies included in this review were consisted of five randomized controlled trials which had respiratory tract infections as the outcome of interest, and three retrospective cohort studies with SARS-CoV-2 infection as the outcome of interest. The study population of the randomized controlled studies were all infants or children who were attending school, thus limited the generalization of the results. Vitamin D supplementation among infants 3-12 months

old showed a decrease in all viral infections. Among students 15-18 years old, it showed a minor increase in risk of influenza A and influenza-like illness. Among students 6-15 years old, influenza A incidence was lower among students who received supplementation. Among children 3-17 years old, supplementation slightly decreased the hazard of non-influenza virus infections, but slightly increased the hazard of influenza virus infections. Among children 1-5 years old, those who received a daily 2,000 IU vitamin D supplementation showed minor decrease in upper respiratory tract infection than those who received a daily 400 IU vitamin D supplementation. Overall, the results from these randomized controlled trials showed that vitamin D supplementation led to a minor decrease in influenza and respiratory tract infection risk. Except for one study which did not clearly indicate details of the study methods [24], the randomized controlled trials showed low risk of bias among all components of the study design [21-23, 25]. Results of these randomized controlled studies must be interpreted in the context of an infant or child population. Future studies must be conducted on a study population which includes older participants.

Overall, two out of the three retrospective cohort studies showed that vitamin D deficiency was linked to increased risk of SARS-CoV-2 infection. After adjusting for covariates, participants whose vitamin D status were deemed deficient or insufficient showed higher odds of SARS-CoV-2 infection compared to those who were vitamin D sufficient [26, 27]. The one exception found no association between vitamin D status and SARS-CoV-2 infection after adjusting for covariates [28]. Based on the Newcastle-Ottawa Scale, these studies showed little risk of bias and good quality with an average score of 7. A couple limitations from these studies are that the time period of the COVID-19 datasets which were used for the studies were limited to about a month at the beginning of the pandemic, where only a limited number of the

population had been potentially exposed with SARS-CoV-2. Therefore, the study findings that were included may not be applicable to the general population. Furthermore, the vitamin D status of the study participants were collected up to several years prior to their SARS-CoV-2 infection. It is possible that their vitamin D status may have changed significantly between the points of plasma vitamin D status data collection and SARS-CoV-2 infection. Clearly, there is lack of literature regarding nutrition and COVID-19 due to the rapidly changing circumstances. Further studies are needed in order to accurately assess the association between the two factors.

The two Vitamin E studies that were included in this review had the common cold and respiratory tract infection as the outcome of interest. The study population for these two studies were men 50-69 years old, and long-term care facility residents [29, 30]. Evidence from these studies indicate daily vitamin E supplementation had little to no effect in reducing common cold or lower and upper respiratory tract infections. In fact, men who engaged in either heavy job activity or heavy exercise during their leisure time had a slightly elevated risk of common cold with vitamin E supplementation. Both studies showed low risk of bias during their quality assessment, but the prospective cohort study lacked controlling for confounders when calculating the effect estimates [29]. Due to the specificity of the study population of these studies, it is recommended that future studies be performed on a more general population, but not limited to elderly participants.

Of the three zinc randomized controlled trials included in the review, two studies provided elemental zinc tablets as the intervention [32, 33], and one study provided zinc fortified water as the intervention [31]. Evidence suggested that zinc supplementation reduced the average common cold episodes and number of participants catching the common cold among children 2-6 years old, but detailed description of the methods used in this study were not specified, making

the study at high risk of bias [33]. The other randomized controlled study was unique in that the participants were actively exposed to rhinovirus via nasal drops instead of relying on naturally occurring infections [32]. However, the studies yielded conflicting results, most likely due to the small number of participants for each trial [32]. Zinc fortified water proved to be an effective way to increase dietary intake of zinc, and thus reduced the risk of common cold accompanied with a runny nose, and other cold related symptoms among children 6-10 years old [31]. These two studies showed low risk of bias but lacked description regarding measures to prevent selective outcome reporting. [31, 32]. Overall, the zinc studies were small in their study size, and it is recommended that future zinc studies be performed on larger sample sizes.

Strengths of this systematic review and meta-analysis include that it is a comprehensive and collective review of the sole effect of a micronutrient on viral infection of both experimental and observational studies, and it synthesized quantitative evidence from randomized controlled trials by calculating the overall effects of micronutrient supplementation. Studies which measured the combined effect of micronutrient supplementation alongside a vaccine or therapeutic treatment were excluded.

