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ORIGINAL ARTICLE

Antioxidant nutrient intakes and corresponding biomarkers associated with the risk of atopic dermatitis in young children

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Background/Objectives: To investigate the association of antioxidant nutritional status with the risk of atopic dermatitis (AD) in young children in a case–control, population-based study.
Subjects/Methods: Identified from preschools by using the Korean version of the International Study of Asthma and Allergies in Childhood (ISAAC). Final analysis included 180 AD (mean age 5.3 ± 0.9 years) and 242 non-AD (mean age 5.2 ± 1.0 years) children. Diet was assessed using a validated semi-quantitative food frequency questionnaire. Fasting blood samples were used for analyses of fat-soluble vitamins (retinol, α-tocopherol, and β-carotene) and vitamin C.
Results: AD was associated negatively with intakes of antioxidant-related nutrients. The adjusted odds ratio (OR) and 95% confidence interval (95% CI) were 0.44 (0.22–0.88) for the highest (vs lowest) quintile of β-carotene. A similar association was observed for dietary vitamin E (OR = 0.33, 95% CI = 0.16–0.67), folic acid (OR = 0.37, 95% CI = 0.18–0.73), and iron (OR = 0.39, 95% CI = 0.19–0.79). Reduced AD risk was found with 1 s.d. increase of serum α-tocopherol [OR = 0.64, 95% CI = 0.41–0.98) and retinol (OR = 0.74, 95% CI = 0.58–0.96) concentrations, and marginally with that of serum β-carotene levels ($P = 0.0749$ for trend). There was no relationship of AD risk with dietary and plasma vitamin C as well as nutrient supplement intake regardless of nutrient type. AD was predicted better by the intake measure than the corresponding blood biomarker regarding vitamin E and β-carotene.
Conclusions: These findings suggest that higher antioxidant nutritional status reduces the risk of AD and that such risk-reduction effects depend on nutrient type.
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Keywords: antioxidant nutrients; atopic dermatitis; young children

Introduction

Atopic dermatitis (AD), a common chronic inflammatory skin disease, is one of the most common disorders in children, and its prevalence has been increasing in both Western countries and Korea. Prevalence rates of AD in Korea were 19.7% in 1995, 27.5% in 2000, and 33.1% in 2006 among elementary school children, and 34.9% in 2003 for preschoolers, according to parental reporting of their child's AD assessment by the Korean version of the International Study of Asthma and Allergies in Childhood (ISAAC) (Oh *et al.*, 2003; Yeon *et al.*, 2005; Son *et al.*, 2007).

These rapid increases are most likely to be a result of changes in environmental influences, although atopic diseases have a clear genetic basis. It has been reported that a reduced barrier function as well as altered immunity are fundamental to the development of AD (Terui, 2009). Reactive oxygen species generated from environmental pollution and solar radiation have been suggested to induce oxidative protein damage in the stratum corneum, which

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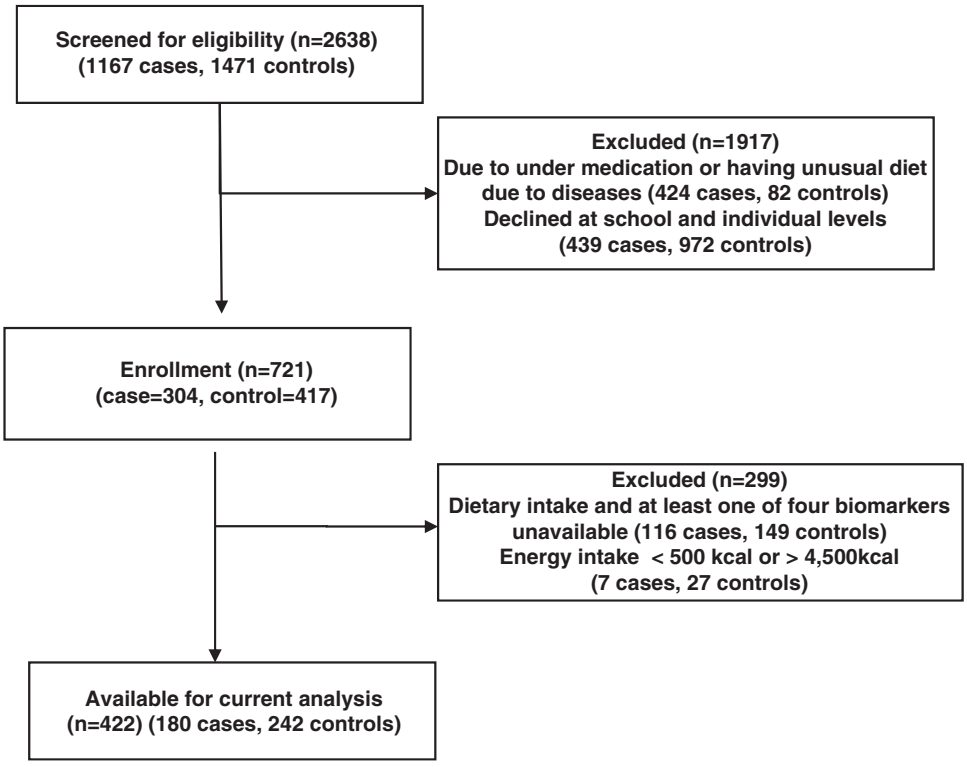

