Effects of diet on the childhood gut microbiome and its implications for atopic dermatitis

To the Editor:

Atopic dermatitis (AD) is a common childhood disorder with significant reductions in the child's quality of life. The composition and diversity of the early gut microbiota has been linked to the development of atopic conditions including AD.[1]

Although the prevalence of atopic diseases is lower in Africa,[2] individuals of African origin who live in Western countries are at a significantly higher risk for atopic conditions like AD and food allergy, and suffer from more severe allergic diseases than do their white counterparts,[3] possibly reflecting an enhanced genetic predisposition to allergy that is kept in check by their ancestral environment.[2] In certain areas of Africa, people still live in an environment enriched in microbes through close daily contact with animals and plants in natural settings. Therefore, determining how their natural environment or diet protects these potentially at-risk populations should shed light on causes of the global rise in allergic conditions. In this study, we characterized gut microbiotas of black South African (Xhosa) children from the remote rural Mqanduli district of the Eastern Cape in association with AD.

Patients with AD were recruited from the Dermatology Department of the Nelson Mandela Academic Hospital in Umtata, in the Eastern Cape. Control (nonallergic, non–food-sensitized) subjects were recruited from the areas surrounding 10 district community health clinics. Detailed clinical data were gathered on all toddlers. The fecal microbiota of these children was analyzed and compared in relation to AD, other clinical variables, and their diet. For details, see this article's Methods section in the Online Repository at www.jacionline.org. This study was approved by the institutional review boards of the University of Cape Town's Faculty of Health Sciences and of Rush University Medical Center.

Eighty-three children were recruited: 36 with AD and 47 controls without AD. AD was significantly associated with food sensitization, but not with allergic rhinitis or wheezing (see Table E1 in this article's Online Repository at www.jacionline.org). Children with AD had a significantly higher daily consumption of total sugar and saturated fat than did controls (see Table E2 in this article's Online Repository at www.jacionline.org). However, being breast-fed at all and the duration of exclusive and total breast-feeding were not associated with AD status (Table E2).

The gut microbiota analysis showed that beta diversity based on Bray-Curtis distance in cases with and without AD is significantly different in terms of both abundance and presence/absence of different operational taxonomic units (OTUs) (see Fig E1, *A*, in this article's Online Repository at www.jacionline.org). However, alpha-diversity indices were not different between cases with and without AD (Fig E1, *B*). Constrained ordination analysis by distance-based redundancy analysis showed the potential major genera driving microbial community differentiation among cases with and without AD. The microbiota of healthy control toddlers at genus level is driven by increased *Prevotella, Xylanibacter*, and *Streptococcus*, and decreased *Faecalibacterium, ClostridiumXI, Ruminococcus, Collinsella, Blautia*, and unclassified *Lachnospiraceae*, whereas increased Bacteroides and *Clostridum XIVa* drive the differentiation of the gut microbiome in rural children with AD. At the OTU level, *Prevotella copri* was significantly increased in control children, whereas *Bacteroides vulgatus* had a significantly higher relative abundance in children with AD (Fig 1).

Food sensitization, allergic rhinitis, or wheezing were not associated with changes in gut bacterial diversity or in the abundance of OTUs. There was no association between gut microbiome and child's age, sex, and breast-feeding.

Among dietary components analyzed, high consumption of both total sugar and protein was associated with decreased alpha diversity of bacteria. High saturated fat intake was associated with lower abundance of *P copri* (0.096 vs 0.041 in low saturated fat and high saturated fat groups, respectively; false discovery rate [FDR] $P = .02$).

For confirmation of the results, the *P copri* abundance was measured in all enrolled children by quantitative PCR designed specifically for this bacterium and was found to be significantly decreased in children with AD (mean ± SD ratio of 1.45 ± 3.94 vs 0.38 ± 0.53; $P < .00001$ in children without and with AD, respectively) (Fig E2). Children with high sugar content in their diet had a significantly lower *P copri* (mean ± SD ratio of 1.04 ± 3.32 vs 0.43 ± 0.52; $P < .0001$ in children with lower and higher than median sugar intake, respectively).