Limitations of this study include the lack of available literature regarding this research topic, especially for vitamin A, B group, E, and trace minerals. Majority of the studies surrounded the association between vitamin C, D and respiratory tract infection risk. Also, the study population of the included studies were performed on subsets of the general population which limits generalizability of the study findings. Finally, due to ethical reasons, all but one randomized controlled trial included in the review relied on the natural occurrence of viral infections instead of assuring exposure to the pathogen to the participants. It is unsure if the participants of the intervention and placebo group had equal chance of infection.

In summary, our study indicates that vitamin C, D, E, and zinc supplementation has modest benefits in reducing respiratory tract infection risk. Associations between other micronutrients and viral infection risk were inconsistent and thus inconclusive. There is an absence of published literature especially in vitamin A, vitamin E, and zinc studies. Further investigations should consider collecting the micronutrient status of interest from the participants and recruit a sizeable sample size from a generalizable study population.

CONFLICTS OF INTEREST

The author declares no conflicts of interests or financial ties to disclose.

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TABLES AND FIGURES

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram illustrating the study identification and selection process.

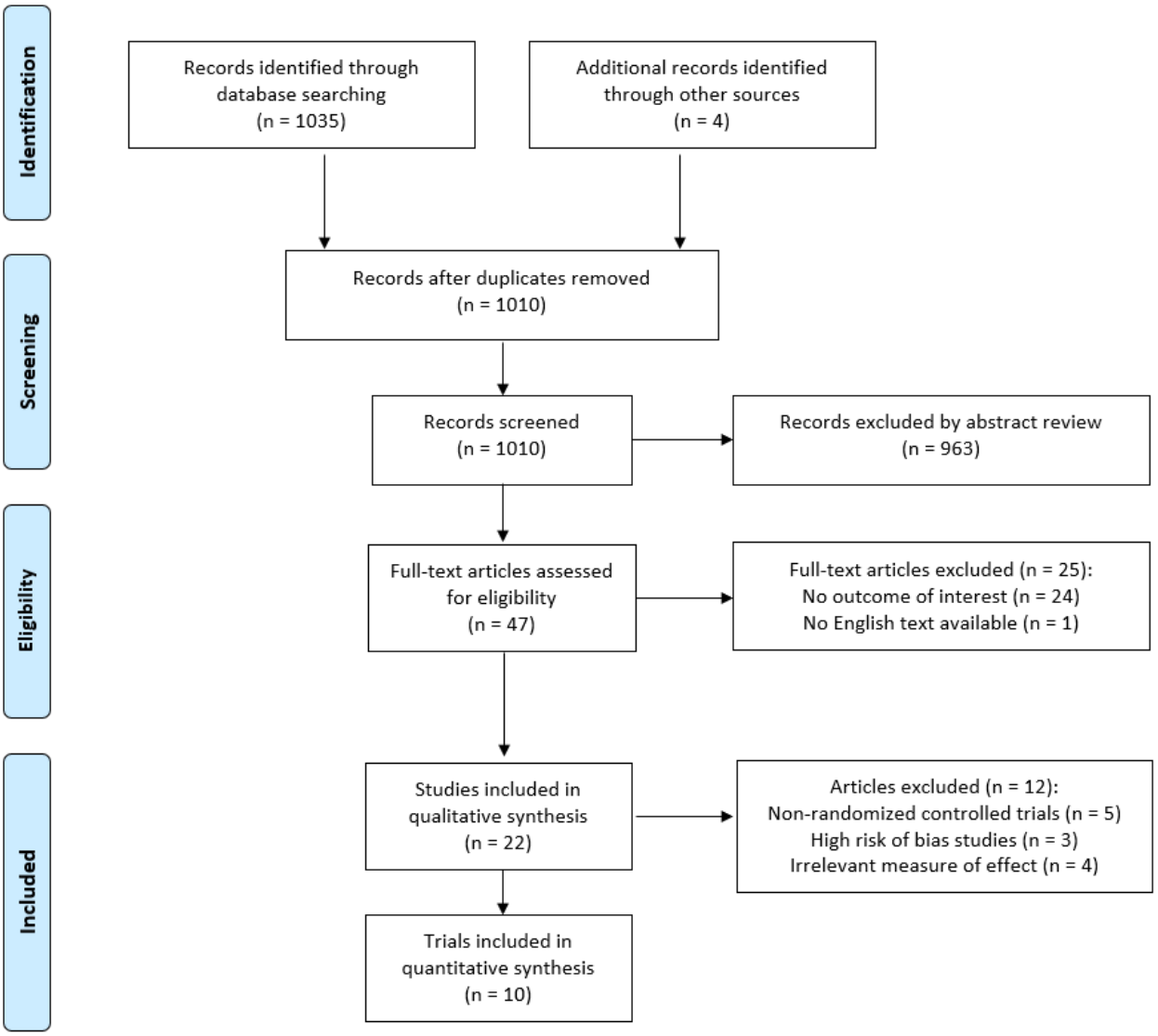

Figure 2. Forest plot of the effects of micronutrient supplementation on viral respiratory tract infection risk.

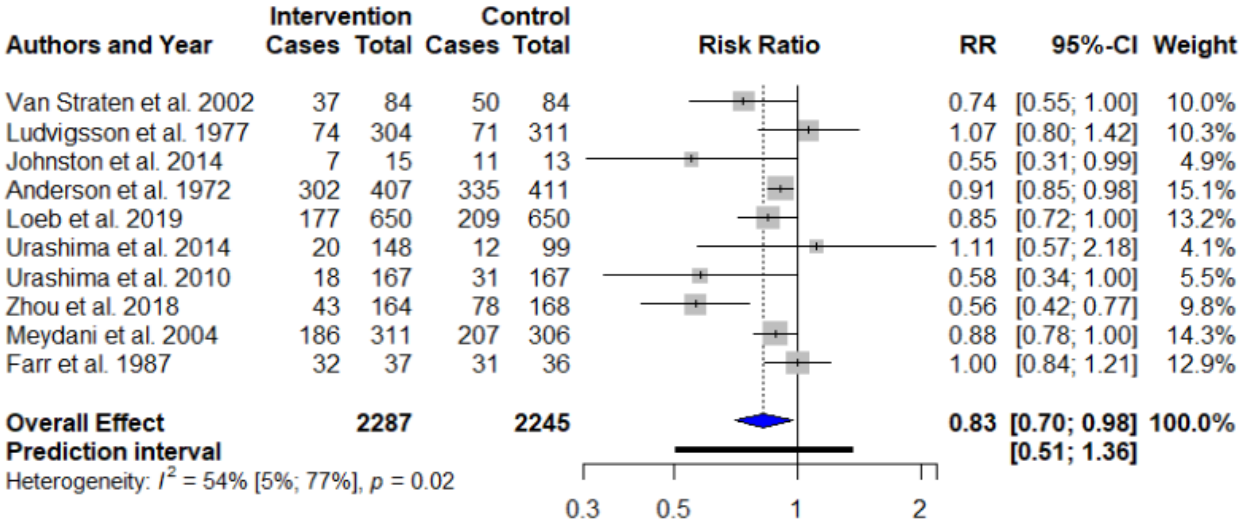

Figure 3. Funnel plot for publication bias assessment.