Figure 1 Sample selection process.

lead to the disruption of barrier function and exacerbation of AD (Niwa *et al*., 2003).

Antioxidant nutrients have been proposed to counteract oxidative stress and inhibit the inflammatory response and are known to be possibly associated with the ability of the individual to restrain the inflammatory response and allergic diseases (Hughes, 2001; Devereux, 2005). The most important antioxidants are vitamin A, C and E, β-carotene, and selenium. Maternal antioxidants have been reported to have a function in reduced asthma of children (Hoppu *et al*., 2005; Martindale *et al*., 2005; Sausenthaler *et al*., 2007), yet there are limited studies regarding subjects' habitual dietary intake and AD in young children. To examine whether antioxidant nutritional status could prevent AD in young children, we conducted a case–control study while assessing antioxidant nutrient intakes and corresponding blood biomarkers.

Methods

The Research Ethics Committee of College of Human Ecology, Kyung Hee University approved the study protocol.

Study population

As shown in Figure 1, subjects were recruited from 2638 preschoolers in large cities (Seoul and Incheon) between May and July 2006 using the Korean version of ISAAC (The International Study of Asthma and Allergies in Childhood (ISAAC), 2009; Oh *et al*., 2003). Cases were those if their parents reported their children as experiencing appearances and disappearances of rashes for at least 6 months or having experienced itchy rashes during the last 12 months, or if their children had been diagnosed with AD by a physician. Controls were recruited from the same preschools where the eligible cases were enrolled in while considering age and gender. Among those subjects, we included the children only if their parents reported that they did not take any medication and had habitual diet unmodified by AD or other diseases, and agreed to take part in the study on behalf of them. A total of 304 AD (case) and the 417 non-AD (control) children met the study criteria. Among those, data analyses of this report included 180 AD and 242 non-AD children with at least one of four biomarkers and an energy intake ranging from 500 to 4500 kcal (Willett and Stampfer, 1986). As a large number of the eligible children were excluded due to missing values of some measures and the difficulty of obtaining fasting blood and urine samples, we compared age, gender, and household income of the children included in this study with those of their counterparts excluded. There was no significant difference of these indices between them. The informed consent was obtained from the parents of the all participants. The household monthly incomes of about 40% of our children and urban Koreans were over 4 million won (3000$), which indicates that our participants were from middle income neighborhoods (Korea National Statistical Office, 2009).

Measurements

Dietary intake. Usual dietary intake was assessed by a modified version of the semi-quantitative food frequency questionnaire (FFQ) with 86 food items with 9 non-overlapping frequency response categories (Lim and Oh, 2002; Shin *et al*., 2007). The caretaker was asked to indicate his/her child's average frequency of consumption and portion size of foods during the previous year. Respective correlation coefficients for reproducibility and validity of this instrument ranged 0.54–0.76 and 0.29–0.57 depending on the nutrient. Using Computer Aided Nutritional Analysis Program II (CAN PRO II) developed by the Korean Nutrition Society, the amount of each food item included in the FFQ was converted into grams, from which the daily intakes of nutrients were calculated.

We also assessed intakes of antioxidant-related nutrients, such as vitamin A, C and E, folic acid, and iron, from supplements. Vitamin A was included as many products had no information on β-carotene, but provided vitamin A contents. We used nutrition supplement information regarding type, specific brand names, and usual daily dose. Information including four different types of nutrition supplements for multiple supplement users was gathered. Doses were calculated using nutrient content information on product labels.

