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Intestinal parasitic infections are related to micronutrient status and body composition in Mexican school-age children: results from a cross-sectional study

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Abstract

Background: Intestinal parasitic infections remain a significant public health issue, particularly in low-resource settings. They have been linked to poor micronutrient status and body composition, which are critical determinants of child health and development. The aim of this cross-sectional study was to determine the relationship between intestinal parasitic infections and micronutrient status, and whether these differ according to the body composition .

Methods: Serum concentrations of zinc, iron, ferritin, vitamins A, E, C, D, folate, B12 and CRP, were determined in 269 school-aged children from rural Mexico. Infection with soil transmitted helminths (STHs) and intestinal protozoa was screened in a fecal sample. Anthropometric and body composition measurements were taken.

Results: Lower ferritin, zinc and vitamin C concentrations were found in children infected with any STHs or *A. lumbricoides* compared to parasite-free children ($p<0.05$). Children infected with any intestinal protozoa, *Endolimax nana* or *Entamoeba coli* had higher concentrations of ferritin and B12 than parasite-free children ($p<0.05$). Vitamin E:lipid concentration was higher in children infected with any intestinal protozoa and *E. nana.* Among the children with high body fat percentage, those infected with STH had lower zinc, and those infected with intestinal protozoa had lower vitamin A than parasite-free children ($p<0.05$).

Conclusion: STH infection was associated with lower concentrations of ferritin, zinc and vitamin C, whereas intestinal protozoa infection with higher concentrations of ferritin, vitamin E:lipids, and B12. These associations differed according to body fat percentage.

Key words: Body Composition, Helminths, Intestinal Parasites, Micronutrients, Protozoa.

Introduction

Globally, intestinal parasitic infections represent a major health burden, contributing to malnutrition and impaired growth in children, particularly in low- and middle-income countries. Intestinal parasite (helminths and protozoa) infections contribute one fourth of the infectious diseases in humans, and they are a recognized worldwide public health problem. (Alum, Rubino, & Ijaz, 2010). Socioeconomic status, health conditions, education, the presence of domestic animals, poor water supply and food hygiene are factors that affect the risk of having intestinal parasites (Black et al., 2013; García, Zavala, Campos-Ponce, & Polman, 2023). Intestinal parasite infections have been associated with malnutrition, and inflammation (Gutiérrez-Jiménez, Luna-Cazáres, & Vidal, 2017; Zavala et al., 2018). Additionally, intestinal parasite infections (5.7%), obesity (3.9 %), and overall micronutrient deficiencies (6.1%) account for more than 15% of the global disability-adjusted-life-years (Black, 2003; Hotez et al., 2014; Ng et al., 2014).

Intestinal parasitic infections affect the nutritional status of the host through several mechanisms, including reducing food intake (anorexia) and nutrient absorption, and increasing the loss of nutrients, leading to malnutrition and higher risk of micronutrient deficiencies (García, Zavala, Campos-Ponce, et al., 2023; Katona & Katona-Apte, 2008). Multiple micronutrient deficiencies negatively impact both disease progression and infection (Bhaskaram, 2002; Katona & Katona-Apte, 2008), and infection in turn may exacerbate malnutrition (Stephenson, Latham, & Ottesen, 2000). Micronutrient deficiencies and intestinal parasitic infections in children both affect growth and cognitive development (Schaible & Stefan, 2007). Most studies that have evaluated the association between poor micronutrient status and intestinal parasitic infection in children have focused on zinc, iron and vitamin A status (Brechje de Gier et al., 2014). The relationship between intestinal parasitic infections and other micronutrients that are essential for an adequate immune response (i.e. vitamins C, D or E) has not been extensively studied (Maggini et al., 2007). Moreover, the available evidence is centered on the relationships between intestinal parasites and micronutrients in undernourished populations; but not in children affected by a high prevalence of overweight and obesity. This is particularly important in transitional countries such as Mexico, where childhood obesity is the current main nutritional problem and where co-existing parasitic infections and micronutrient deficiencies are both public health problems.