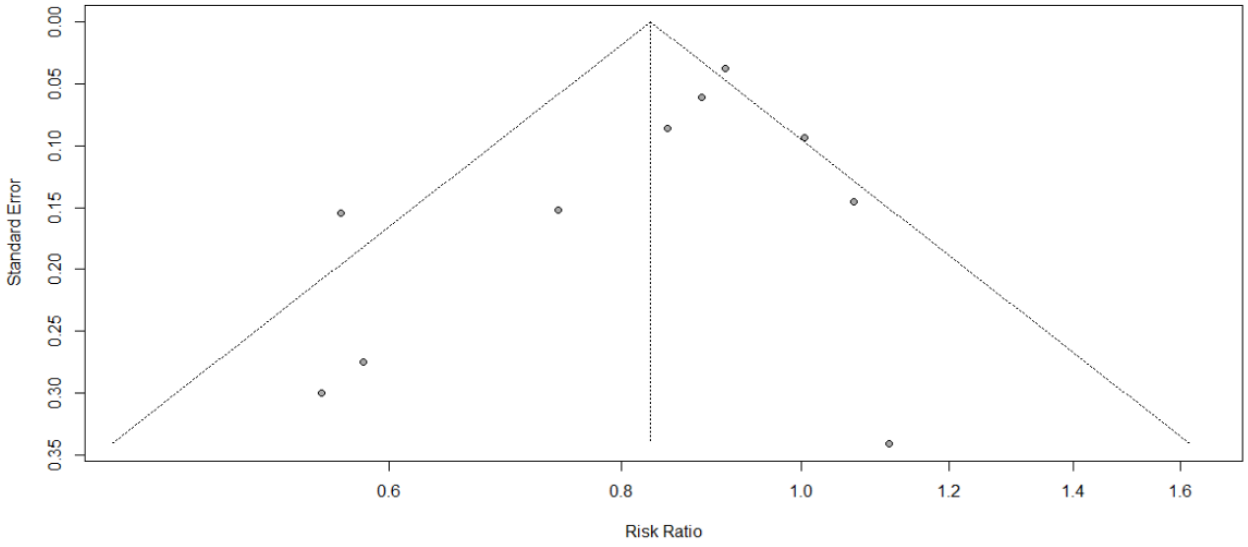

Table 1.
Characteristics of Published Studies Included in the Systematic Review

Nutrient	Author, year	Study Design	Country	Number of males (%)	Age (Average) (Intervention/Control)	Population Type	Sample size (Intervention/Control)	Outcome	Study Duration
Vitamin A									
	Dollimore 1997	Randomized Controlled Trial	Ghana	12,812 (50.3)	11.7 (months)	Children	25,443	Measles	2 years
	Humphrey 2006	Randomized Controlled Trial	Zimbabwe	0 (0)	24.4/24.0	Mothers and neonates	9,562 (4,802/4,760)	HIV	2 years
	Long 2007	Randomized Controlled Trial	Mexico	63 (49.6)	6-18 (months)	Children < 2 years	127 (57/70)	Norovirus	3 months
	Huang 2020	Cross-sectional	US	0 (0)	35.9/38.2	Women 18-59 years old	13,452	HPV	14 years
Vitamin C									
	Van Straten 2002	Randomized Controlled Trial	UK	27 (16.1)	47.7/48.5	General population	168 (84/84)	Cold	60 days
	Ludvigsson 1977	Randomized Controlled Trial	Sweden	316 (51.4)	9.3/9.3	Children 9 years old	615 (304/311)	Upper respiratory tract infection	7 weeks
	Anderson 1972	Randomized Controlled Trial	Canada	357 (43.6)	N/A	General population	818 (407/411)	Cold	4 months
	Johnston 2014	Randomized Controlled Trial	US	30 (100)	23/23.2	Men 18-35 years old	28 (15/13)	Cold	8 weeks
	Sasazuki 2006	Randomized Controlled Trial	Japan	86 (35.2)	58.7/56.3	Adults 40-69 years old	244 (124/120)	Cold	5 years
Vitamin D									
	Loeb 2019	Randomized Controlled Trial	Vietnam	621 (52.2)	8.6/8.4	Children 3-17 years old	1,300 (650/650)	Influenza virus infection Non-influenza virus infection	1 year
	Urashima 2014	Randomized Controlled Trial	Japan	163 (66)	N/A	Students 15-18 years old	246 (148/99)	Influenza A Influenza-like illness	2 months
	Aglipay 2017	Randomized Controlled Trial	Canada	703 (57.5)	2.70/2.76	Children 1-5 years old	703 (349/354)	Upper respiratory tract infection	5 years (winter months only)
	Zhou 2018	Randomized Controlled Trial	China	173 (52.1)	7.7/8 (months)	Infants 3-12 months old	332 (164/168)	Influenza A	4 months
	Urashima 2010	Randomized Controlled Trial	Japan	242 (56.2)	10/10.4	Students 6-15 years old	334 (167/167)	Influenza A and B	4 months
	Meltzer 2020	Retrospective Cohort	US	123 (25)	45.9/51	Hospital patients who tested for COVID-19	489 (172/317)	SARS-CoV-2 Infection	39 days
	Mezron 2020	Retrospective Cohort	Israel	3,234 (41.4)	35.6/47.4	General population who tested for COVID-19	7,807 (782/7,025)	SARS-CoV-2 Infection	3 months
	Hastie 2020	Retrospective Cohort	UK	168,656 (48.4)	49/49	General population	348,598	SARS-CoV-2 Infection	1 month
Vitamin E									
	Hemila 2003	Prospective Cohort	Finland	3,127 (100)	N/A	Men 50-69 years old	3,127	Cold	2 years
	Meydani 2004	Randomized Controlled Trial	US	169 (27.3)	84.9/84.5	Long-term care facility residents 65-103 years old	617 (311/306)	Respiratory tract infections	1 year
Zinc									
	Kujinga 2018	Randomized Controlled Trial	Kenya	84 (46)	47/46 (months)	Children 2-6 years old	184 (90/94)	Cold	6 months
	Farr 1987	Randomized Controlled Trial	US	Trial 1: 13 (40.6) Trial 2: 22 (50)	Trial 1: 21.4/20.6 Trial 2: 21.1/21.1	Adults	Trial 1: 32 (16/16) Trial 2: 44 (23/21)	Cold	Trial 1: 5 days Trial 2: 7 days
	Vakili 2009	Randomized Controlled Trial	Iran	100 (50)	93.7/93.1 (months)	Children 78-120 months old	200 (100/100)	Cold	5 months

