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Effects of a dietary supplement on the incidence of acute respiratory infections in susceptible adults: a randomized controlled trial

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Abstract

Introduction: **although supplementation of specific micronutrients may improve immunologic factors, few studies about the combination of micronutrients with plant extracts on the occurrence of acute respiratory infections (ARI) have been published.**

Objectives: **to assess the effect of a nutritional supplement with micronutrients and plant extracts on the incidence of ARI in susceptible adults between January and April, 2012.**

Methods: **a randomized, double-blind, parallel, placebo-controlled clinical trial was performed. Participants were adults susceptible to ARI who were healthy at the time of evaluation, signed informed consent forms and were not taking medication. They completed a medical history; weight, height, vital signs and laboratory analyses were assessed. Subjects were randomly assigned for consumption of the supplement or a placebo, for a 90 days period. Subjects made daily diary entries indicating the presence ARI symptoms. Those who became ill notified researchers and the attending physician confirmed the presence of an infection. Fisher's exact test was used to compare the proportion of ill subjects between groups. Relative risk and risk difference were also calculated ($p<0.05$ significant).**

Results: **of 59 included subjects, 45 (25 women) completed the study (21 in the supplemented group and 24 in the placebo group). There were no significant differences at baseline between groups. After the intervention, the supplemented group had a lower incidence of ARI compared with the placebo group (57.1% vs. 91.7%, $p=0.013$, RR = 0.62, 95%CI 0.42, 0.92).**

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EFECTOS DE UN SUPLEMENTO NUTRICIONAL EN LA INCIDENCIA DE INFECCIONES RESPIRATORIAS AGUDAS EN ADULTOS SUSCEPTIBLES: UN ENSAYO CLÍNICO CONTROLADO

Resumen

Introducción: **aunque la suplementación de micronutrientes específicos puede mejorar determinados factores inmunológicos, han sido publicados pocos estudios sobre la combinación de micronutrientes con extractos herbales y la incidencia de infecciones respiratorias agudas (ARI) .**

Objetivos: **evaluar el efecto de un suplemento alimenticio con micronutrientes y extractos herbales en la incidencia de ARI en adultos susceptibles, en enero-abril de 2012.**

Métodos: **se realizó un ensayo clínico paralelo, aleatorizado, doble ciego, controlado con placebo. Se incluyeron adultos susceptibles a ARI, sanos en el momento de la evaluación, que firmaron un consentimiento informado y que no tomaban medicamentos. Completaron una historia clínica y se evaluó: peso, talla, signos vitales y de laboratorio. Se asignaron aleatoriamente para consumir durante 90 días el suplemento o un placebo. Los sujetos registraron diariamente si presentaban o no síntomas de ARI en un diario. En caso de enfermedad, se lo notificaron a los investigadores y el médico responsable confirmó la presencia de infección. Se utilizó la prueba exacta de Fisher para comparar la proporción de enfermos entre los grupos y se calculó el riesgo relativo y la diferencia de riesgos ($p<0,05$ significativa).**

Resultados: **de 59 sujetos incluidos, 45 (25 mujeres) completaron el estudio (21 del grupo suplementado y 24 del placebo). No hubo diferencias significativas al inicio entre grupos. Al finalizar la intervención, el grupo suplementado tuvo una menor incidencia de ARI en comparación con el placebo (57,1% *vs* 91,7%, $p=0,013$, RR = 0,62, IC95% 0,42, 0,92).**

Discussion: **the consumption of a supplement with vitamins, minerals and plant extracts may decrease the incidence of ARI in susceptible adults.**

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Key words: *Respiratory tract infection. Incidence. Dietary supplements. Micronutrients. Plant extracts.*

Discusión: **en conclusión, el consumo de un suplemento a base de micronutrientes y extractos herbales puede disminuir la incidencia de ARI en adultos susceptibles.**

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Palabras clave: *Infecciones respiratorias. Incidencia. Suplementos dietéticos. Micronutrientes. Extractos herbales.*

Abbreviations

95%IC: 95% confidence interval.
ARI: Acute respiratory infections.
BMI: Body Mass Index.
CONAQUIC: National Association of Clinical Chemists.
DBP: Diastolic blood pressure.
HR: Heart rate.
IDEA: Instituto Diagnóstico Especializado Arboledas.
IPAQ: International Physical Activity Questionnaire.
ISAK: International Society for the Advancement of Kinanthropometry.
PACAL: Quality Assurance for Laboratories Program.
PREVECAL: Internal Evaluation Quality Program.
PSS: Perceived Stress Scale.
SBP: Systolic blood pressure.

