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Micronutrient Deficiencies Are Associated with Impaired Immune Response and Higher Burden of Respiratory Infections in Elderly Ecuadorians[1,2]

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Abstract

The proportion of the Latin American population above age 65 y is expected to rise substantially. To better define the prevalence of infectious diseases and micronutrient deficiencies, assess immunological status, and evaluate associations between nutritional status and infection, we performed a cross-sectional study of elderly Ecuadorians in a low-income peri-urban community in Quito, Ecuador. Culturally adapted questionnaires, delayed type hypersensitivity (DTH) skin response, micronutrient, and immunological assays were performed in randomly selected Ecuadorians aged ≥65 y. Multiple linear and logistic regression models were developed to assess relationships between micronutrient concentrations and history of infection, DTH, and immune function. Participants ($n = 352$; mean age ± SD, 74.4 ± 6.4 y) recalled recent episodes of colds/influenza-like syndromes (62.8%), cough (61.0%), urinary tract infection (37.9%), diarrhea (32.2%), fever (24.1%), and pneumonia (3.5%). A prospective substudy of respiratory infections (RI) in 203 elderly revealed similar findings. Colds and pneumonia occurred in 42.8 and 7.9% of participants, respectively, during 737 person-weeks of observation (3.6 ± 1.1 wk per person). Anemia and micronutrient deficiencies, especially for vitamins C, D, B-6, and B-12 and folic acid and zinc, were common. Plasma vitamin C was associated with interferon-γ (IFNγ) ($P < 0.01$) and zinc with IFNγ and interleukin-2 (each $P < 0.0001$). RI history was associated with any micronutrient deficiency ($P < 0.001$). The burden of infectious diseases, micronutrient deficiencies, and anemia was substantial in this elderly Ecuadorian population. Deficiencies of essential vitamins and minerals place these elderly adults at risk for infections through their negative impact on immune function. J. Nutr. 139: 113–119, 2009.

Introduction

There has been a steady increase in the proportion of the elderly population in both developed and developing countries during the last few decades. As a result of decreased infant mortality, lower fertility rates, and improved longevity, developing country populations will soon undergo rapid and unparalleled changes in their age structure that will result in a substantial increase in the percentage of the aged population (1). The increase in the proportion of the population over 60 y of age in developing countries is estimated to be 1.5 times faster than that in developed countries (1).

In Latin America and the Caribbean, the number of people aged ≥60 y is projected to increase from ~40 million in 2004 to 100 million in 2025 (2). Because many parts of Latin America are poor, the elderly in Latin American countries are likely to have more infectious and chronic diseases, greater disability, and fewer resources available for their health care needs. Unlike many developed nations, few of the limited health resources of developing countries have been devoted to the elderly, despite their diminished capacity to fight disease without adequate nutritional, economic, and psychosocial support. They therefore

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risk living their lives burdened by disease and disability, which threatens to put an even greater strain on the limited health care resources of their countries.

The elderly in Latin America suffer from the same age-associated physiological, psychological, social, and economic changes that adversely affect the nutritional and immunological status of older individuals in industrialized nations. The incidence of chronic medical conditions such as cardiovascular disease, type 2 diabetes mellitus, malignancy, arthritis, autoimmune disorders, and infections increases with age (3–5). Aging is associated with impaired regulation of the immune system (6–9), contributing to a higher incidence of morbidity and mortality from infectious, autoimmune, and neoplastic diseases. Prospective studies indicate greater morbidity and mortality in elderly subjects with low delayed-type hypersensitivity (DTH)[11] responses, an in vivo measure of cell-mediated immune responses (10–13). Subtle subclinical deficiencies of micronutrients, such as zinc, selenium, and vitamin E, and inadequate macronutrient intake contribute to the decline in immune function in the elderly (14–17).

Limited information is available on the nutritional, immunological, and general health status of the aged in Latin America. Preliminary evidence from a previous, smaller study of 145 elderly in Quito, Ecuador revealed inadequate intake of protein and multiple micronutrients, including vitamins B-6, B-12, and D, folate, iron, and zinc (18). Infectious diseases were a major cause of morbidity and of physician or hospital visits for the elderly in this study. To better define the prevalence of infectious diseases and micronutrient deficiencies, determine the immunological status of the elderly using DTH skin responses, and evaluate the association between nutritional status and infection, we performed a cross-sectional survey of elderly Ecuadorians living in a peri-urban slum community in Quito, Ecuador.