Table 2.
Effect Estimates of Published Studies Included in the Systematic Review

Nutrient	Author year	Type of intervention/exposure	Outcome	Effect measure	Effect measure estimate [95% CI]	Subgroup analysis
Vitamin A						
	Dollimore 1997	Vitamin A (100,000 IU/month) or (200,000 IU/month)	Measles	IRR	0.82	
	Long 2007	Vitamin A (20,000 IU/2 months for infants <12 months, 45,000 IU/2 months for infants >12 months)	All norovirus episodes	RR	0.81 [0.57, 1.15]	
			Norovirus GI episodes	RR	1.13 [0.68, 1.87]	
			Norovirus GII episodes	RR	0.51 [0.27, 0.97]	
	Humphrey 2006	Vitamin A (400,000 IU)	HIV	HR	1.09 [0.85, 1.38]	
	Huang 2020	Dietary Vitamin A Intake (log2 mcg increment)	HPV	OR	0.97 [0.92, 1.02]	
Vitamin C						
	Van Straten 2002	Vitamin C (1,000 mg/day)	Cold	RR	0.74 [0.55, 1.00]	
	Ludvigsson 1977	Vitamin C (1,000 mg/day)	Upper respiratory tract infection	RR	1.09 [0.80, 1.42]	
	Johnston 2014	Vitamin C (1,000 mg/day)	Cold	RR	0.55 [0.33, 0.94]	
			Cold episodes	RR	0.66 [0.27, 1.55]	
	Anderson 1972	Vitamin C (1,000 mg/day), If sick, (4,000 mg/day)	Cold	RR	0.91 [0.85, 0.98]	
	Sasazuki 2006	Vitamin C (500 mg/day) AND β-carotene (15 mg/day)/ Vitamin C (50 mg/day) AND β-carotene (0 mg/day)	Cold	RR	0.93 [0.65, 1.34]	
Vitamin D						
	Loeb 2019	Vitamin D (14,000 IU/wk)	Non-Influenza virus infection	HR	0.76 [0.61, 0.94]	
			Influenza virus infection	HR	1.18 [0.79, 1.77]	
			All viral infections	HR	0.81 [0.67, 0.99]	
	Urashima 2014	Vitamin D (2,000 IU/day)	Influenza A	RR	1.11 [0.57, 2.18]	
			Influenza-like Illness	RR	1.26 [0.72, 2.14]	
	Aglipay 2017	Vitamin D (2,000 IU/day)/(400 IU/day)	Upper respiratory tract infection	IRR	0.97 [0.8, 1.16]	
	Urashima 2010	Vitamin D (1,200 IU/day)	Influenza A	RR	0.58 [0.34, 0.99]	
			Influenza B	RR	1.39 [0.9, 2.15]	
	Zhou 2018	Vitamin D (1,200 IU/day)/(400 IU/day)	Influenza A	RR	0.56	
	Meltzer 2020	Vitamin D < 20 ng/mL	SARS-CoV-2 Infection	RR	1.77 [1.12, 2.81]	
	Mezron 2020	Vitamin D < 20 ng/mL	SARS-CoV-2 Infection	OR	1.58 [1.13, 2.09]	
		Vitamin D < 30 ng/mL	SARS-CoV-2 Infection	OR	1.5 [1.13, 1.98]	
	Hastie 2020	Vitamin D < 10 ng/mL	SARS-CoV-2 Infection	OR	0.88 [0.72, 1.08]	
		Vitamin D < 20 ng/mL	SARS-CoV-2 Infection	OR	0.92 [0.71, 1.21]	
Vitamin E						
	Meydani 2004	Vitamin E (200 IU/day)	All respiratory tract infections	RR	0.88 [0.76, 1.00]	
			Lower respiratory tract infections	RR	0.99 [0.78, 1.23]	
			Upper respiratory tract infections	RR	0.84 [0.69, 1.00]	
			Cold	RR	0.83 [0.67, 1.00]	
	Hemila 2003	Vitamin E (50 mg/day)	Cold	RR	1.08 [0.99, 1.18]	Heavy job activity
			Cold	RR	1.1 [0.96, 1.27]	Heavy exercise at leisure
		Vitamin E (50 mg/day) & β-Carotene (20 mg/day)	Cold	RR	0.95 [0.87, 1.04]	Heavy job activity
			Cold	RR	1.21 [1.06, 1.38]	Heavy exercise at leisure