Introduction

Acute respiratory infections (ARI) are those that affect the respiratory system and include the common cold, flu, pharyngitis, sinusitis, laryngitis, bronchitis and pneumonia. They are one of the leading reasons for which medical care is given worldwide and can be caused by both viruses and bacteria. These infections are more prevalent during the winter season and usually last fewer than 15 days. On average, adults have 2-3 episodes per year. They present with symptoms which may include cough, rhinorrhea, nasal obstruction, sore throat, hoarseness, difficulty breathing or fever (in some cases).[1-2] Some factors which may facilitate the spread of the causative agents of ARI are: contact with infected people, overcrowded conditions (at home or work), poor ventilation in crowded rooms, sudden changes in temperature, smoking, stress, fatigue, emotional disturbances, physical activity, alcohol consumption, and diet.[3-6] The cost of treating ARI is high, as these infections affect people's sense of well being, their productivity and rates of absenteeism. In addition, treating ARI generates costs associated with medication and hospitalization[2].

Dietary supplements are used to complement nutrient needs and therefore, they may be useful to strengthen the immune system. Various degrees of micronutrient deficiency, even of individual micronutrients, are known to markedly impair immune function. The extent to which these deficiencies contribute to clinically significant infections is unclear. Nevertheless, several studies indicate that adequate micronutrient supplementation may improve various immunologic factors[7].

Besides micronutrients, dietary supplements focused on immunity may be also based on plant extracts or on a combination of micronutrients with plant extracts. Although the individual effects of each of these ingredients have been documented[7], few studies have demonstrated the effect of the consumption of a combination of micronutrients and plant extracts on the occurrence of ARI[8].

Objectives

To evaluate the effects of a supplement with vitamins, minerals and plant extracts on the incidence of ARI in susceptible adults.

Methods

Study design, subjects and sample collection

We conducted a randomized, parallel, double-blind, placebo-controlled clinical trial from January to April, 2012 (without considering the recruitment period, which lasted 2 weeks approximately). Although it may seem that the supplementation period lasted for 4 months, consumption of the product was six days of the week (from Monday to Saturday), so the duration of the study was adjusted for 90 days of actual consumption. No important changes occurred to methods after trial commencement. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the Committees on Research Ethics and Biosafety of the *Centro Universitario de Ciencias de la Salud* from *Universidad de Guadalajara* (CI-01112). Also, this clinical trial was registered in the local research record from the health authority (*Registro Estatal de Investigación, Secretaría de Salud*, 19/UG_JAL/2012) and in ClinicalTrials.gov (NCT02210156, https://www.clinicaltrials.gov/ct2/show/NCT02210156).

Two authors (BOMF and PFM) invited to participate all staff who worked at the *Centro de Respuesta a Empresarios Omnilife* department (Omnilife's Entrepreneur Support Department or CREO by its initials in Spanish), the company's call center. We selected this department within Omnilife since it has a high incidence of ARI as reported internally by the company's medical service (accounting for 55% of doctor's visits within the department in 2010). Some of the factors that may increase the risk of ARI in this particular population are: overcrowding, frequent contact with people with ARI episodes, lack of ventilation[3], among others, and hence, they represent a high number of individuals susceptible to ARI.

We held a preliminary session with the department's entire staff to explain the procedures; we asked subjects interested to participate in the study to sign an informed consent form and provide a clinical history. The latter included an assessment of stress, using the Perceived Stress Scale (in which higher scores indicate higher levels of stress)[9-10], and physical activity, using the International Physical Activity Questionnaire or IPAQ (in which physical activity is categorized in three levels: light, moderate and vigorous)[11-12].

In a second session the same week, participants visited the company's medical service for measurements of their weight and height in accordance with the International Society for the Advancement of Kinanthropometry (ISAK)[13], control laboratory tests (with a 12-hour fast) and a medical examination to rule out the presence of infections.

Weight measurements were taken using a Tanita BC553 Ironman Inner Scan Body Fat Scale (capacity of 150 kg, accuracy to 0.1 kg). Height measurements were made using a Seca 206 Stadiometer (accuracy to 0.1 cm, measuring range to 220 cm). Both measurements were performed by two certified and standardized anthropometrists (ISAK Level II). Body mass index (BMI) was subsequently calculated using both variables as follows[14]: BMI = weight in kg/(height in m)^2.