Methods

Study site and population. This cross sectional study was conducted in 2 consecutive rainy seasons from September 2003 to December 2004 in poor peri-urban areas in northwestern Quito, at an altitude of 2800 meters above sea level. The study area had an estimated population of 19,000 and, based on electoral results, 5% (~950 individuals) were above the age of 65 y. The 3 study neighborhoods, which included only residents of low socioeconomic status (mean monthly income was US$54, which is <50% of the basic income in Ecuador), were located on a hillside and were structurally similar, with 1 main paved road and electricity present in all homes. More than 40% of the elderly individuals in these neighborhoods were illiterate. Some households had a municipal source of potable water and sewerage. Inhabitants of this community were originally from small cities and rural areas of Ecuador. This area of Quito was typical of poor, under-resourced neighborhoods within the city. This population is similar to those one would encounter in peri-urban areas in other Andean countries, such as Puno, Peru and La Paz, Bolivia.

Screening and enrollment. The Ethical Committee of the Corporación Ecuatoriana de Biotecnología and the Tufts-New England Medical Center Institutional Review Board approved the protocol and informed consent form. During meetings at the field work station, each subject was given detailed information on the study objectives and procedures. If the potential study participant was deaf, we used the assistance of a relative to help the participant understand the study purpose and procedures. After potential participants had an opportunity to ask questions, written informed consent was obtained. If the participant could not read, the form was read to them in the presence of a literate family member. The participant was then asked to place an X on the signature line and the form was cosigned by a witness.

To identify eligible elderly people, we conducted a census in the 3 neighborhoods. During household visits, we provided potential study candidates ($n = 413$) with detailed information on the study and a subset of participants ($n = 352$) were selected, using a random numbers table, to participate. Eligibility criteria included age ≥65 y, mental competence, and willingness to provide written informed consent. Participants' age was verified with their national identification card. The study nurses determined mental competence by means of a brief set of simple questions related to memory and cognitive skills for daily decision making.

Study procedures. Questionnaires and study procedures were performed by trained nurses at the field work stations, which were located in each of the 3 study neighborhoods and were accessible to all study participants. Anthropometric measurements, DTH, and phlebotomy for micronutrient, hematological, and immunological assays were performed at the field work stations.

Participants were interviewed to assess their social, economic, housing, sanitation, and general health status using a Spanish version of the Cross Cultural Research on the Nutrition of Older Subjects questionnaire (19), which has been used in Guatemala. The questionnaire was adapted by including Spanish idioms used in Ecuador and reviewed to ensure that they were relevant and culturally appropriate. A pilot test was conducted to assess the adequacy of words and questions for the local culture. The questionnaire was finalized using the pilot study results (18). A week-long pilot study with 30 elderly participants was performed to allow study personnel to familiarize themselves with the study procedures and to finalize data collection instruments. The results of the pilot study were not included in the final analysis.

Active surveillance of respiratory infections substudy. To assess accuracy of infection data collected through recall by participants, 203 elderly participants were randomly selected from the study neighborhoods to participate in a substudy of the incidence of respiratory infections (RI). After obtaining informed consent, participants were evaluated at a study clinic based in a local community center once per week during a 5-wk period from September until early October 2004. During the visit, a history of RI symptoms during the previous week was obtained and then the participants were examined for signs of respiratory and eye infections. Using standardized definitions of infections (20), the field physicians determined diagnoses of RI and conjunctivitis based on their history and physical examination findings.

Anthropometry. Anthropometric measurements were taken as described by Gross (19); these included weight, height, knee height, and waist circumference. Weight was recorded to the nearest 0.1 kg using a Detecto scale with a minimal amount of clothing. Height was measured to the nearest 0.1 cm using a steel fiberglass measuring tape affixed to a wooden rod, with a sturdy straight edge used as a headpiece. Because it can be difficult to measure standing height in some elderly individuals, we also measured knee height to the nearest 0.1 cm, using a special knee-height anthropometer (21). We used knee height in the BMI calculation for 23 women (10% of female participants) and 17 men (13% of male participants) who were not able to stand fully erect. Waist circumference was assessed with a nonstretch measuring tape between the upper edge of the right iliac crest and the umbilicus and was recorded twice to the nearest 0.1 cm.