Our detailed microbiome study in this group of children from rural communities of South Africa has revealed differences in the gut microbiota of toddlers with AD that are associated with the diet. Previous studies comparing the gut microbiota of children living in natural rural settings with those living in urban environments has shown a link between lower abundance of the *Prevotella* genus and urban living.[4] We found that *P copri* was significantly decreased in children with AD, suggesting a protective role for these bacteria against AD. The markedly high abundance of *Prevotella* in rural populations has been largely attributed to the high consumption of complex carbohydrates.[4] It has been hypothesized on the basis of high amount of short chain fatty acids in the stool of African children that the intestinal microbiota coevolved with diets rich in polysaccharides in rural children.[4] *P copri* possesses a number of enzymes essential for utilization of complex polysaccharides[5] and produces a significant amount of succinate. Lower amounts of succinate in stool samples of infants are associated with developing AD.[6] Thus, production of succinate in the gut may be a process through which *P copri* plays a protective role against AD. Increased intake of simple (refined) sugars, especially in children who have low abundance of *P copri*, may result in uncontrolled sugar metabolism. There are several lines of evidence showing a link between high sugar intake and atopic diseases in childhood.[7] Another mechanism that can link diet to gut microbiome and allergic diseases is epigenetics. Epigenetic modifications of DNA are dynamic processes and are known to be heavily impacted by external factors such as diet. High glucose in diet can induce long-lasting activating epigenetic changes in the promoter of the nuclear factor-κB gene[8] and result in persistent glucose intolerance.[8] These studies show that epigenetic modifications can occur during early development in response to diet and result in reprogramming of the gut mucosal immunity, which can directly impact the gut microbiome.[9]

Another dietary factor that was linked to AD and lower relative abundance of *P copri* was higher consumption of saturated fat. Dietary fat intake has been shown to be positively associated with allergic diseases.[7] This finding calls for future studies to investigate the underlying mechanism of this link as it relates to microbiota.

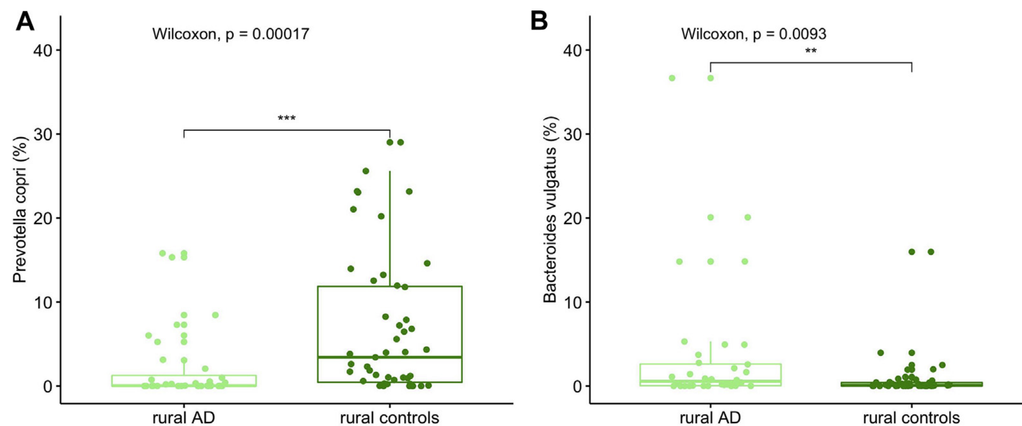

FIG 1. A, The relative abundance of *P copri* was significantly higher in control children without AD than in children with AD. **B,** The relative abundance of *B Vulgatus* was significantly higher in children with AD than in controls. Both *P* values remain significant after removing outliers for these analyses.

The strength of our study is the unique setting of these children living in an environment enriched in microbes through close daily contact with animals and plants in natural settings. However, the cross-sectional nature of this study limits its interpretation in entailing the complex associations of diet, environmental changes, microbiome, and atopy. The use of one 24-hour diet recall to assess nutrient intake that may not reflect the children's regular dietary habit is another limitation. Furthermore, we did not have direct assessment for malnutrition and its link to the gut microbiome.

Here, we have identified 2 species of bacteria that seem to be linked to AD and diet, and can explain some of the unsolved chain of events in the pathogenesis of this condition. However, much research is needed to uncover the whole story behind this major increase in allergic diseases and the huge impact it has around the globe.