The medical examination was performed by the attending physician and a nurse. It consisted of an assessment of vital signs following procedures described in the literature[15] and a physical examination of bodily organs and systems. Temperature was measured using a Microlife MT 1931 digital thermometer (measuring range from 32 to 43.9°C, accuracy to 0.1°C). Heart and respiratory rates were measured using a 3M Littmann Classic II S.E. stethoscope. Blood pressure readings were taken using a 3M Littmann Classic II S.E. stethoscope and an Aneroid Sphygmomanometer ce0483 (18-300 mmHg measurement range, accuracy to 2 mmHg).

Laboratory tests were conducted with the support of an external laboratory (*Instituto Diagnóstico Especializado Arboledas*, IDEA by its initials in Spanish), which is certified by the Quality Assurance for Laboratories Program (PACAL), and the Internal Evaluation Quality Program (PREVECAL); in addition, its staff is certified in clinical diagnosis by the National Association of Clinical Chemists (CONAQUIC). Included were a blood test of six items (fasting glucose, uric acid, creatinine, blood urea nitrogen, cholesterol and triglycerides) analyzed by the spectrophotometric method, a complete blood count with a flow cytometry/microscopy analysis of platelets, and a spectroscopy analysis to assess liver function.

Following these preliminary assessments, a selection was made by the main investigator (BOMF) of healthy adult subjects (without active respiratory infections; chronic respiratory disease requiring treatment other than bronchodilators at the time of assessment; fever; diseases affecting the immune system like autoimmune illness, diabetes, etc.; cardiovascular disorders like uncontrolled hypertension; neurological disorders like epilepsy; and allergies to any components of the product), no currently pregnant or nursing, who presented no abnormalities in their laboratory tests (active infections, immunosuppression, renal or hepatic impairment), and who did not take drugs that affect the immune system (anti-inflammatory drugs, antibiotics, steroids) (see details in Fig. 1). In total, 59 subjects were included (mean age of 26.6 ± 5.9) years old, 55.9% women). We based sample size determination on a previous study by Barringer and colleagues[8]. According to this study, we needed 29 subjects per supplementation group to detect a prevalence difference of 35%, with an alpha value of 0.05 and a power of 80%.

Following selection of the sample group, subjects were requested to suspend the consumption of any supplement used to strengthen the immune system (if that was the case), and to remain the suspension until the end of the study. This was supervised with the help of a small following questionnaire, applied at the end of each month during the study.

Intervention and follow-up

After stratification by sex, the main investigator (BOMF) used a random permutation number list to randomly assign participants by balanced blocks into two groups: one which received the supplement (n=30), whose nutritional composition and ingredients are shown in table I, and a control group who received a placebo, which consisted in a mixture of maltodextrins, colors and flavors; (n=29).

Every day from Monday to Saturday during the intervention period (90 days of actual consumption), two dietitians prepared the products (one preparer per product) by dissolving two envelopes of the product in 240 ml of water in a disposable cup. In order to maintain blinding, these dietitians then gave the prepared cups to two different supervisors who in turn gave the product to each participant for consumption (once-daily doses). The supervisors recorded product administration and consumption for each participant on a control form. The preparation and delivery of the pro-

Table I
Nutritional information and ingredients of the supplement

Nutriment	*Amount Per Serving (20 ml envelope)*
Calories (kcal)	0.0
Protein (g)	0.0
Fat (g)	0.0
Carbohydrates (g)	0.0
of which sugars (g)	0.0
Dietary fiber (g)	0.5
Sodium (mg)	4.0
βcarotene (mg)	0.6
Calcium (mg)	98.0
Magnesium (mg)	39.0
Niacin (Equivalents to Nicotinic acid) (mg)	20.0
Vitamin A (Equivalents to Retinol) (μg)	1000.0
Vitamin B1 (thiamine) (mg)	1.5
Vitamin B2 (riboflavin) (mg)	1.7
Vitamin B6 (pyridoxine) (mg)	1.9
Vitamin B12 (cobalamin) (μg)	5.9
Vitamin C (ascorbic acid) (mg)	117.0
Vitamin A (Equiv. to α tocopherol) (mg)	15.0
Zinc (mg)	14.0
Pantothenic acid (mg)	9.8
Biotin (μg)	146.0
Copper (μg)	490.0
Manganese (μg)	490.0
Selenium (μg)	48.0
Vitamin D (Cholecalciferol) (μg)	4.9