DTH skin response. DTH was assessed using the Mantoux technique with 4 recall antigens (22): tetanus toxoid, tuberculin, *Candida albicans*, and *Trichophyton mentagrophytes*, as well as a glycerin control administered on the volar surface of the right arm. The same investigator evaluated the DTH response for all participants. We measured the vertical and horizontal diameters of induration after 24 and 48 h and considered the reaction positive when the mean value was ≥5 mm. Means for each antigen were calculated and a composite score, based on the results of all of the antigens in each participant, was determined.

[11] Abbreviations used: DTH, delayed type hypersensitivity; IFNγ, interferon-γ; IL-2, interleukin-2; PHA, phytohemagglutinin; RI, respiratory infection.

Laboratory procedures. A 10-mL venous blood sample was drawn from each participant, after an overnight fast, into an EDTA-treated tube and a tube without anticoagulant. Samples were immediately transported to the laboratory and centrifuged. Plasma samples for vitamin C were promptly deproteinized using perchloric acid and EDTA. Serum or plasma was collected in plastic tubes, frozen at −20°C, and shipped to Boston, MA for analysis of B vitamins; vitamins A, C, D and E; and calcium, zinc, iron, and copper according to standard procedures at the Nutritional Evaluation Laboratory of the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University as previously described (18). Full details of the derivation of the reference ranges are described in Sempértegui et al. (18).

Lymphocyte proliferation and cytokine measurements were conducted using a modified whole-blood assay (23,24) phytohemagglutinin (PHA) was used to stimulate the whole blood as follows. In each well, 100 μL of a PHA 200 mg/L solution was added to 900 μL of blood. We measured PHA-stimulated interleukin-2 (IL-2) and interferon-γ (IFNγ) production in culture supernatant (25,26) using commercially available ELISA kits (R&D Systems). Complete blood counts and a blood smear were done to determine the white blood cell differential. Participants were asked to provide a fecal sample, which was analyzed for ova and parasites by saline wet mounts under 40× microscopy.

Statistical analysis. The sample size was based on the assumptions that nutritional deficiencies are prevalent in poor elderly Ecuadorians living in Quito, that these nutritional deficiencies contribute to the age-associated decline in T cell-mediated function, and that this results in an increase in infectious diseases. We therefore hypothesized that elderly Ecuadorians with more severe nutrient deficiencies would have the lowest T cell-mediated function and the highest prevalence of infectious diseases. As data on prevalence of infectious diseases in this population were not available, variability observed in DTH was used for sample size calculation. A sample of 350 participants would yield an 80% chance that the hypothesis of a 0 population correlation coefficient or partial correlation coefficient would be rejected at the 0.05 level, as long as the magnitude of the underlying correlation coefficient was ≥0.15.

Data entry and management were done using Epi-Info software, version 6.04d (CDC, Atlanta, GA). Statistical analyses were performed using SPSS, version 11.5. Descriptive statistics for anthropometric measurements, BMI, DTH response, and health profile survey responses were calculated globally and by sex. BMI was calculated as kg/m^2 (27). Weight was classified as underweight (BMI <20), recommended (BMI 20–24.9), and overweight (BMI ≥25). RI incidence was calculated by dividing the number of diagnoses by person-weeks of observation. Differences in means and percentages between male and female participants were evaluated by Student's *t* and chi-squared tests, respectively. Values in the text are means ± SD.