The combined prevalence of overweight and obesity in Mexican school-age children is among the highest in the world (~34%) (Cuevas-Nasu et al., 2017). In addition, the prevalence of overall micronutrient deficiencies is high, especially among children (Rivera Dommarco, 2012). For the micronutrients on which there is national data, the most prevalent

deficiencies in school-age Mexican children (5 to 11 years) are iron (9.3%; serum ferritin <15 µg/L) and zinc (23.6%;<65 µg/dL) deficiencies (Shamah-Levy et al., 2012; Villalpando et al., 2015). Also, intestinal parasitic infections in children have been reported to range from 30% to 70%, being highest in the southern states of the country (Morales-Espinoza et al., 2003; Zavala et al., 2020). Infection with intestinal parasites, in particular G. lamblia and STH, is highly associated with dual burden households (co-existing overweight/obesity and stunting) (Campos Ponce et al., 2012). We recently found that Mexican children with moderate to heavy infection with E. coli had higher body fat and higher reported food intake compared to non-infected children (Zavala et al., 2016).

The aim of this study was to determine the relationships between micronutrient status and intestinal parasitic infections, and whether these differ across the range of body composition in children.

Materials and methods

Study population and design

A cross-sectional study was conducted in 269 school-aged children (6-10 y) recruited from two rural communities (Santa Cruz and Santa María Begoña), State of Querétaro, Mexico. All children from 2 local schools were invited to participate in the study. Parents or caretakers received oral and written information about their participation and the children's participation in the study. Written signed informed consent was obtained before entry into this research. Children also provided their assent to participate. The study was conducted according to the guidelines of the Declaration of Helsinki, and the study protocol was approved by the Bioethics Committee of the School of Natural Sciences at the Universidad Autónoma de Querétaro (UAQ) (protocol # FNN-2014-02).

Children who received deworming treatment in the last 4 months or had a physical or mental disability were excluded. Children that met the age criteria, that lived in one of the two communities and whose parents signed consent forms were included in the study. Parents or caretakers were asked to attend their local health clinic to answer a medical history and a socioeconomic status questionnaire previously validated. The complete research process is illustrated in Figure 1.

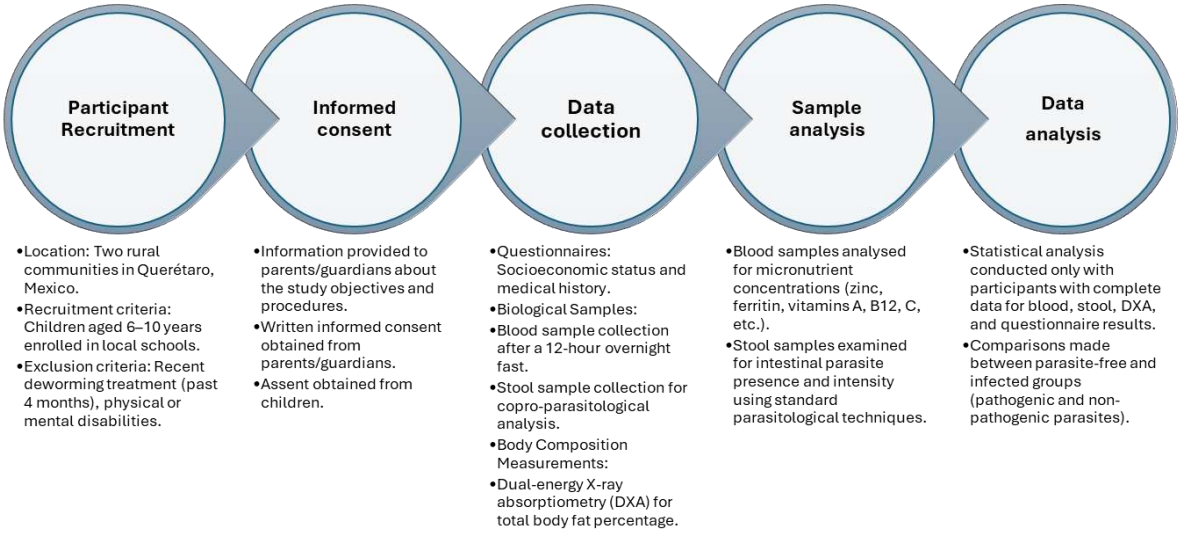

Figure 1. Flow chart of the research procedures

Blood sample collection

Children's parents/caretakers were instructed not to feed the children after dinner (8:00 PM) one day before the blood sample collection to ensure a 12 h fasting before the blood sample was collected by venipuncture in the early morning. The samples were stored in a cooler and transported the same morning to the Human Nutrition Laboratory, at UAQ. Plasma and serum were separated by centrifugation at 1800–2000 rpm for 15 min (Beckman Allegra 21R, Palo Alto, CA, USA), and aliquots stored at −70 °C for later analysis. All the biochemical analyses were performed in duplicate by trained personnel at the Human Nutrition Laboratory, UAQ, except vitamin B12 and folate that were measured at the measured at the U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS) Western Human Nutrition Research Center in Davis, California.