Ingredients: water, fruit juice concentrate (apple, orange, pineapple and white grape), calcium chloride, magnesium chloride, vitamin E acetate, ascorbic acid, citric acid, zinc gluconate, masking flavor, acesulfame K, sucralose, vitamin A palmitate, acacia gum, xanthan gum, nicotinamide, aloe vera gel powder (*Aloe vera*), potassium sorbate, calcium pantothenate, gotu kola (*Centella asiatica*), guarana (*Paullinia cupana*), kelp (*Laminaria fruitescens*) kola nut (*Cola nitida*), schizandra (*Schisandra chinensis*), sodium benzoate, beta-carotene, chlorella (*Chlorella vulgaris*), spirulina (*Spirulina maxima*), wheat grass (*Triticum sp*), parsley (*Petroselinum sativum*), manganese gluconate, copper gluconate, pyridoxine hydrochloride, riboflavin 5-phosphate sodium, hawthorn berries (*Crataegus monogyna*), suma (*Pfaffia paniculata*), vitamin D, thiamine mononitrate, echinacea (*Echinacea angustifolia*), chamomile (*Matricaria chamomilla*), stevia, biotin, sodium selenite, vitamin B12.
Information obtained from the product label in Mexico (Omniplus Supreme®).

duct was carried out during two work shifts (morning and afternoon), depending on each participant's work schedule. The delivery of the products was performed in hours between meals (mid-morning and mid-afternoon), so product consumption was independent from food consumption.

Simultaneously, each week the supervisors provided each participant with an ARI symptom and side effect diary to be filled in daily (indicating whether or not the symptom in question had presented). Participants returned their filled-in diaries to the supervisors the following week. In case of ARI symptoms, subjects were requested not to self-medicate and to notify the principal researcher and attending physician, who would make a clinical assessment to confirm the presence or absence of ARI and provide any needed medical treatment. In such cases, participants were not to be excluded from the study, but treatment provided to them was documented.

Following each 30-day consumption period, assessments of physical activity, weight, and vital signs were again made. A follow-up health status questionnaire was also administered at these times. At the end of the supplementation period, laboratory control tests were repeated and subjects were also asked about their health perception at that time compared to how they felt at the beginning of the study.

From these assessments, we compared between supplementation groups: perceived health status at the end of the study compared with that at the beginning; incidence (number of subjects reporting at least one ARI episode during supplementation period), mean number and mean duration of ARI episodes; absenteeism at work; drug use and the number of subjects who reported more than one episode of ARI. All of these factors, except for perceived health at endpoint vs. baseline and the incidence of ARI, were also compared only between ill subjects in the supplemented group vs. control group patients. From these measurements, our primary outcome was the incidence of ARI episodes.

Statistical analysis

Quantitative variables are expressed as mean ± standard deviation. Qualitative variables are expressed as frequency (%). Fisher's exact test was used to compare distribution percentages of qualitative variables between groups and to compare the total proportion of ill subjects across intervention groups using intent-to-treat and per-population concepts[16]. The risk ratio and the risk difference were also calculated for episodes of ARI with 95%CI. To compare the mean of quantitative variables between treatments, the Mann-Whitney U test was used. Statistical analyses were run using the IBM SPSS Statistics program version 22 for Windows (International Bussiness Machines Corporation, U.S.A.) and Open Epi version 3.01 (Dean AG, Sulli-

van KM & Soe MM, Open Source Epidemiologic Statistics for Public Health, www.OpenEpi.com); p<0.05 was considered as significant.

Results

Subject characteristics

Figure 1 shows the flow of subjects throughout the study. During the study, there was a loss of 14 subjects (23.7%) at follow-up: 9 from the supplemented group and five from the placebo group. Four of the 14 excluded subjects reported some degree of discomfort: two experienced gastrointestinal discomfort (one in the placebo group) and two reported headaches (of the intervention group). Those with gastrointestinal disturbances had reported before the presence of gastritis, reflux and/or irritable bowel syndrome in their clinical history. In the case of people with headaches, one did not have previous records, while the other entered the company in the same month the trial began, so we do not have this information. The final sample was comprised of 45 subjects (25 women, 24 in placebo group and 21 in supplement group).

The initial assessment revealed no statistically significant differences between groups regarding characteristics such as age, weight, height, BMI, physical activity, perceived stress, smoking, alcohol consumption or vital signs. However, diastolic blood pressure was significantly lower in the supplemented group, although this difference was not clinically significant (Table II). There were no differences between groups in mean laboratory values or significant changes in these variables during the period of the study (data not shown).