Biochemical analysis. Fasting blood samples were collected for analyses of fat-soluble vitamins (retinol, α-tocopherol, and β-carotene), vitamin C, and total IgE concentrations. Serum fat-soluble vitamin levels were measured simultaneously using a method reported by Talwar *et al*. (1998). Briefly, fat-soluble vitamins were extracted with hexane and analyzed by High performance liquid chromatography (HPLC) using a reverse-phase C18 column (Nucleosil ODS1, (Supelco), 3.2 × 250 mm, Sigma-Aldrich Corp., St Louis, MO, USA) and a solvent mixture of methanol:acetonitrile: tetrahydrofuran (75:20:5) containing 0.01% ascorbic acid. Absorbance for retinol, α-tocopherol, and β-carotene was measured at 325 nm, 290 nm, and 450 nm, respectively, using two internal standards, retinol acetate for retinol and tocopherol acetate for α-tocopherol and β-carotene.

For the analysis of vitamin C, heparin-treated plasma samples were deproteinized with freshly prepared 10% metaphosphoric acid (w/v) immediately after blood collection and separation, and stored at −80 °C until further analyzed. Vitamin C was quantified by HPLC using a reverse-phase C18 column (Cosmosil 5C18-AR-II, 4.6 × 150 mm, Nacalai Tesque Inc., Kyoto, Japan) and a mobile phase containing 5 mM cetyltrimethylammonium bromide and 50 mM potassium dihydrogen phosphate (pH 4.5) with a UV detection at 264 nm, as described by Esteve *et al*. (1997). Total serum IgE levels were measured by using commercially available kits (UniCAP; Phadia, Uppsala, Sweden). Total cholesterol and triglyceride concentrations were determined by enzymatic-colorimetric assays using commercial kits (Bio Clinical System Corp., Anyang, Korea).

Other variables. The severity of AD was evaluated by a pediatrician using the objective components of Scoring Atopic Dermatitis (SCORAD) index ranging from 0 to 83 points (European Task Force on Atopic Dermatitis, 1993). The severity of AD was subsequently categorized into mild (<15 SCORAD points), moderate (15–40 SCORAD points), and severe (>40 SCORAD points).

We measured child's birth weight, breastfeeding, and allergic history; parental education and allergic history; and household monthly income using a questionnaire. Parents who had experienced AD, asthma, or rhinitis were regarded as having a history of atopic disease. Trained graduate students majoring in nutrition measured the children's height and weight following recommended standard procedures (World Health Organization, 1995). Body mass index (BMI) was calculated by using height and weight measures.

Data collection. The survey instrument had been pretested between May to July 2006, and was revised with minor modification. The study collected data from the caretakers on behalf of the children between September 2006 and January 2007.

Statistical analysis. Statistical analyses were conducted with SAS for Windows (Version 9.1). The children were divided into five groups on the basis of the levels of total energy and nutrient residuals and compared higher intakes (Q2–Q5) with a low intake (Q1). Nutrient residuals were obtained by adjusting for total energy using a linear regression model in which total energy intake was the independent variable and each nutrient was the dependent variable (Willett and Stampfer, 1986). Serum α-tocopherol concentration was divided by the sum of serum cholesterol and triglycerides concentrations to adjust for serum lipid (Thurnham *et al*., 1986).

Associations of AD risk with the background characteristics of subjects were assessed by χ^2 test and *t*-test as appropriate (Cody and Smith, 2005). Multivariate analyses were performed adjusting key covariates such as monthly household income, parental histories of allergic diseases, and the child's age, gender, and BMI (Dunder *et al*., 2001; Tricon *et al*., 2006; Sausenthaler *et al*., 2007). The model for nutrient intakes were also adjusted for supplement (dichotomous variable [yes/no]) and total energy intake, as suggested elsewhere (Willett and Stampfer, 1986). Where there was a group difference of $P<0.05$, the sites of significant effects were determined by odds ratios (ORs) and 95% confident intervals (CIs).