Multiple linear or logistic regression models were developed to assess the relationship between micronutrient concentrations likely to have an effect on immune function (vitamins A, B-6, C, and E and iron and zinc) and the main outcome measures: DTH, IL-2, IFNγ, history of diarrhea, pneumonia, common cold, and RI (defined as a history of either common cold or pneumonia). Potential confounders for models in which diarrhea was an outcome included age, sex, housing score (based on a composite score of type of walls, floors, and roofs, number of bedrooms, kitchen location, cooking fuel, food storage, drinking water source, and toilet facilities), education, income, BMI, and fecal carriage of pathogenic (*Giardia lamblia*, *Ascaris lumbricoides*, *Trichuris trichiura*, *Hymenolepsis nana*, *Balantidium coli*, and *Strongyloides stercoralis*) or non-pathogenic parasites (e.g. *Entamoeba coli*, *E. dispar*, *Chylomastix mesnili*). The RI model included the same variables, with the exception of the fecal parasites, and also included a variable for crowding (defined as the number of persons living in the household divided by the number of bedrooms). Response variables were log transformed to improve model fit, as needed.

Results

There were 225 women and 127 men enrolled. The majority of the participants, 95.2%, were enrolled in 2 sequential rainy seasons. The age of the participants was 74.4 ± 6.4 y. Male participants were older and had higher educational levels than female participants, although few men and no women had attended secondary school (**Table 1**). A greater percentage of men than women were currently married, whereas more women were divorced. Most participants were Catholic and had originated from rural areas of Ecuador. Overall, 11% currently smoked some form of tobacco and 21% had smoked in the past for more than 1 y. Only 7.7% acknowledged current consumption of alcoholic beverages, although 50% used to occasionally drink, 10% most days, and only 4.6% daily. Women were significantly more likely than men to be overweight and to have a high waist circumference.

Recall of recent infections. During the previous month, only 12% of participants denied having had 1 of the following: diarrhea, fever, cough, cold or influenza-like syndrome, pneumonia, or urinary tract infection. Of those who recalled 1 of these syndromes in the last month, 19% had 1 episode, 25% had 2 episodes, and 44% had 3 or more. Participants recalled having had colds or influenza-like syndromes (62.8%), cough (61.0%), urinary tract infection (37.9%), diarrhea (32.2%), fever (24.1%), and pneumonia (3.5%). Overall, 42% had seen a health care provider, 25% had spent time in bed, and 4.6% had been hospitalized overnight because of illness in the last 3 mo. Common types of infections that prompted visits to health workers were pneumonia (0.85%), cough (1.7%), cold or flu (0.85%), urinary tract or kidney infection (1.1%), and diarrhea (0.85%). Common infectious diseases that resulted in spending time in bed at home included colds or flu (4.8%) and pneumonia (0.85%).

TABLE 1 Demographic characteristics and anthropometric measurements of elderly Ecuadorians[1]

Variable	Men	Women	*P*-value
n	127	225	
Age, *y*	75.8 ± 6.5	73.7 ± 6.1	0.003
(range)	(65 – 94)	(65–97)	
Education level, %			
No school	29.4	52.5	<0.0001
<3 y primary school	23.0	24.2	0.8
3–6 y primary	42.9	22.9	<0.0001
Some secondary	4.0	0	
Completed secondary	0.8	0	
Marital status, %			
Married	69.8	37.5	<0.0001
Single	2.4	7.6	0.07
Widowed	22.8	49.6	<0.0001
Divorced or separated	5.6	4.5	0.62
Religion, *% Catholic*	91.3	91.0	0.4
Birthplace, *% rural*	69.0	73.9	0.31
Anthropometrics			
Weight, *kg*	59.9 ± 9.5	54.0 ± 9.80	<0.0001
Height, *cm*	156 ± 7	144 ± 6	<0.0001
BMI, *kg/m^2*	24.8 ± 3.2	26.1 ± 4.0	0.0037
Underweight: BMI <20, %	2.7	4.5	0.444
Normal: BMI 20–24.9, %	57.3	39.8	0.003
Overweight: BMI ≥25, %	40.0	55.7	0.008
High waist circumference,[2] %	8.8	46.7	<0.0001

[1] Values are mean ± SD or %.
[2] Men >102 cm; women >88 cm.

Substudy of active RI surveillance. There were 203 participants (65.5% women) in the RI substudy who were observed for a 3.6 ± 1.1 wk per person for a total of 737 person-weeks of observation. Nearly one-third (29.6%) had a RI or acute conjunctivitis. The common cold was the most frequently encountered RI, occurring at a rate of 115 per 1000 person-weeks of observation, followed by conjunctivitis (71 episodes), pharyngitis (53 episodes), pneumonia (38 episodes), bronchitis (7 episodes), otitis media (5 episodes), and 2 cases of asthma per 1000 person-weeks of observation. When all RI were combined (pharyngitis, common cold, otitis, bronchitis, and pneumonia), 50.5% of the elderly had some type of RI in the preceding month. Common colds and pneumonia occurred in 42.8 and 7.9% of participants, respectively.