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REFERENCES

1. Bjorksten B, Sepp E, Julge K, Voor T, Mikelsaar M. Allergy development and the intestinal microflora during the first year of life. J Allergy Clin Immunol 2001;108:516-20.
2. Laurence C, van der Merwe L, Zhang G, Le Souef P, Levin M. Association between pro-inflammatory alleles and allergic phenotypes in Xhosa adolescents. Pediatr Allergy Immunol 2018;29:311-7.
3. Mahdavinia M, Fox SR, Smith BM, James C, Palmisano EL, Mohammed A, et al. Racial differences in food allergy phenotype and health care utilization among US children. J Allergy Clin Immunol Pract 2017;5:352-7.e1.
4. De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci U S A 2010;107:14691-6.
5. Dodd D, Mackie RI, Cann IK. Xylan degradation, a metabolic property shared by rumen and human colonic Bacteroidetes. Mol Microbiol 2011;79:292-304.
6. Kim HK, Rutten NB, Besseling-van der Vaart I, Niers LE, Choi YH, Rijkers GT, et al. Probiotic supplementation influences faecal short chain fatty acids in infants at high risk for eczema. Benef Microbes 2015;6:783-90.
7. Barros R, Moreira A, Padrao P, Teixeira VH, Carvalho P, Delgado L, et al. Dietary patterns and asthma prevalence, incidence and control. Clin Exp Allergy 2015;45:1673-80.
8. El-Osta A, Brasacchio D, Yao D, Pocai A, Jones PL, Roeder RG, et al. Transient high glucose causes persistent epigenetic changes and altered gene expression during subsequent normoglycemia. J Exp Med 2008;205:2409-17.
9. Paparo L, di Costanzo M, di Scala C, Cosenza L, Leone L, Nocerino R, et al. The influence of early life nutrition on epigenetic regulatory mechanisms of the immune system. Nutrients 2014;6:4706-19.

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METHODS

Subjects

Control (nonallergic, non–food-sensitized) subjects were recruited from randomly selected creches in the Cape Town metropole. Patients with AD were recruited from the Nelson Mandela Academic Hospital in the Eastern Cape. AD was diagnosed by a dermatologist on the basis of diagnostic criteria proposed by the American Academy of Dermatology,[E1] including essential features of pruritus and eczematous rash with typical morphology and age-specific patterns, and a chronic or relapsing history.

Diet assessments

We administered detailed standardized questionnaires to obtain data on infant feeding patterns including breast-feeding history and duration and food introductions. Food consumption in children was assessed with one 24-hour dietary recall, completed on paper at the visit. This was administered by study staff trained in the multiple pass method, including first inquiring to ascertain a quick list of foods consumed, followed by forgotten foods and a detailed description of each food. This inquiry was done using culturally appropriate portion size illustrations to obtain accurate quantities. The individual food items from these completed 24-hour recalls were entered into the Food Processor Nutrition software program (created by Elizabeth Stewart, Hands and Associates, Salem, Ore), a nutrient analysis program using the USDA Standard Reference database, manufacturer's data, restaurant data, and data from literature sources to determine nutrient values for individual food items. Data were entered by registered dietitians, generating key nutrients of interest, including total fat, saturated fat, protein, fiber, and total sugar.

Allergic sensitization assessments

We evaluated all children by skin prick test for sensitivity to common allergenic foods including cow's milk, eggs, wheat, soy, peanut, fish, and hazelnut. Skin prick tests were done using standardized solutions from ALK Abelo (Thermo Fisher). Positive controls (10 mg/mL histamine) and negative controls (saline) were also done. Children were considered food sensitized if they developed a wheal diameter greater than or equal to 3 mm greater than that with the negative control in response to at least 1 allergen.

Fecal collection

Parents collected the early morning stool from the children's diaper and brought it to the study site within 2 hours of collection. Study staff scooped 2 to 3 walnut-sized pieces of stool with a sterile spatula into a clear Gaspack bag and transferred them to a -20°C freezer within 2 hours of being passed. Samples were transferred to a -80°C freezer within 24 hours of being passed.