Micronutrient status evaluation

Vitamins A and E (α-tocopherol) were measured simultaneously in serum using reverse phase high pressure liquid chromatography (HPLC) (Mod 2996, Waters Associates, Milford, MA, USA) using corresponding U.S. Pharmacopeia (USP) certified retinol and alpha-tocopherol standards. Vitamin A status was considered deficient with serum retinol concentrations <10 μg/dL and low if <20 μg/dL.(Gibson, 2005) Vitamin E deficiency was defined as serum alpha-tocopherol <3 μg/mL and as low status at concentrations <5 μg/mL.(Fares et al., 2011) Using the vitamin E:lipids ratio, vitamin E deficiency was classified as <0.8 mg/g.(Drewel et al., 2006) Serum vitamin C was analyzed using reverse phase HPLC, with an ascorbic acid USP standard, and a cut point for deficiency of <2 μg/mL and low status

as <4 µg/mL.(Gibson, 2005) Serum vitamin D was determined by a commercial 25(OH)-Vitamin D direct ELISA kit (Immundiagnostik AG, Bensheim, Germany) and a microplate spectrophotometer (Multiskcan Ascent, Thermo Electron Corporation, MA, USA). Vitamin D deficiency was defined as <20 ng/mL and insufficient status if <30 ng/mL.(LeBlanc et al., 2014) Plasma vitamin B12 and folate concentrations were measured by enzyme-based immunoanalysis using a Cobas® e41 analyzer (Roche, Switzerland) and the reagents vitamin B12 (theoretical normality values 95%CI: 191-663 pg/mL) and folate III (4.6-18.7 ng/mL). Low serum folate was defined as <4 ng/mL and high as >20 ng/mL (the highest limit of detection of the instrument) and low and marginal B-12 concentrations were defined as <200 ng/mL and between 200 and 300 ng/mL, respectively.(Brito et al., 2015)

Serum ferritin concentrations were determined by a commercial ELISA kit (ab108837, Abcam, UK) and a microplate spectrophotometer (Multiskan Ascent, Thermo Electron Corporation, MA, USA). Low iron stores were defined as serum ferritin <15 µg/L. Total serum iron concentrations were measured using a commercial kit (Iron Ferrozine, Elitech, Sées, France). Low iron status was defined as <60 µg/dL and iron deficiency as <45 µg/dL (Organization, 2011). Zinc concentrations were measured in serum by atomic absorption spectrometry (AAnalyst 7000, Perkin Elmer Instruments, Norwalk, CT, USA), using its corresponding standard (Perkin Elmer) and deficiency was defined as serum zinc <65 µg/dL (Hotz, Peerson, & Brown, 2003).

High sensitive C-reactive protein (hsCRP) was measured in plasma using commercial ELISA kits (High Sensitivity C-Reactive Protein ELISA Kit, Bioquant) and analyzed in a Multiskan Ascent microplate photometer (Thermo Electron Corporationn, Ma, EUA).

Anthropometry and body composition evaluation

Children and one parent or caretaker were transported from their local communities to the Nutrition Clinic at the UAQ. Weight and height were measured by trained personnel following the World Health Organization (WHO) procedures. Weight was measured using a calibrated digital scale (SECA Mod. 813, Hamburg, Germany) and height with a stadiometer (SECA Mod 206, Hamburg, Germany). Underweight was defined as BMI two standard deviations (SD) below, and overweight and obesity as one and two SDs above, the WHO reference median of the BMI-for-age z-scores for children aged 5 to 19 years, respectively. Stunting was defined as two SDs below the WHO reference median for height-for-age (Onis, 2006).

Whole body composition was measured to determine body fat percentage by a certified technician using dual-energy X-ray absorptiometry (DXA) (Hologic Mod Explorer, Bedford, MA, USA). Children were asked to remove any

jewelry or anything metal, and once in the machine, they were asked to remain still for the duration of the scanning. Total body fat percent was obtained from the DXA absorptiometry values; high body fat for girls was considered to be above 30%, and for boys, above 25% (Ellis, Abrams, & Wong, 1997).