Micronutrient blood and serum concentrations. The mean concentrations of vitamins A and D and of iron were significantly lower in women than in men (**Table 2**). In contrast, male participants had significantly lower concentrations of vitamins C, E, and B-6 and folate than women. Substantial deficiencies (≥5%) were noted for vitamins C, D, B-6, and B-12 and folate, zinc, and copper (Table 2). Men were more likely than women to be deficient in vitamins C, D, and B-6 and tended to have lower folate ($P = 0.06$).

DTH responses, hematological measurements, and immunological assays. More than 50% of participants had positive DTH responses to *Candida* and tuberculin, whereas only 22 and 16% responded to *Trichophyton* and tetanus toxoid, respectively (**Table 3**). The mean diameter of induration was significantly greater at 48 h relative to 24 h for *Candida* and tuberculin but not for the other antigens tested. Men and women did not differ in total white blood cell count (6876/μL ± 1992 for men vs. 6588/μL ± 1810 for women), IFNγ (4889 ± 5224 ng/L supernatant for men vs. 5464 ± 5789 ng/L supernatant for women), and IL-2 (3375 ± 4382 ng/L supernatant for men vs. 4022 ± 4796 ng/L supernatant for women). Hemoglobin tended to be higher in men (157 ± 29.1 g/L) than in women (136 ± 25.8 g/L) ($P = 0.08$). Using a standard cutoff point for anemia at sea level (men <120 g/L and women <115 g/L), 17.3% of men and 27.1% of women were anemic ($P = 0.038$). However, because Quito is at 2800 m above sea level, the altitude-adjusted threshold for anemia was higher (men <136 g/L and women <131 g/L) (28,29). Using this cutoff point, 29.9% of men and 39.6% of women were anemic ($P = 0.08$).

Stool parasites. Of 343 participants (97.4%) for whom stool samples were submitted, 6.4% were positive for potentially pathogenic parasites. Although 36% of the participants had *E. histolytica/E. dispar*, specialized tests were not performed to distinguish between these species. Because there were no correlations between the presence of these organisms and blood/fecal leukocytes and a history of diarrhea in the last month, they most likely had the nonpathogenic *E. dispar*. Whereas 36.7% had no parasites in stool specimens, 63.3% had 1 or more parasites (pathogenic and nonpathogenic combined) and 42.6% had 2 or more. Sixty-one percent of the elderly had 1 or more nonpathogenic parasites identified in their fecal specimens.

Logistic and linear regression analysis. None of the potential confounding variables, specific micronutrient deficiencies, or the presence of any type of micronutrient deficiency were associated with a history of recent diarrhea or pneumonia in logistic regression models. However, a history of RI (defined as either pneumonia or cold) and a cold were strongly associated with the presence of any micronutrient deficiency ($P < 0.001$) and were inversely associated with education ($P = 0.03$) (**Table 4**).

Plasma vitamin C and IFNγ were associated ($P < 0.01$) and serum zinc and IFNγ were associated ($P < 0.0001$) in linear models, adjusting for age, sex, and BMI (**Table 5**). Serum iron and IL-2 concentrations were also positively associated ($P = 0.02$), as were serum zinc and IL-2 ($P < 0.0001$).