Table II
Subjects characteristics according to treatment group[a]

Variables	*Intention-to-treat population*		*Per-protocol population*	
	Intervention group (n=30)	*Placebo (n=29)*	*Intervention group (n=21)*	*Placebo (n=24)*
Age (years)	26.1 ± 6.3	27.2 ± 5.4	25.8 ± 5.9	26.8 ± 5.8
Sex				
Women	17 (56.7)	16 (55.2)	12 (57.1)	13 (54.2)
Men	13 (43.3)	13 (44.8)	9 (42.9)	11 (45.8)
Weight (kg)	71.1 ± 16.4	67.5 ± 13.5‡	69.6 ± 17.4	66.4 ± 13.1§
Height (cm)	165.9 ± 7.3	164.6 ± 8.4	165.6 ± 6.4	164.7 ± 8.2
BMI (kg/m^2)	25.8 ± 5.4	24.8 ± 4.0‡	25.3 ± 5.9	24.3 ±3.7§
SPB (mmHg)	105.7 ± 13.0	110.4 ± 11.0‡	103.8 ± 12.8*	110.9 ± 10.8§
DBP (mmHg)	69.3 ± 8.3**	75.0 ± 7.5‡	68.6 ± 8.5**	74.8 ± 7.3§
HR (beats/min)	72.7 ± 8.6	72.5 ± 7.6‡	73.4 ± 9.2	73.2 ± 7.9§
Smoking				
Active	6 (20.0)	9 (31.0)	3 (14.3)	8 (33.3)
Passive	8 (26.7)	5 (17.2)	5 (23.8)	5 (20.8)
Non-smoker	16 (53.3)	15 (51.7)	13 (61.9)	11 (45.8)
Regular alcohol consumption				
Yes	26 (86.7)	23 (79.3)	17 (81.0)	18 (75.0)
No	4 (13.3)	6 (20.7)	4 (19.0)	6 (25.0)
Physical activity (IPAQ)				
Medium-Intense	26 (86.7)	20 (69.0)	19 (90.5)	18 (75.0)
Low	4 (13.3)	9 (31.0)	2 (9.5)	6 (25.0)
Stress (PSS)	18.3 ± 6.4	18.9 ± 5.5	18.8 ± 7.3	17.9 ± 4.9

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; IPAQ, International Physical Activity Questionnaire; PSS, Perceived Stress Scale.
[a]Data are presented as mean ± standard deviation or number of subjects (percentage).
*U Mann-Whitney test, p <0.05, **p<0.01.
‡n=28; §n=23.

Incidence of acute respiratory infections (ARI)

A total of 53 confirmed episodes were reported: 30.2% from the supplemented group (15 cases in 21 subjects) and 69.8% from the placebo group (37 cases in 24 subjects) (data not shown). Of the 45 subjects who completed the study, 34 (75.6%) reported at least one episode of respiratory disease.

Data on ARI episodes are presented in table III. The supplemented group had a significantly lower incidence of ARI than the placebo group (57.1% vs. 91.7%, p=0.013). We also observed that they had a 34.5% lower incidence of ARI (95%CI -10.7, -58.4) and were 38% less susceptible to ARI (RR 0.62, 95%CI 0.42, 0.92) than the placebo group.

In addition, ARI episodes in the supplemented group were fewer in number and of lesser duration (0.8 ± 0.8 vs. 1.5 ± 1.1 for the number of infections per group and 5.6 ± 7.5 vs. 10.7 ± 9.0 for the mean duration of episodes in days). However, after adjusting the analysis for subjects who reported at least one episode of ARI (n=34, 12 in the intervention group and 22 in the placebo group), we found no differences between groups nor between the mean number of ARI episodes (1.3 ± 0.7 in the intervention group vs. 1.7 ± 1.0 in the placebo group) or their duration (9.8 ± 7.5 the supplemented group vs. 11.7 ± 8.8 in the placebo group). No differences related to the other assessed variables were found between groups (Table IV).

Symptoms reported by subjects with ARI are presented in table V. In the intervention group, the most frequent symptoms were headache (100%) and sore throat (91.7%), while the least common were earache (16.7%) and plugged ears (25%). In the placebo group, rhinorrhea (95.5%), sneezing (95.5%) and stuffy nose (90.9%), were the most common symptoms, while plugged ears (40.9%), sinusitis (40.9%) and chest pain (50%) were the least frequent ones. These last symptoms could be considered more severe than those observed in the intervention group.