Evaluation of intestinal parasitic infections

A single stool sample was collected from each participant and analyzed on the day of collection. A direct copro-parasitological test consisting of a wet mount with iodine staining of slides was performed to determine the presence of protozoan intestinal parasites, as described by WHO (WHO, 1991). These protozoa included Entamoeba coli (E.coli), *Endolimax nana* (*E. nana*), *Balantidium coli* (*B. coli*), *Entamoeba histolytica, Iodamoeba Bütschlii* and *Giardia lamblia.* Two Kato-Katz smears (2 x 41.7g) were made according to standard procedures to determine the presence and number of eggs of soil transmitted helminths (STHs).(Willcox & Coura, 1991) These parasites included *Ascaris lumbricoides* (*A. lumbricoides*), *Trichuris trichiura* (*T. trichiura*) and hookworm. The intensity of infection was characterized following WHO recommendations. Light- and moderate-intensity infection respectively were defined as 1-4999 and >5000 epg feces for *A. lumbricoides*; 1–999 and ≥1000 epg for T. trichiura; and; 1-1999 and ≥2000 epg for hookworm. Children with no intestinal protozoa or STH eggs were categorized as parasite-free. Those infected with more than one species of intestinal parasite were combined and categorized as having "multiple infections".

Statistical Analysis

The Shapiro-Wilk test was used to check normality of the distributions of the variables. Chi- squared tests were performed to compare the proportion of children with parasitic infections versus parasite-free children and to compare children with and without micronutrient deficiencies. After checking linearity and normal distribution of the dependent variables, one way, multivariate analyses of co-variance (MANCOVA) were used to compare the concentrations of micronutrients in children infected with any STH, any intestinal protozoa or specific parasites with prevalence above 10% (i.e. *A. lumbricoides, E. coli, B. coli* and *E. nana*) versus parasite-free children and CRP concentration. Interactions were also assessed through MANCOVA defining the absence/presence of infection as factor (independent variable); micronutrient concentrations as the response (dependent variable) and considering the category of normal/high body fat percent as covariate. Both the MANCOVAs to compare groups and to assess interactions were adjusted by age (months), gender (male/female), mother's education level (years of education) and CRP concentrations. A p value <0.05 was considered statistically significant. We analyzed the interactions between body

fat and each micronutrient, and conducted post hoc analysis to further investigate the interaction for those micronutrients with a significative interaction term. Serum folate concentrations were not incorporated in the regression models because most of the values were above the limit of detection of the assay, affecting the distribution of the variable. All statistical analyses were performed using SPSS 21.0 (SPSS, /Chicago IL) and the figure plotted using Graph Pad Prism 8 (Graph Pad Software Inc, San Diego, CA).

Results

A total of 269 children (8.0 ± 1.6 y, 47% girls) participated in the study (**Table 1**). The most common micronutrient deficiencies were vitamin D (19.3%) and zinc (15.2%). The prevalences of vitamin B-12 and vitamin E deficiencies were very low (1.1% and 0.4%, respectively). No child had folate deficiency and most had high folate (84.8%). No deficiencies were observed for vitamin A, vitamin C or iron. A total of 18.4% of the children were overweight, 9.6% obese, 51% had high body fat, 2% were underweight and 4% were stunted. C-reactive protein concentration was directly related to iron (β=-0.13, 95% C.I.=-0.20 to -.006 p <0.001) and zinc (β=-2.23, 95% C.I.=0.14 to 4.32 p=0.036) and negatively to ferritin (β= 0.01, 95% C.I.=0.01 to 0.02 p <0.001).