Discussion

This cross-sectional study of elderly Ecuadorians residing in a peri-urban community in Quito, characterized by low socioeconomic status, revealed a high burden of infectious diseases, especially RI, and numerous deficiencies of essential vitamins and minerals. DTH testing revealed that a minority of the study population mounted responses to tetanus toxoid and *Trichophyton*, whereas more than one-half responded to tuberculin and *Candida* test antigens. Although the majority of the elderly participants had normal white blood cell and platelet counts, a moderate proportion was anemic. Given the high-altitude

TABLE 2 Circulating vitamin and trace mineral concentrations in elderly Ecuadorians stratified by gender[1]

Micronutrient	Reference range[2]	Men ($n = 125$)	Women ($n = 224$)	Men	Women
				% deficient	
Serum vitamin A, *μmol/L*	1.05–3.14	1.92 ± 0.48*	1.78 ± 0.43	0.8	4.1
Plasma vitamin D, *nmol/L*	39.0–98.7	57.1 ± 15.7**	49.3 ± 13.6	18.8¶	9.4
Serum vitamin E, *μmol/L*	11.6–41.8	27.4 ± 7.0†	30.4 ± 9.1	0	0
Plasma vitamin C, *μmol/L*	11.4–125	11.4 ± 9.1**	17.0 ± 10.2	59.8**	32.6
Plasma vitamin B-6, *nmol/L*	30–117	42.6 ± 20.0†	52.3 ± 28.5	27	16.1
Plasma vitamin B-12, *pmol/L*	184–884	300.4 ± 157.7	325.5 ± 169.9	20.5	19.6
Plasma folate, *nmol/L*	11.3–68.0	13.13 ± 4.7††	15.0 ± 6.1	37	27.4
Serum copper, *μmol/L*	Female 13.2–24.0 Male 13.2–21.7	18.5 ± 5.2	19.4 ± 5.3	16.7	12.4
Serum iron, *μmol/L*	Female 8.95–30.4 Male 11.6–31.3	23.4 ± 9.0**	19.9 ± 8.2	4	5
Serum zinc, *μmol/L*	10.7–19.9	11.6 ± 3.4	11.4 ± 3.6	41.3	45.4

[1] Values are mean ± SD. *$P = 0.004$, **$P < 0.001$, †$P = 0.001$, ††$P = 0.003$, ¶$P = 0.02$.
[2] 18.

TABLE 3 Delayed type hypersensitivity response to 4 test antigens at 24 and 48 h in elderly Ecuadorians[1]

	Diameter of induration[1]		Timepoint	
Antigen	24 h ($n = 346$)	48 h ($n = 343$)	24 h	48 h
	mm		*% Positive*	
Tetanus toxoid	1.94 ± 3.76	2.03 ± 4.40	15.6	15.7
Tuberculin	7.69 ± 5.90	9.08 ± 7.15*	59.5	61.5
Glycerin	0.04 ± 0.32	0.02 ± 0.24	0	0
Candida	5.97 ± 3.67	6.54 ± 4.28*	64.6	65.4
Trichophyton	3.43 ± 3.40	3.27 ± 3.56	22.8	22
Total diameter of induration, *mm*	19.0 ± 9.05	21.0 ± 10.2*		
Total positive responses, *n*/person	1.61 ± 0.96	1.64 ± 0.97		

[1] Values are mean ± SD or %. *Different from 24 h, $P < 0.05$.

setting where this study occurred, these participants are at risk for morbidity associated with decreased functional capacity secondary to the anemia.

We found several sex-specific differences in demographic, anthropometric, and nutritional measures in this elderly population. More men than women had at least 3–6 y of primary education. Some men had attended secondary school. In contrast, more than one-half of the women had not attended any school and none had attended secondary school. More women than men were overweight based on BMI and waist circumference. Men were more likely than women to be deficient in vitamins B-6, C, and D, and folate.

DTH testing demonstrated that the elderly Ecuadorians had substantially lower numbers of positive responses and mean diameters of induration for common skin test antigens than has been observed in elderly in the United States (30). However, DTH responses to tuberculin were common. This is not surprising given the relatively high prevalence of tuberculosis in Andean populations. In contrast to the tuberculin results, only a minority of the study participants mounted DTH responses to tetanus toxoid. Similarly, in a study of United States war veterans, elderly participants were significantly more likely than younger participants to have negative tetanus skin test responses (31). This finding suggests that there may be a loss of tetanus reactions with ageing. Alternatively, the elders living in these relatively poor communities, many of whom had migrated to Quito from rural farming communities, may never have been immunized with tetanus toxoid. These elderly Ecuadorians may therefore be at risk for the acquisition of tetanus.