Results

Background characteristics

There was no group difference of selected general characteristics except for child and parental histories of allergic diseases as well as child's total IgE and cholesterol levels (Table 1). Parents of the children with AD had experienced

Table 1 General characteristics of children with and without AD[a]

Characteristic[b]	*With AD (n = 180)*	*Without AD (n = 242)*	P[c]
Family history			
Atopic history of mother			
Asthma (%)	9 (5.0)	13 (5.4)	0.8650
Rhinitis (%)	43 (23.9)	57 (23.6)	0.9362
AD (%)	23 (12.8)	14 (5.8)	0.0120
Atopic history of father			
Asthma (%)	8 (4.4)	6 (2.5)	0.2649
Rhinitis (%)	41 (22.8)	35 (14.5)	0.0279
AD (%)	22 (12.2)	17 (7.0)	0.0683
Father's education (⩾16 years)	101 (57.1)	138 (57.7)	0.8899
Mother's education (⩾16 years)	82 (46.1)	99 (41.1)	0.3082
Monthly income ($\geq 4 \times 10^6$ Won ≈ 3000 US $)	66 (37.9)	93 (39.6)	0.7360
Child			
Girls	81 (45.3)	119 (49.2)	0.1500
Breastfeeding (>3 months)	75 (41.9)	84 (35.0)	0.3353
Supplement intake	73 (40.6)	113 (46.7)	0.2090
Birth weight (kg)	3.3 ± 0.7	3.3 ± 0.9	0.7619
Height (cm)	115.3 ± 7.8	115.0 ± 8.1	0.7070
Weight (kg)	20.8 ± 3.8	20.3 ± 3.8	0.1640
Body mass index (kg/m^2)	15.5 ± 1.5	15.2 ± 1.7	0.0615
Age (years)	5.3 ± 0.9	5.2 ± 1.0	0.5271
Current exposure to smoking at home (yes)	28 (16.6)	33 (14.6)	0.5926
Total IgE	278.6 ± 436.2	187.2 ± 309.9	0.0048
Total cholesterol in blood	156.0 ± 25.7	150.1 ± 23.6	0.0497
Triglycerides in blood	86.8 ± 47.5	84.6 ± 48.5	0.7046
Diagnosis of asthma or rhinitis, lifetime	54 (30.5)	44 (18.4)	0.0041

Abbreviation: AD, atopic dermatitis.
[a]Values are numbers (percents) or means ± s.d.
[b]Number studied varied by characteristics (children with and without AD) are as follows: father's education (177/239), mother's education (178/241), monthly income (174/235), gender (179/242), birth weight (172/234), current smoking exposure at home (169/226), diagnosis of asthma or rhinitis, lifetime (177/239), total IgE, total cholesterol, total triglycerides (174/228), and others (180/242).
[c]χ^2 test for categorical variables and Student's *t*-test for continuous variables.

AD (for mothers) and rhinitis (for fathers) more frequently than those of the children without AD. Likewise, AD children had more other allergic diseases and higher total cholesterol levels than non-AD children. Total serum IgE levels, showing marked variation among children, were significantly higher in the AD children. Mean BMI tended to be higher in the AD group at $P = 0.0615$. The severity of AD diagnosed by the SCORAD index presented that most AD children were mild cases (88%) and only single case was with severe AD (data not shown).

Relation between nutrient intakes and AD

Dietary energy intake was below the estimated energy requirement for 49–55% of the children (Table 2). For dietary folic acid, 40–54% of the children were below the estimated average requirement (EAR), respectively. Regarding dietary iron and vitamin A and C, ~16–22% of the children were below the EARs. When supplemental nutrient intakes were considered, there was a decrease in the proportions of the children whose nutrient intakes were below the EARs.

Antioxidant-related nutrient intakes were significantly associated with AD after controlling for potential confounders (Table 2). Compared with those in the lowest quintile, children in the highest quintile of dietary vitamin E (OR = 0.33, 95% CI = 0.16–0.67) and β-carotene (OR = 0.44, 95% CI = 0.22–0.88) were likely to have lower risk of AD. Reduced risk was also found regarding iron (OR = 0.39, 95% CI = 0.19–0.79) and folic acid (OR = 0.37, 95% CI = 0.18–0.73) from diet. Conversely, intakes of vitamin C and micronutrients from supplements had no association with AD risk. Intakes of iron, vitamin E, and folic acid from both diet and supplement were associated with lower likelihood of AD.

Relation between blood antioxidant concentrations and AD

With adjustment of potential confounders, serum α-tocopherol and retinol showed a negative association with AD, indicating ORs of 0.64. (95% CI = 0.41–0.98) and 0.74 (95% CI = 0.58–0.96), respectively, with 1 s.d. increase (Table 3). At marginal significance ($P = 0.0749$ for trend), serum β-carotene concentrations (OR = 0.76, 95% CI = 0.57–1.03) were related to reduced risk of AD. Plasma vitamin C concentrations did not differ between the groups.