Table 1. Main characteristics of the population (n=269)

Variable	Mean (SD[a])	Proportion children with micronutrient deficiency (n)
Age (years)	8.0 (1.6)	NA
Anthropometry and body composition		
Weight (kg)	27.6 (8.2)	NA
Height (cm)	126.1 (9.9)	NA
Height-for-age (Z-Score)	-0.6 (1.0)	NA
BMI-for-age (Z-Score)	0.3 (1.3)	NA
Total body fat (%)	4.0 (2.7)	NA

Biochemical measurements

Ferritin (µg/L)	99.0 (51.3)	NA
Iron (µg/dL)	92.1 (24.5)	0% (0)
Zinc (µg/dL)	74.2 (11.2)	15.2% (41)
Retinol (µg/dL)	32.5 (6.7)	0% (0)
Vitamin D (ng/mL)	17.9 (3.1)	19.3% (52)
Vitamin E (µg/mL)	2.7 (1.0)	NA
Vitamin E lipids ratio (mg/g)	2.7 (1.0)	.04% (1)
Vitamin C (µg/mL)	7.6 (1.8)	0% (0)
Vitamin B-12 (pg/mL)	662.3 (258.1)	1.1% (3)
C-reactive protein (mg/L)	0.97 (1.6)	NA

[a] SD=Standard Deviation

Intestinal infections were common, with 60% of children being infected with at least one intestinal parasite and 46% with at least one protozoan. Among the protozoans, *E. coli (*19 %), *E. nana* (16%) and *B. coli* (12%) were the most common. The prevalence of any STH infection was 19%; *A. lumbricoides* was the most prevalent (16.4%). Multiple-intestinal parasitic infections affected 14.5%.

Children infected with any intestinal parasite exhibited higher serum concentrations of vitamin B12 and ferritin compared to parasite-free children (Table 2). Those infected with any STH or *A. lumbricoides* had lower concentrations of plasma ferritin, zinc, and serum vitamin C. Conversely, children infected with intestinal protozoa showed higher levels of serum ferritin, vitamin B12, and vitamin E:lipids compared to parasite-free peers. Specifically, *E. coli*-infected children had higher serum ferritin retinol and vitamin B12 concentrations, while those infected with *E. nana* had higher levels of serum ferritin, retinol vitamin E:lipids, and vitamin B12. Finally, children with multiple infections demonstrated lower serum ferritin but higher vitamin B12 concentrations compared to parasite-free children ($p<0.05$)."

1 **Table 2.** Serum or plasma micronutrient concentrations by parasite infection group (n=269)[a]

Micronutrient	Parasite-Free (107)	Infected [b] (162)	Soil transmitted helminths (52)	*A. lumbricoides* (44)	Intestinal Protozoa (123)	*E. coli* (52)	*B. coli* (33)	*E. nana* (44)	Multiple infection [c] (39)
Ferritin (µg/L)	98.88 ± 52.83	99.54 ± 50.67*	92.22 ± 45.32*	93.64 ± 48.28*	101.03 ± 51.20*	101.53 ± 54.66*	92.31 ± 35.96	108.56 ± 64.76*	93.91 ± 54.61*
Iron (µg/dL)	89.07 ± 28.10	94.67 ± 31.92	91.73 ± 29.12	93.70 ± 29.23	95.96 ± 32.26	98.71 ± 26.17	92.94 ±37.65	100.58 ± 33.33	100.27 ± 27.56
Zinc (mg/L)	0.75 ± 0.09	0.74 ± 0.11	0.71 ±0.12*	0.70 ± 0.12*	0.75 ± 0.10	0.77 ± 0.10	0.73 ± 0.13	0.75 ± 0.11	0.75 ± 0.11
Retinol (µg/dL)	32.59 ± 7.67	32.98 ± 6.01	32.72 ± 5.49	32.85 ± 5.56	32.91 ± 6.03	32.85 ±5.89*	32.75 ± 6.55	33.43 ± 5.42*	32.46 ±4.21
Vitamin D (ng/mL)	17.40 ± 3.32	18.25 ± 2.93	18.17 ± 2.75	18.22 ± 2.70	18.24 ± 3.03	18.38 ± 2.75	18.34 ± 3.70	17.52 ± 5.28	18.11 ± 3.84
Vitamin E (µg/mL)	5.35 ± 1.66	5.34 ± 1.66	5.11 ± 1.53	5.24 ± 1.53	5.46 1.98	5.27 ± 1.59	5.36 ± 1.71	5.57 ± 1.77	5.65 ± 1.57
Vitamin E lipids (µg/mL)	2.67 ± 0.97	2.72 ± 1.03	2.64 ± 1.08	2.74 ± 1.11	2.74 ± 1.02*	2.66 ± 1.14	2.66 ± 0.97	2.83 ± 1.06*	2.69 ± 1.06
Vitamin C (mg/mL)	7.81 ± 1.91	7.57 ± 1.81	7.16 ± 1.92*	7.09 ± 1.92*	7.73 ± 1.74	7.72 ± 1.64	7.34 ± 2.26	7.81 ± 1.86	7.89 ± 2.01
Vitamin B-12 (pg/mL)	633.22 ± 204.92	678.13 ± 289.48*	646.39 ± 241.59	641.42 ± 237.54	699.75 ± 302.84*	719.88 ± 325.19*	612.62 ± 170.41	727.93 ± 335.64*	648.42 ± 260.66*