Recall of RI during the last month showed a substantial burden of cold and influenza-like syndrome and a small but meaningful number of recent episodes of pneumonia. As impaired memory may limit the ability of elders to recall recent events, the similar levels of RI, especially common cold and pneumonia, in the prospective active surveillance substudy suggests that recall bias did not adversely impact the infection history data, as collected with the modified Cross Cultural Research on the Nutrition of Older Subjects questionnaire. In fact, the active surveillance substudy revealed even higher rates of pneumonia than recall; 7.9% of participants had pneumonia during the period of observation, yielding an incidence of 38 episodes of pneumonia per 1000 person-weeks of observation, whereas 3.5% recalled having had pneumonia during the last month. Although this difference is relatively small, active surveillance yielded pneumonia rates that were more than double the recall rates. This suggests that the burden of pneumonia in this elderly Ecuadorian population is substantial. Given the morbidity and mortality from pneumonia in the aged (5), the negative impact of pneumonia in this poor Latin American population is likely to be substantial. However, in contrast to the pneumonia results, the rate of recall was higher for common colds/influenza-like illness than the rates in the prospective study.

Measurements of micronutrient concentrations in blood samples revealed substantial deficiencies of vitamins C, D, B-6, and B-12 and folic acid and zinc. Vitamin C and zinc and IFNγ were associated, as were zinc, iron, and IL-2. The essentiality of these nutrients for normal function of the immune response is well recognized. Both IL-2 and IFNγ play critical roles in resistance to infectious diseases. Although we did not observe an association between any of the individual micronutrients with a history of recent pneumonia, influenza-like illness, cold, or diarrhea, the presence of some type of micronutrient deficiency and a history of recent infection were associated. Subclinical

TABLE 4 Logistic regression analysis for RI and diarrhea in elderly Ecuadorians

	RI		Pneumonia		Cold		Diarrhea	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Sex, *male*	0.83	0.46, 1.48	2.44	0.57, 10.4	0.82	0.46, 1.45	1.15	0.64, 2.05
Age, *y*	0.97	0.93, 1.02	0.91	0.80, 1.04	0.97	0.93, 1.02	0.97	0.93, 1.01
BMI, *kg/m*^{2}	0.97	0.90, 1.03	1.12	0.96, 1.32	0.96	0.90, 1.03	1.01	0.94, 1.08
Income, *US dollars/mo*	1.00	0.99, 1.00	0.98	0.95, 1.00	1.00	0.99, 1.00	1.00	0.99, 1.00
Housing score[1]	1.00	0.93, 1.08	1.12	0.93, 1.35	0.99	0.92, 1.07	1.02	0.95, 1.10
IL-2, *ng/L*	1.00	1.00, 1.00	1.00	1.00, 1.00	1.00	1.00, 1.00	1.00	1.00, 1.00
IFNγ, *ng/L*	1.00	1.00, 1.00	1.00	1.00, 1.00	1.00	1.00, 1.00	1.00	1.00, 1.00
Education, *y*	0.74[2]	0.56, 0.97	1.07	0.51, 2.23	0.74[2]	0.56, 0.98	0.84	0.63, 1.12
DTH score at 48h, *mm*	0.98	0.95, 1.00	0.95	0.89, 1.02	0.98	0.95, 1.00	1.00	0.97, 1.02
Crowding[3]	0.87	0.70, 1.07	0.78	0.40, 1.51	0.89	0.72, 1.09		
Any micronutrient deficiency[4]	4.00[5]	1.76, 9.06	1.51	0.17, 13.7	3.30[5]	1.51, 7.21	1.04	0.47, 2.28

[1] Housing score based on a composite score of type of walls, floors, and roofs, number of bedrooms, kitchen location, cooking fuel, food storage, drinking water source, and toilet facilities.
[2] $P = 0.03$.
[3] Ratio of number of persons to the number of rooms in the participant's home.
[4] Defined as the presence of a deficient blood or plasma level of any of the following micronutrients: vitamin C, vitamin E, pyridoxal 5′-phosphate (PLP), folate, iron, or zinc.
[5] $P < 0.001$.