Zinc

Vakili 2009	Elemental Zinc (10 mg/day)	Average cold occurrence	RR	0.55
Farr 1987	Elemental Zinc (184 mg/day)	Rhinovirus type-13	RR	0.77 [0.52, 1.16]
	Elemental Zinc (1st day: 207 mg, remainder: 184 mg/day)	Rhinovirus type-39	RR	1.07 [0.75, 1.57]
Kujinga 2018	Zinc fortified water	Cold episodes	RR	0.91 [0.83, 0.99]

Table 3.

Assessment of Potential Biases in Published Randomized Controlled Trials

Nutrient	First Author, Year	Random Sequence Generation	Allocation Concealment	Selective Reporting	Blinding (Participants and Personnel)	Blinding (Outcome Assessment)	Incomplete Outcome Data	Other Sources of Bias
Vitamin A								
	Dollimore 1997	High	High	Unclear	Unclear	Low	High	High
	Humphrey 2006	Unclear	Low	Unclear	Unclear	Low	Low	Low
	Long 2007	Low	Low	Low	Low	Low	Low	Low
Vitamin C								
	Van Straten 2002	Low	Low	Low	Low	Unclear	Low	Unclear
	Ludvigsson 1977	Low	Low	Unclear	Unclear	Low	Low	Low
	Anderson 1972	Low	Low	Unclear	Low	Unclear	High	High
	Johnston 2014	Low	Low	Unclear	Low	Unclear	Low	Low
	Sasazuki 2006	Low	Unclear	Unclear	Unclear	Unclear	High	High
Vitamin D								
	Loeb 2019	Low	Low	Low	Low	Low	Low	Low
	Urashima 2014	Low	Low	Low	Low	Low	Low	Low
	Aglipay 2017	Low	Low	Unclear	Low	Low	Low	Low
	Zhou 2018	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Unclear
	Urashima 2010	Low	Low	Low	Low	Low	High	Low
Vitamin E								
	Meydani 2004	Low	Low	Low	Low	Low	Low	Low
Zinc								
	Kujinga 2018	Low	Low	Unclear	Low	Unclear	Low	Unclear
	Farr 1987	Low	Low	Unclear	Low	Low	Low	Low
	Vakili 2009	High	High	Unclear	Unclear	Unclear	Low	Unclear

Table 4

Quality Assessment for Published Observational Studies

Nutrient	Author/Year	Representativeness	Selection of non-exposed cohort	Ascertainment of exposure	Demonstration of outcome of interest presence at start of study	Comparability of cohorts	Outcome assessment	Follow-up length	Follow-up adequacy	Total
Vitamin D	Meltzer 2020	c	a	a	b	a,b	b	a	b	7
	Merzon 2020	a	a	a	b	a,b	b	a	c	7
	Hastie 2020	a	a	a	b	a,b	b	a	c	7
Vitamin E	Hemila 2003	b	a	b	a	c	b	a	b	7
		Representativeness	Sample size	Non-respondents	Ascertainment of exposure	Comparability	Assessment of outcome	Statistical test		
Vitamin A	Huang 2020	b	a	a	b	b	a	a		7