Relation between antioxidant nutrient intakes and biomarkers

Figure 2 shows the dose–response distribution of the mean concentrations of antioxidant nutrients in blood according to the quintiles of corresponding nutrient intakes. There was no linear relationship between intake and biomarker variables in children both with and without AD.

Relation of antioxidant nutrients in diet and blood with AD

As antioxidant nutrients in diet and blood showed significant associations with AD in separate analyses and the intake and biomarker variables were not correlated with each other, we examined the independent association of antioxidant nutrient from diet and blood in AD while including both variables in the same analytic model. Intakes of vitamin E (OR = 0.68, 95% CI = 0.49, 0.95) and β-carotene (OR = 0.69, 95% CI = 0.50–0.93) but not their biomarkers were significantly related to a lower risk of AD (Table 4). On the other hand, serum retinol concentrations, but not dietary retinol, tended to be related to AD risk ($P = 0.0741$ for trend). Vitamin C levels from both diet and blood were not related to AD risk.

Discussions

To identify nutrient intakes related to AD, this study included children who had no medication and no dietary change due to diseases. As a result, a considerable proportion

Table 2 Comparison of quintiles (Q) with the lowest quintile (Q1) for each nutrient intake by likelihood of AD

Variable	*With AD (n = 168)*		*Without AD (n = 224)*		*Compared with Q1*[a]				P *for trend*[b]
	Mean	*s.e.m.*	*Mean*	*s.e.m.*	*Q2*	*Q3*	*Q4*	*Q5*	
	(% <DRI)		*(% <DRI)*		*OR 95% CI*	*OR 95% CI*	*OR 95% CI*	*OR 95% CI*	
Energy (kJ)	6276 (54.8)[c]	208	6586 (48.7)	195	0.94 0.50, 1.80	1.07 0.55, 2.10	0.59 0.29, 1.20	0.76 0.39, 1.47	0.20
Iron									
Diet (mg)	9.0 (19.1)	0.4	9.9 (16.5)	0.3	0.99 0.50, 1.99	0.52 0.25, 1.07	0.85 0.42, 1.74	0.39 0.19, 0.79	0.01
Suppl (mg)[d]	0.4	0.2	0.5	0.2				0.51 0.19, 1.35	0.18
Total (mg)	9.4 (17.9)	0.4	10.4 (15.6)	0.4	1.08 0.53, 2.16	0.54 0.26, 1.09	0.82 0.40, 1.67	0.30 0.15, 0.63	0.00
Zinc									
Diet (mg)	7.4 (4.2)	0.3	7.8 (6.3)	0.2	1.02 0.52, 2.00	1.65 0.85, 3.20	1.20 0.62, 2.34	0.66 0.32, 1.34	0.52
Suppl (mg)[e]	0.6	0.1	0.7	0.1				1.26 0.69, 2.30	0.42
Total (mg)	8.1 (3.0)	0.3	8.6 (4.9)	0.3	0.78 0.39, 1.56	1.44 0.75, 2.79	1.23 0.62, 2.46	1.06 0.51, 2.21	0.48
Vitamin A									
Diet (μg RE)	432.6 (19.1)	21.5	502.3 (16.1)	21.4	0.78 0.40, 1.54	0.74 0.37, 1.47	0.72 0.36, 1.44	1.51 0.78, 2.91	0.05
Suppl (μg RE)[f]	201.4	28.4	234.8	28.7			1.03 0.49, 2.17	0.81 0.40, 1.64	0.60
Total (μg RE)	634.0 (11.3)	34.4	737.1 (11.6)	33.8	0.98 0.49, 1.96	0.82 0.41, 1.62	0.58 0.28, 1.18	0.59 0.28, 1.25	0.08
Retinol (μg)	228.3	12.5	241.5	11.2	1.50 0.77, 2.95	1.39 0.70, 2.78	0.72 0.36, 1.44	1.51 0.78, 2.91	0.89
β-carotene (μg)	1399	98.5	1706	93.4	0.70 0.34, 1.41	0.45 0.22, 0.93	0.64 0.32, 1.29	0.44 0.22, 0.88	0.03
Vitamin C									
Diet (mg)	68.1 (22.0)	4.4	74.6 (21.0)	4.0	0.60 0.30, 1.18	0.62 0.30, 1.28	0.62 0.31, 1.26	0.53 0.27, 1.06	0.14
Suppl (mg)[g]	51.6	18.2	16.7	3.6			1.26 0.56, 2.86	1.44 0.71, 2.90	0.31
Total (mg)	119.8 (14.3)	18.4	91.3 (14.3)	5.6	0.46 0.23, 0.94	0.57 0.28, 1.16	0.52 0.26, 1.03	0.79 0.40, 1.55	0.64
Folic acid									
Diet (μg)	169.1 (54.2)	6.9	196.4 (39.7)	6.8	0.90 0.45, 1.79	0.58 0.30, 1.15	0.51 0.25, 1.02	0.37 0.18, 0.73	0.00
Suppl (μg)[h]	57.6	8.7	73.9	9.5			1.88 0.65, 5.42	0.88 0.47, 1.64	0.73
Total (μg)	226.7 (30.9)	10.5	270.3 (28.1)	11.4	0.49 0.24, 0.98	0.58 0.29, 1.16	0.37 0.18, 0.75	0.33 0.16, 0.70	0.01
Vitamin E									
Diet (mg α-TE)	8.3	0.4	10.2	0.5	0.56 0.27,1.13	0.39 0.19, 0.83	0.40 0.19, 0.82	0.33 0.16, 0.67	0.00
Suppl (mg α-E)[i]	4.3	0.6	5.5	0.7	1.09 0.51, 2.33	0.83 0.41, 1.67			0.66
Total (mg α-TE)	12.6	0.7	15.7	0.8	0.67 0.34, 1.35	0.60 0.30, 1.22	0.49 0.25, 0.98	0.46 0.22, 0.96	0.03