[a] MANCOVA, Mean ± Standard Deviation, adjusted by age, sex, mother's educational level and C-reactive protein concentration [b] Infected with any intestinal parasite [c] Infection with 2 or more intestinal parasites; * Significantly different from parasite-free with MANCOVA ($p<0.05$)

2

Of the micronutrients studied, a significant interaction with body fat and parasitic infection was observed only with zinc and vitamin A. Total body fat percentage interacted with STHs infection in relation to serum zinc concentrations and with intestinal protozoa in relation to serum retinol concentration (*p* value < 0.05) **(Figure 2).** Among the children with high body fat percentage, those infected with STH had lower serum zinc concentrations than parasite-free children, while those infected with intestinal protozoa had lower serum retinol than parasite-free children (p<0.05). In addition, among the children with normal body fat percentage, those infected with STH had similar serum zinc concentrations compared to parasite-free children and those infected with intestinal protozoa had higher vitamin A concentration than parasite-free children (p<0.05).

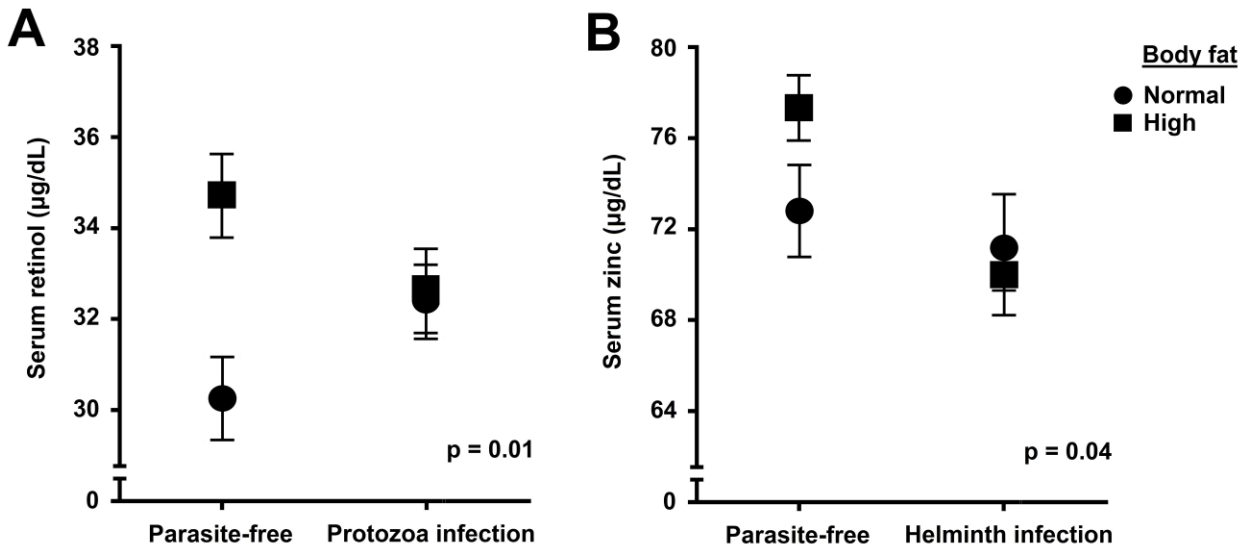

Fig. 2 Interaction between high/normal body fat with A) helminth infection and serum zinc; B) protozoan infection and serum retinol concentrations. Lines represent 95% confidence interval