SUPPLEMENTARY MATERIAL

Search Terms

Pubmed – 757 Results

Intervention/Exposure

("Vitamin A"[Mesh] OR "Vitamin B Complex"[Mesh] OR "Thiamine"[Mesh] OR "Vitamin B 6"[Mesh] OR "Folic Acid"[Mesh] OR "Vitamin B 12"[Mesh] OR "Ascorbic Acid"[Mesh] OR "Vitamin D"[Mesh] OR "Vitamin E"[Mesh] OR "Copper"[Mesh] OR "Iron"[Mesh] OR "Zinc"[Mesh] OR "Selenium"[Mesh] OR "Magnesium"[Mesh] OR "Fatty Acids, Omega-3"[Mesh] OR "Docosahexaenoic Acids"[Mesh] OR "alpha-Linolenic Acid"[Mesh] OR "Eicosapentaenoic Acid"[Mesh])

Outcome

("Virus Diseases"[Mesh] OR "Virulence"[Mesh])

Study Type

("Clinical Trial" [Publication Type] OR "Observational Study" [Publication Type])

Combined Search Keyword

("Vitamin A"[Mesh] OR "Vitamin B Complex"[Mesh] OR "Thiamine"[Mesh] OR "Vitamin B 6"[Mesh] OR "Folic Acid"[Mesh] OR "Vitamin B 12"[Mesh] OR "Ascorbic Acid"[Mesh] OR "Vitamin D"[Mesh] OR "Vitamin E"[Mesh] OR "Copper"[Mesh] OR "Iron"[Mesh] OR "Zinc"[Mesh] OR "Selenium"[Mesh] OR "Magnesium"[Mesh] OR "Fatty Acids, Omega-3"[Mesh] OR "Docosahexaenoic Acids"[Mesh] OR "alpha-Linolenic Acid"[Mesh] OR "Eicosapentaenoic Acid"[Mesh]) AND ("Virus Diseases"[Mesh] OR "Virulence"[Mesh]) AND ("Clinical Trial" [Publication Type] OR "Observational Study" [Publication Type])

Embase – 308 Results

Intervention/Exposure

'retinol'/exp OR ‘thiamine’/exp OR 'pyridoxine'/exp OR 'folic acid'/exp OR 'cyanocobalamin'/exp OR 'vitamin B complex'/exp OR 'ascorbic acid'/exp OR 'vitamin D'/exp OR 'alpha tocopherol'/exp OR 'copper'/exp OR 'iron'/exp OR 'zinc'/exp OR 'selenium'/exp OR 'magnesium'/exp OR 'omega 3 fatty acid'/exp OR 'docosahexaenoic acid'/exp OR 'linolenic acid'/exp

Outcome

'virus infection'/exp OR 'virus virulence'/exp OR ‘virus infectivity’/exp OR ‘virus’

Study Type

('intervention study'/exp OR 'observational study'/exp)

Combined Search Keywords

('retinol'/exp OR 'retinol' OR 'thiamine'/exp OR 'thiamine' OR 'pyridoxine'/exp OR 'pyridoxine' OR 'folic acid'/exp OR 'folic acid' OR 'cyanocobalamin'/exp OR 'cyanocobalamin' OR 'vitamin b complex'/exp OR 'vitamin b complex' OR 'ascorbic acid'/exp OR 'ascorbic acid' OR 'vitamin d'/exp OR 'vitamin d' OR 'alpha tocopherol'/exp OR 'alpha tocopherol' OR 'copper'/exp OR 'copper' OR 'iron'/exp OR 'iron' OR 'zinc'/exp OR 'zinc' OR 'selenium'/exp OR 'selenium' OR 'magnesium'/exp OR 'magnesium' OR 'omega 3 fatty acid'/exp OR 'omega 3 fatty acid' OR 'docosahexaenoic acid'/exp OR 'docosahexaenoic acid' OR 'linolenic acid'/exp OR 'linolenic acid') AND ('virus infection'/exp OR 'virus infection' OR 'virus virulence'/exp OR 'virus virulence' OR 'virus infectivity'/exp OR 'virus infectivity' OR 'virus'/exp OR 'virus') AND ('intervention study'/exp OR 'intervention study' OR 'observational study'/exp OR 'observational study')