However, although symptoms were more frequently reported by subjects in the placebo group, there were no statistically significant differences between groups, except for the watery eyes symptom, which was more common in the placebo group (86.4% vs 50%).

When the subjects were asked to subjectively rate their health status at the end of the study compared to baseline (Table VI), no statistically significant differences were found between groups (p=0.064). However, subjects who had consumed the product tended to feel better compared with the placebo group.

Discussion

Our results show that the incidence of clinically diagnosed ARI was significantly lower within a group of susceptible adults who were given the supplement during part of the winter and early spring (90 days of actual consumption), compared with the other group of susceptible adults who were given a placebo (57.1% vs. 91.7%). Thus, subjects who received the supplement were 38% less susceptible to develop ARI episodes.

Micronutrients are important for immune system regulation. Dietary supplementation with a combination of nutrients may increase the immune response because they correct any deficiencies in a safe and positive way from a cost-benefit perspective[17].

Specifically, the supplement used in this study is a calorie-free dietary supplement, sweetened with non-caloric sweeteners such as stevia (calorie-free sweetener from the plant *Stevia rebaudiana,* whose sweetening power is 300 times higher than that of sugar)[18] and contains vitamins (vitamin C, vitamin A, vitamin E, among others), minerals (selenium, zinc, etc.), as well as plant extracts used to strengthen the immune system or that have an adaptogenic effect (increasing the body's resistance to stress), such as *Gotu kola*[10], *Laminaria*[20-21], *Schizandra*[22], *Chrorella*[23], *Espirulina*[24-25], *Equinacea*[26-27], in addition to others.

Of the micronutrients present in the supplement, those which could further affect the immune response are vitamin B12, pyridoxine, vitamin C, vitamin A, zinc, vitamin E, selenium, vitamin D and copper[4,6,28-32]. Antioxidants and minerals may counteract the damage

Table III
Incidence of acute respiratory infections (ARI) episodes[a]

	Intent-to-treat population			*Per-protocol population*		
	ARI	*Healthy*	*Total*	*ARI*	*Healthy*	*Total*
Intervention group	15 (50.0)*	15 (50.0)	30 (100.0)	12 (57.1)*	9 (42.9)	21 (100.0)
Placebo	24 (82.8)	5 (17.2)	29 (100.0)	22 (91.7)	2 (8.3)	24 (100.0)
Total	39 (100.0)	20 (100.0)	59 (100.0)	34 (100%)	11 (100%)	45 (100.0)
Risk ratio	0.60 (95%CI 0.41, 0.90)			0.62 (95%CI 0.42, 0.92)		
Risk difference	-32.8% (95%CI -55.3, -10.2)			-34.5% (95%CI -58.4, -10.7)		

[a]Data are presented as number of subjects (percentage), otherwise indicated.
Fisher's exact test:*p<0.05.

Table IV
Acute respiratory infections (ARI) characteristics[a]

	Intent-to-treat population		*Per-protocol population*	
	Intervention group (n=30)	*Placebo (n=29)*	*Intervention group (n=21)*	*Placebo (n=24)*
Analysis per supplementation group				
Mean episodes of ARI	0.7 ±0.8††	1.6±1.5	0.8 ± 0.8††	1.5 ±1.1
Mean duration (days)	4.6 ± 6.8†	10.9 ± 11.9	5.6 ± 7.5†	10.7 ± 9.0
Subjects with more than one ARI episode				
Yes	21 (70.0)	16 (55.2)	12 (57.1)	11 (45.8)
No	9 (30.0)	13 (44.8)	9 (42.9)	13 (54.2)
Absenteeism				
Yes	5 (16.7)	5 (17.2)	4 (19.0)	5 (20.8)
No	25 (83.3)	24 (82.8)	17 (81.0)	19 (79.2)
Episode-related medication				
Yes	14 (46.7)	21 (72.4)	11 (52.4)	19 (79.2)
No	16 (53.3)	8 (27.6)	10 (47.6)	5 (20.8)
Analysis in subjects who experienced at least one ARI				
Mean episodes of ARI	1.3 ± 0.6	1.9 ± 1.5	1.3 ± 0.7	1.7 ± 1.0
Mean duration (days)	9.2 ± 7.1	13.2 ± 11.8	9.8 ± 7.5	11.7 ± 8.8
Subjects with more than one ARI episode				
Yes	6 (40.0)	11 (45.8)	3 (25.0)	9 (40.9)
No	9 (60.0)	13 (54.2)	9 (75.0)	13 (59.1)
Absenteeism				
Yes	5 (33.3)	5 (20.8)	4 (33.3)	5 (22.7)
No	10 (66.7)	19 (79.2)	8 (66.7)	17 (77.3)
Episode-related medication				
Yes	14 (93.3)	21 (87.5)	11 (91.7)	19 (79.2)
No	1(6.7)	3 (12.5)	1 (8.3)	3 (13.6)