Discussion

The present study aimed to evaluate the relationship between intestinal parasites and micronutrient status in a population of school-aged children in Mexico, where the prevalence of overweight and obesity is high,lower

concentrations of ferritin, zinc, and vitamin C were found in children infected with *A. lumbricoides* which may be explained by different physiological and immunological pathways. For instance, impaired micronutrient absorption has been related to reduced gastrointestinal function, to damage of the gut mucosa and to competition for available micronutrients when infected by STHs (Hesham, Edariah, & Norhayati, 2004). In addition, lower intake of micronutrients and food has been associated with STH infection, due to symptoms such as abdominal pain and loss of appetite (Crompton & Nesheim, 2002; Greichus & Greichus, 1980; Northrop et al., 1987; Stephenson, Latham, & Ottesen, 2000; Zavala et al., 2017). However, the possibility that children with lower ferritin, zinc and vitamin C concentrations are more likely to be infected should also be considered. Iron, zinc and vitamin C are immuno-modulators and play a role in the immune system responses that occur at mucosal membranes (Liu et al., 2016; Maggini, Wenzlaff, & Hornig, 2010). Additionally, vitamin C is a exogenous antioxidant, necessary for collagen synthesis required for the adequate function of the epithelial barrier (Liu et al., 2016; Maggini, Wenzlaff, & Hornig, 2010). The association found between STH infection and lower plasma zinc and ferritin concentrations is in line with several studies in different age-groups and populations that have found similar associations (Adebara, Ernest, & Ojuawo, 2011; G. O. Arinola et al., 2015; de Gier et al., 2015). In contrast, there are no available studies evaluating the relationship between vitamin C and STHs. No association between any of the STH infections and serum vitamin E was found. According to a systematic review, few available studies have addressed the relationship between vitamin E and STH infection.(B. de Gier et al., 2014).

The higher concentrations of circulating ferritin, vitamin B-12 and vitamin E:lipids found in children infected with intestinal protozoa may be explained by possible a higher intake of animal source foods. In a previous study from the same cohort, we observed that children infected with *E.coli* have higher intakes of meat, poultry, fish and eggs which are the main sources of vitamin B-12 and bioavailable iron in the diet (Zavala et al., 2017). To the best of our knowledge, only one study has evaluated the association between intestinal protozoa and micronutrient concentrations. In that study no association was observed between *E. coli* infection and serum iron, retinol, zinc or vitamin B-12 concentrations in the children (Boeke et al., 2010). Since it has been shown that *E. coli* infection is associated with higher food intake, discrepancy among studies may be attributed to differences in food group intake (i.e. seafood, meat, egg and legumes), food quality, availability and affordability. Is important to highlight that despite the retinol associations with *E. coli* and *E. nana* being significant they may not be biologically or clinically relevant..

Given the distinct pathogenic profiles of the parasites studied, our findings require careful interpretation. *A. lumbricoides* and *B. coli*, which are recognized as pathogenic, were associated with lower serum concentrations of ferritin, zinc, and vitamin C, supporting their known impact on nutrient absorption and host health. In contrast, *E. coli* and *E. nana*, generally considered non-pathogenic, were associated with higher serum levels of vitamin B12 and ferritin. This may reflect differences in dietary intake or host responses among infected children, as previously reported in studies linking *E. coli* infection to higher consumption of animal-source foods (Zavala et al., 2017). These findings highlight the importance of differentiating between pathogenic and non-pathogenic parasites when interpreting the health and nutritional impacts of intestinal infections. Further research is warranted to explore whether these associations are mediated by dietary, immunological, or microbiota-related mechanisms.

Plasma concentrations of zinc were sensitive to STH infections in children with high body fat. The lower zinc concentrations in the STH infected children that had high body fat content might be caused by zinc malabsorption associated with both obesity and STH infection (Ganiyu Olatunbosun Arinola et al., 2015). In addition, as zinc is necessary for immune function, children with lower zinc concentrations are more likely to become infected with intestinal parasites (Brown et al., 2004; Maares & Haase, 2016). Thus, obesity together with low zinc concentrations may have a larger weakening effect on the immune response and defense against STHs.

The interaction between serum retinol and intestinal protozoa infection differed by body fat content. In the children with high body fat, the lower serum retinol concentrations in the intestinal protozoa infected children might be attributed to an inflammation process, specifically a T-helper type 1 (Th1) pro-inflammatory response, associated to both infection and obesity (Kirkpatrick et al., 2002). A Th1 response has been associated with lower serum retinol concentrations (García, 2012; Thurnham et al., 2003; Zavala et al., 2013). In contrast, in the children with normal body fat, the higher serum retinol concentrations observed in the protozoa infected children might be attributed to a higher food intake (Zavala et al., 2017).