16S rRNA gene sequencing

Standard procedures for metagenomic DNA extraction and 16S rRNA gene sequencing were used. In brief, metagenomic DNA from human stool samples was extracted using a PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, Calif). The 16S rRNA V1-3 hypervariable region was amplified using primers 27F and 534R (27F: 5′-AGAGTTTGATCCTGGCTCAG-3′ and 534R: 5′-ATTACCGCGGCTGCTGG-3′). The oligonucleotides containing the 16S primer sequences also contained an adaptor sequence for the Illumina sequencing platform. A barcode sequence unique to each sample was embedded within each of the forward and reverse oligonucleotides used to create the amplicons (dual tags). The uniquely barcoded amplicons from multiple samples were pooled and sequenced on the Illumina MiSeq sequencing platform. Illumina's software handled initial processing of all the raw sequencing data. One mismatch in primer and zero mismatch in barcodes was applied to assign read pairs to the appropriate sample within a pool of samples. Barcodes and primers were removed from the reads via Trimmomatic software. Reads were further processed, removing the sequences with low quality (average quality <35) and ambiguous bases (N's). Paired reads from the same amplicon were assembled and joined using the FLASH algorithm. Chimeric amplicons were removed using UChime software.[E2] OTUs were generated with the remaining amplicons. OTUs were clustered at 97% similarity by using the USEARCH algorithm,[E3] and then taxonomic assignment was done using the Ribosomal Database Project (RDB). Species identification used BLAST searches against the NCBI 16S ribosomal RNA (Bacteria and Archaea) database.

Microbiome statistical analysis

Downstream analyses including alpha and beta diversity estimations were conducted using R package 3.3.3, with a sampling average depth of 21,000 reads per sample for all samples. All samples were rarefied to 6985 reads. Alpha diversity was first calculated as represented by the following: (1) Observed Richness, which was the number of species; (2) Shannon Diversity Index, which was calculated as Σ piln(pi), where pi denotes the proportional abundance of species; and (3) Simpson Diversity Index, which was calculated as (1-Σ(pi2) using the Phyloseq package.[E4,E5] Univariate testing for differential diversity or abundance between 2 or more groups was computed using the Wilcoxon and Kruskal-Wallis tests, respectively, corrected for multiple comparisons for the relative abundances of different genera and OTUs. We also calculated the Spearman correlation coefficient. Beta diversity was analyzed using the Bray-Curtis distance for community abundance and Jaccard distance for community presence/absence. We have performed the permutational multivariate ANOVA using the distance matrices (ADONIS) and the analysis of group similarities on the relative abundances. Those tests compare the intragroup distances to the intergroup distances in a permutation scheme. Analysis of group similarities is usually used to test whether that the mean rank similarity between samples within a group is the same as the mean rank similarity between samples in other groups, whereas ADONIS is used for testing the hypothesis of no difference between 2 or more groups based on the analysis and partitioning sums of square of distances. Distance-based redundancy analysis with the canonical analysis of principal coordinates (Capscale function) shows the potential genera driving community differentiation by the linear relationship between community structures and environmental factors. These functions are implemented in the Vegan package. The data are reported at 2 taxonomic levels: at the 97% similarity using the USEARCH algorithm referred to as OTU and at the taxonomic level of genus.

Quantitative PCR

Quantitative PCR was performed on a Viia 7 Real-Time PCR system (Applied Biosystems, Carlsbad, Calif) using Fast SYBR Green Mix (Applied Biosystems, Foster City, Calif). Universal 16S primers using the primers described by Nadkarni et al (F: 5′-TCCTACGGGAGGCAGCAGT-3′, R: 5′-GGACTACCAGGGTATCTAATCCTGTT-3′) were used to quantify the total bacterial DNA concentrations. *P copri*–specific quantitative PCR primers (5′-ACTGACGCTGAAGCTCGAAA-3′′and 5′-GAAGGGCGTCATTGTCTC CA-3′) designed by Lauren Petersen (a member of the Weinstock lab) were used to quantify *P copri*. Briefly, DNA was diluted 1:10,000 in ultrapure DNAse-free water, and mixed 1:4 with Fast SYBR Green master mix containing the appropriate primers for a total volume of 25 μL. Each well was then mixed, sealed, and centrifuged briefly. Samples were then cycled 40 times at 95°C for 1 second and 60°C for 45 seconds. Samples were run in triplicate. Known positive and negative controls were included as well. The number of *P copri* 16S genes was compared to the total number of bacterial 16S genes determined with the Universal Nadkarni primers for analysis and the ratio of this bacterium was calculated and used for analysis.