TABLE 5 Linear regression analysis for IL-2 and INFγ in elderly Ecuadorians

	IL-2			INFγ		
	β[1]	SE[2]	*P*	β[1]	SE[2]	*P*
Age, *y*	−48.9	46.3	0.29	3.0	57.2	0.96
Sex, *male*	−474.4	614.6	0.44	−423.0	759.7	0.58
BMI, *kg/m*^{2}	−1.5	71.7	0.98	44.4	88.7	0.62
Vitamin E, μ*mol/L*	−1.0	0.74	0.17	−0.68	0.91	0.46
Vitamin C, μ*mol/L*	2144.1	1577.6	0.18	5345.1	1950.1	0.007
PLP, *nmol/L*	−19.5	11.5	0.09	−19.8	14.2	0.16
Folate, *nmol/L*	−1.1	109.6	0.99	112.5	135.5	0.41
Iron, μ*mol/L*	−15.1	6.4	0.02	−3.7	7.95	0.65
Zinc, μ*mol/L*	75.5	13.3	<0.0001	84.2	16.4	<0.0001

[1] The β coefficient is the estimated variation of the dependent variable (IL-2 or INFγ) by the effect of the independent variables.
[2] SE, Standard error.

micronutrient deficiencies in general, and that of zinc in particular, have been associated with impairment of immune function (15,32) and increased risk of infection in the elderly population (15,33–35). However, the current study did not demonstrate an association of zinc deficiency with infections. This may have stemmed from the use of recall as the primary method for determining recent infection history or from an insufficient sample size. Given the anticipated rapid growth of the elderly portion of the total population in Latin America during the next few decades (2), there is a need to focus resources on more rigorous, prospective evaluations including interventions to improve the health of the elderly in Latin America.

We found a significant association between recall of a RI (either pneumonia or the common cold) and the presence of some type of micronutrient deficiency. Given the proportion of elderly men and women with micronutrient deficiencies, this finding suggests that inadequate nutrition places elderly Ecuadorians at increased risk for infection. A prior preliminary study conducted by our group in a similar elderly population in Quito revealed that dietary intake of total energy, protein, and several micronutrients, including vitamins A, D, B-6, and B-12 as well as zinc, was substantially below the reference range for usual dietary intake (18). Based on the findings of this earlier study and the present study, the overall nutritional status of the elderly in this peri-urban, marginal community appears suboptimal. Given the well-described immunosenescence associated with aging (36,37) and deficiencies of essential micronutrients such as zinc and vitamins C, D, and B-6, which play important roles in immune function, this population is at high risk for morbidity, and potentially mortality, from infectious diseases.

The fecal parasitology results suggest that the majority of the elderly Ecuadorians in this study population are living in a "contaminated" environment due to inadequate hygiene, sanitation, water sources, or contaminated food sources. Consequently, in addition to the high burden of RI, they are at increased risk for food- and water-borne disease.

Because, with the exception of the RI substudy, this was a cross-sectional study, the study design limited our ability to interpret some of the findings. For instance, each participant was interviewed, examined, and had blood work conducted at only 1 point in time so we cannot comment on longitudinal changes in immune function or micronutrient status over time. We also were limited to noting associations between micronutrient deficiencies and a history of illness such as diarrhea and thus were unable to determine the directionality of causation. Because we staggered enrolment of study participants over a 15-mo period, there is a possibility that there were seasonal fluctuations in the types of local foods available that might have influenced the micronutrient status of the participants. However, by virtue of its location near the equator, agricultural production is relatively stable throughout the year in Quito and its surrounding rural areas, which provide food to the city. In addition, most participants were enrolled and had their micronutrient and immunological status evaluated during the rainy season. Due to the small number enrolled in the sunny season, it was not feasible to evaluate the potential impact of seasonal fluctuations in food availability on the micronutrient status of the elderly in this study.

Given the evidence of substantial deficits in essential nutrients, along with high infection rates, this marginalized elderly population in Ecuador would benefit from improvements in overall food security and sanitation as well as focused interventions to address specific micronutrient deficiencies. In view of the rising proportion of the population that is aged in Ecuador and other countries in Latin Americ, and the anticipated increased health care costs associated with this demographic transition, there is a need to develop and test low-cost, simple nutritional and behavior change interventions to improve nutritional status, strengthen immune function, and minimize the risk of acquisition and severity of infectious diseases of the elderly in this region.

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