As far as we know, this is the first study assessing the relationship between micronutrients and intestinal parasites while taking into consideration the interaction with body composition. These findings and possible underlying mechanisms that may lead to the observed differences, especially those between serum zinc and serum retinol concentrations, parasitic infections and body fat need to be further explored. Some methodological limitations of our study need to be addressed. Due to the cross-sectional study design, we were unable to determine the causal relationship between intestinal parasitic infections, micronutrient concentrations and body composition. In this

population the most prevalent STH was *A. lumbricoides* with 44 cases, followed by hookworm with only 6 cases, thus the association between STH and micronutrients in this study are mostly driven by *A. lumbricoides* infection, which should be taken into consideration while interpreting the results. Parasitic infection prevalence was determined by parasitological examination of one stool sample per individual which may underestimate the number of infected children (Staat et al., 2011). However, the prevalence of the studied intestinal parasites was high and similar to that reported previously in rural Mexico (Quihui-Cota & Morales-Figueroa, 2012). Our analyses and interpretations were limited for several micronutrients because of high status (i.e. folate, vitamin E) or because of absence of deficiency (i.e. vitamin B-12), affecting our ability to detect associations or interactions. Also, we did not measure all the biochemical markers available to determine status, but this study covered a large number of vitamins and minerals as it was intended to contribute to the knowledge of overall micronutrient deficiencies.

It is important to consider that Mexico has moved from a situation where a high prevalence of micronutrient deficiencies was a major concern to one where status of many has improved. For example, iron deficiency (serum ferritin <15 µg/L) has gone from 13% to 9.3% in school-aged children in a period of 6 years.23,24 The prevalence of marginal and deficient vitamin B12 status also has been shown to be much lower in recent published data than two decades ago (Anaya-Loyola et al., 2019). Improvement has been achieved by the success of national programs such as Prospera, and voluntary fortification of foods (such as ready-to-eat cereals, dairy products among others) and flour fortification with iron, zinc and folic acid. Although there have been improvements in the micronutrient status of the Mexican population, overweight and obesity have increased, but we also found a high proportion of children with elevated folate status. None of them presented folate values <4.41 ng/mL; 99.7% of them had values above 13.2 ng/mL, being 84.8% above the upper limit of the standard curve for the assay (>19.9 ng/mL). Our finding on excessive folate represents an alarm for public health in Mexico and this issue should be addressed in the near future. In the same sense, the prevalence of parasitic infections remains high. While some associations with micronutrient status were observed, the low prevalence of deficiency of some micronutrients and the situation of folate repletion likely made it difficult to detect more associations of the parasites on status that might occur in more micronutrient deficient populations.

Conclusion

In conclusion, STH and intestinal protozoa have different and opposite associations with micronutrient concentrations. STH infection was associated with lower concentrations of zinc and vitamin C and intestinal protozoa infection with

higher concentrations of iron and vitamin B12. The associations between zinc and vitamin A status with intestinal parasites differed by body fat percentage.Interestingly, non-pathogenic parasites like *E. coli* and *E. nana* were linked to higher serum levels of vitamin B12 and ferritin. While these parasites are not considered harmful, they may still influence gut microbiome composition, appetite, and dietary intake, potentially altering nutrient absorption or metabolism.Further studies with better epidemiological design are needed to explore the relationship between multiple micronutrients and parasites in populations with co-existing high prevalence of micronutrient deficiencies and overweight/obesity.

Statements and Declarations

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Competing interest: None

Authors contributions: OPG, GAZ, MCP and JLR designed and conceived the study. MC and AB conducted laboratory analyses at UAQ in Querétaro, Mexico, and at the WHNRC in Davis, USA, respectively. OPG and GAZ performed statistical analyses. OPG, GAZ, MCP, CMD, AB, KP, JLR and LHA interpreted data and provided intellectual input to the drafts of the manuscript. All authors were involved in writing the paper. All authors read and approved the final version of the manuscript. OPG has final responsibility for all parts of the manuscript.

Data availability: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethical approval: The study was conducted according to the guidelines of the Declaration of Helsinki, and the study protocol was approved by the Bioethics Committee of the School of Natural Sciences at the Universidad Autónoma de Querétaro (UAQ) (protocol # FNN-2014-02). The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Consent to participate: Informed consent was obtained from all parents of children that participants in the study.

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