REFERENCES

E1. Eichenfield LF, Tom WL, Chamlin SL, Feldman SR, Hanifin JM, Simpson EL, et al. Guidelines of care for the management of atopic dermatitis: section 1. Diagnosis and assessment of atopic dermatitis. J Am Acad Dermatol 2014;70:338-51.
E2. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 2011;27:2194-200.
E3. Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 2010;26:2460-1.
E4. McMurdie PJ. Holmes S. phyloseq: Import, share, and analyze microbiome census data using R 2017. Available at: https://joey711.github.io/phyloseq/.
E5. McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 2013;8:e61217.

A

Anosim (Analysis of Similarity) assessing the similarity of the gut bacterial communities

Beta-diversity tests	D_Bray Dissimilarity		D_Jaccard Presence/Absence	
	R (correlation coef.)	p-value	R (correlation coef.)	p-value
At OTU level	0.19	0.002**	0.18	0.001**
At Genus level	0.19	0.001**	0.14	0.001**

Adonis (Analysis of Dissimilarity)

Variable	D_Bray			D_Jaccard		
	F.Model	R2	p-value	F.Model	R2	p-value
At OTU level	4.92	0.06	0.00099***	3.6	0.04	0.00099***
At Genus level	7.7	0.087	0.00099***	4.5	0.05	0.00099***

B

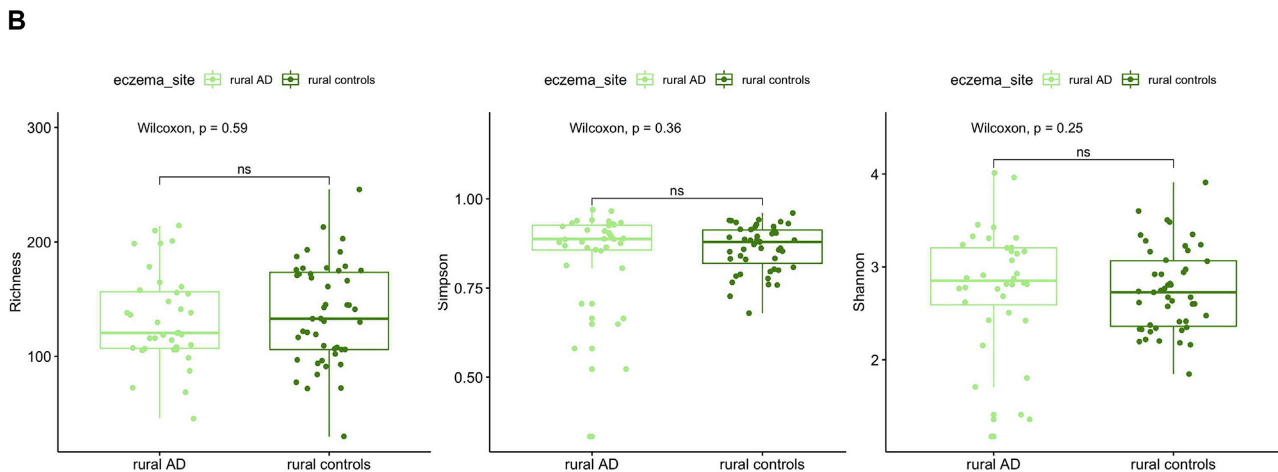

FIG E1. A, There were significant differences from multivariate dispersion (variance) at the OTU level (both ADONIS and ANOSIM, $P < .001$). Beta diversity analysis showed that cases with and without AD are significantly different in terms of both abundance and presence/absence of different OTUs and genera. **B,** Microbiome alpha-diversity indices showed that the alpha-diversity indices are not statistically different between AD and controls. *ANOSIM,* Analysis of group similarities.