[a]Data are presented as mean ± standard deviation or number of subjects (percentage).
† U Mann-Whitney test: $p<0.05$; ††$p<0.01$.

caused by free radicals produced for pathogen removal. They may also regulate transcription factors in immune function that are sensitive to redox and improve the production of cytokines and prostaglandins. In addition, adequate intake and supplementation of pyridoxine, vitamin B12, C and E, as well as selenium, zinc and copper, promote both the pro-inflammatory immune response mediated by T helper (Th) cells 1 and innate immunity (present from birth; the first line of defense, including skin, mucous, stomach acidity, etc.). This is because they promote sufficient production of proinflammatory cytokines, which help to maintain an effective immune response. Moreover, vitamins A and D play an important role in both types of immunity (innate and adaptive, the latter of which is acquired throughout life, e.g. after immunization or successfully fighting infection) and promote an anti-inflammatory Th2 cell-mediated immune response[6,31-32].

Few studies have evaluated the effects of multivitamins and/or multiminerals on the incidence of respiratory infections. Regarding the effectiveness of combinations of micronutrients in preventing ARI, Stephen and Avenell[17] conducted a systematic review on the effectiveness of the combination of at least two micronutrients (taken orally, intravenously or as an injection) in the incidence of respiratory disease. Among their findings, the authors noted that supplemented subjects under the age of 65 experienced statistically significant fewer episodes of illness than subjects who took a placebo (weighted mean difference= -1.2, 95%CI -2.08, -0.32, p = 0.008), yet there was no difference in the number of subjects who had at least

Table V
Symptoms in subjects with ARI episodes, according to supplementation group[a]

Symptoms	Intent-to-treat population		Per-protocol population	
	Intervention group (n=14)[b]	Placebo (n=24)	Intervention group (n=12)	Placebo (n=22)
Rhinorrhea	11 (78.6)	23 (95.8)	9 (75.0)	21 (95.5)
Stuffy nose	10 (71.4)	22 (91.7)	8 (66.7)	20 (90.9)
Sneezing	11 (78.6)	23 (95.8)	9 (75.0)	21 (95.5)
Post-nasal drip	3 (21.4)	12 (50.0)	3 (25.0)	12 (54.5)
Sinusitis	5 (35.7)	11 (45.8)	5 (41.7)	9 (40.9)
Watery eyes	7 (50.0)	21 (87.5)	6 (50.0)	19 (86.4)*
Scratchy/sore throat	13 (92.9)	22 (91.7)	11 (91.7)	20 (90.9)
Cough	10 (71.4)	19 (72.9)	8 (66.7)	18 (81.8)
Phlegm/mucous	9 (64.3)	17 (70.8)	7 (58.3)	16 (72.7)
Hoarse voice	9 (64.3)	15 (62.5)	7 (58.3)	14 (63.6)
Chest pain	7 (50.0)	12 (50.0)	7 (58.3)	11 (50.0)
Fever	6 (42.9)	14 (58.3)	5 (41.7)	13 (59.1)
Headache	14 (100.0)	21 (87.5)	12 (100.0)	19 (86.4)
Muscle ache	8 (57.1)	17 (70.8)	7 (58.3)	16 (72.7)
Fatigue or tiredness	9 (64.3)	19 (79.2)	7 (58.3)	18 (81.8)
Sleeplessness	6 (42.9)	15 (62.5)	5 (41.7)	14 (63.6)
Earache	3 (21.4)	12 (50.0)	2 (16.7)	11 (50.0)
Plugged ears	3 (21.4)	9 (37.5)	3 (25.0)	9 (40.9)

[a] Data are presented as frequency (percentage).
[b] One subject did not specified symptoms presented.
Fisher's exact test:*p<0.05.