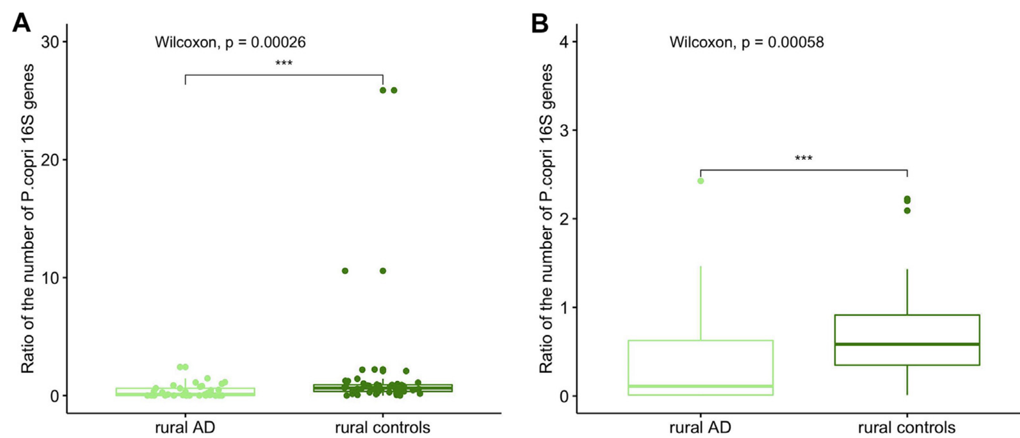

FIG E2. **A,** The ratio of *P copri* was significantly higher in controls compared with controls cases. **B,** The analysis remained significant after excluding the 4 outliers. y-axis: The ratio is that of the number of *P copri* 16S genes to the number of 16S genes of all the bacteria in the sample identified using the Universal Nadkarni primers.

TABLE E1. Demographic and clinical characteristics of 83 toddlers recruited from rural areas of South Africa

Characteristic	With AD (n = 36)	Without AD (n = 47)	*P* value
Sex			.41
Male	19	27	
Female	17	20	
Age (mo), mean ± SD	21.0 ± 7.3	22.33 ± 6.8	.84
Wheezing	0	1 (3.7)	.73
Allergic rhinitis	3 (8.3)	2 (4.2)	.14
Food sensitized, n (%)			
At least 1 food	19 (52.9)	0	.0001
Cow milk	3 (8.3)	0	.03
Egg	11 (30.6)	0	.0001
Peanut	7 (19.4)	0	.0001
Hazelnut	0	0	.21
Wheat	0	0	—
Soy	0	0	.17
Fish	0	0	—
Polysensitized	2 (5.5)	0	.01

TABLE E2. Dietary intake and breast-feeding history in 83 toddlers recruited from rural areas of South Africa

Characteristic	With AD (n = 36)	Without AD (n = 47)	*P* value
Breast-feeding at all	76.1%	79.1%	.73
Duration of exclusive breast-feeding (mo), mean ± SD	2.93 ± 2.33	3.18 ± 2.43	.67
Total duration of breast-feeding (mo), mean ± SD	5.77 ± 4.8	6.69 ± 7.36	.72
Energy (kcal)	855.8 (436.9)	860.2 (610.0)	.976
Carbohydrate, % of energy	61.7 (10.3)	64.8 (15.4)	.261
Total fiber intake (g)	5.7 (6.3)	6.2 (7.2)	.702
Soluble fiber (g)	0.2 (0.4)	0.2 (0.5)	.094
Sugar (g)	47.6 (39.2)	27.2 (35.8)	.007
Fat intake (g)	26.9 (10.4)	21.3 (12.3)	
Fat, % of energy	28.7 (10.1)	25.5 (12.7)	.092
Saturated fat (g)	8.5 (12.5)	3.9 (8.9)	.003
Omega-3 fatty acids (mg)	205.0 (290.0)	180.0 (330.0)	.763
Protein intake (g)	24.0 (13.2)	23.7 (11.5)	.67
Protein, % of energy	11.9 (4.0)	11.2 (3.7)	.266
Calcium (mg)	632.6 (705.4)	414.4 (613.9)	.402
Vitamin C (mg)	47.5 (61.7)	39.4 (52.8)	.397
Thiamine (mg)	0.9 (1.0)	0.9 (0.7)	.996
Iron (mg)	8.4 (14.8)	10.0 (11.0)	.281
Iodine (μg)	14.6 (53.0)	27.6 (87.0)	.85

Data for dietary intake variables are expressed as median (interquartile range) unless otherwise noted; nutrient data expressed as intake per day.