Table VI
Health perception at the end of the study[a]

	Intervention group (n=21)	Placebo (n=24)	Total (n=45)
Better	9 (42.9)	4 (16.7)	13 (100.0)
Same	12 (57.1)	19 (72.9)	31 (100.0)
Worse	0 (0.0)	1 (4.2)	1 (100.0)

[a]Data are presented as number of subjects (percentage).
Fisher's exact test (excluding the only subject with "worse" health perception): p=0.064.

one episode of illness (RR= 0.81, 95%CI 0.65-1.00, p=0.6). The results of our study showed that the supplemented group presented fewer subjects who had at least one episode of illness compared with the placebo group (57.1% vs. 91.7%, p=0.013; RR 0.62, 95%CI 0.42-0.92).

One of the analyzed studies in this systematic review was that performed by Barringer et al[8]. This study is very similar to ours in terms of methods and reported results, although they included 150 adults from 45 years old and older (completers n=130), they did not exclude diabetic patients, they did not only focus in respiratory tract infections, and supplementation lasted for one year. In their results, authors reported that 86% of the sample had a respiratory infection (upper tract, lower tract or influenza type). The incidence of infections and absenteeism was significantly higher in the placebo group than in the supplemented group (73% vs 43% and 57% vs 21%, respectively). When adjusting for age, participants younger than 65 years old experienced an apparent reduction in infection because of vitamin supplementation (78% in the placebo group vs 43% in the supplemented group, relative risk, 0.55, 95%CI 0.38, 0.78). This incidence of infection is very similar to that observed in our study, although in our case, absenteeism did not differ between groups, maybe because of length differences between studies (one year vs. 90 days of actual consumption).

Another study, performed by Rösler *et al*.[7] is very similar to ours since the authors investigated the effect of a combination of vitamins, minerals and plant extracts on the incidence and duration of influenza infections.

The study was completed by 80 subjects (from 100 initial participants), of whom 38 took the supplement and 42 took the placebo for 12 weeks. The authors found that the supplemented group had 1.6 episodes of influenza during the supplementation period (60 cases in 38 subjects), while the control group had 2.7 (113 cases in 42 subjects, p=0.0003). In addition, the mean infection duration was also significantly lower for the supplemented group at 10.0 ± 6.7 days vs. the control group at 28.5 ±13.6 days (p=0.0003). The authors concluded that this supplement was associated with a significant reduction in the incidence of influenza infections. These results are very similar to those reported in our study, although in comparison, the number of infections was higher in the intervention group (1.6 vs. 0.8 reported in our population), as was the duration of episodes of illness (10 vs. 5.6 days).

The beneficial effects of multivitamin and multimineral supplementation has also been observed in diabetic patients[33], patients with leprosy[32], critical patients[34] and children[35-36], although conflicting results were also found in this specific population[37-39] as well as in older adults[40-41].

Among the strengths of our study are: its captive population, and in consequence, a higher degree of supervision of the consumption of the product and following procedures, and a higher degree of control for the timely detection of respiratory infection episodes, although it is important to notice that supplementation adherence is crucial to extrapolate results to the rest of the population, as we can assume with any supplementation study. Another strength of this study is that it is one of the few that reports the effectiveness of taking supplements with more than two micronutrients together with plant extracts in order to prevent acute respiratory infections. It thus shows the advantages of ingesting the enough amount of micronutrients in terms of benefiting and strengthening the immune system.

Weaknesses of this study include its final sample size and the losses experienced during follow-up, which has also been reported in other intervention studies[7]. As well, one of the disadvantages of studying the effect of micronutrients combined with herbal extracts, is that it is difficult to prove which specific ingredients are the responsible for the ARI's incidence reduction, or in fact, if this combination is the responsible one. From our point of view, and taking into consideration the evidence of the participation of micronutrients and herbal extracts in the immune system function[4,6,10,20-32], it may be possible that the combination of ingredients and the synergy between them is responsible for ARI's incidence reduction, although more studies are needed to respond to this complex issue. In addition, no immunological parameters to corroborate or monitor episodes of infection were evaluated, since the aim of our study was to assess the clinical application of the product and its effectiveness in preventing ARI episodes. So, future studies are justified to comprehend the immunological mechanisms affected by consumption of the supplement. We would also like to contrast the effectiveness of the product with that of others available on the market and to analyze its effects on populations presenting other characteristics (immunosuppression, recurrent respiratory diseases, the elderly, diabetes, etc.). Similarly, it would be interesting to assess the effectiveness of the product in periods of supplementation exceeding 90 days, and during a time of the year when the incidence of ARI is lower.

Finally and as a conclusion, the consumption of a supplement comprising vitamins, minerals and plant extracts may decrease the incidence of ARI in susceptible adults.

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