Abbreviations: AD, atopic dermatitis; CI, confidence interval; OR, odds ratio; Suppl, supplement.
[a]Adjusted for monthly household income, parental histories of allergic diseases, and the child's age, gender, body mass index, supplement intake (yes/no), and total energy intake.
[b]Obtained from trend test (Wald) in logistic regression models.
[c]Estimated average requirements for age and gender specific Korean children for all variables except for energy (estimated energy requirement).
[d]Q1 = nonusers, Q5 [$n = 107$, mean ± s.d. (range) = 2.6 ± 2.0 (1.2–15.0) mg].
[e]Q1 = nonusers, Q5 [$n = 25$, mean ± s.d. (range) = 7.5 ± 9.2 (0.2–36.0) mg].
[f]Q1 = nonusers, Q4 [$n = 59$, mean ± s.d. (range) = 320.1 ± 110.9 (70.8–390.0) μg RE], Q5 [$n = 72$, mean ± s.d. (range) = 938.1 ± 427.8 (420.0–2340.0) μg RE].
[g]Q1 = nonusers, Q4 [$n = 47$, mean ± s.d. (range) = 10.1 ± 2.2 (5.0–15.6) mg], Q5 [$n = 89$, mean ± s.d. (range) = 134.2 ± 316.5 (16.6–2500.0) mg].
[h]Q1 = nonusers, Q4 [$n = 19$, mean ± s.d. (range) = 42.0 ± 42.1 (5.0–120.0) μg], Q5 [$n = 103$, mean ± s.d. (range) = 247.0 ± 141.1 (130.0–780.0) μg].
[i]Q1 = nonusers, Q4 [$n = 55$, mean ± s.d. (range) = 6.7 ± 2.4 (1.6–8.3) α-TE], Q5 [$n = 77$, mean ± s.d. (range) = 20.5 ± 10.5 (9.0–50.0) α-TE].

Table 3 The means of antioxidant nutrient concentrations and the associations between AD and 1 s.d. increase of antioxidant nutrient concentrations in blood

Variable	*With AD*		*Without AD*		*Adjusted demographics only*[a]		*Fully adjusted*[b]	
	Mean	*s.d.*	*Mean*	*s.d.*	*OR (95% CI)*	P *for trend*[c]	*OR (95% CI)*	P *for trend*[d]
Serum retinol (umol/l)	1.58	0.64	1.71	0.72	0.78 (0.61, 0.99)	0.0406	0.74 (0.58, 0.96)	0.0222
Serum β-carotene (umol/l)	0.22	0.25	0.30	0.48	0.76 (0.58, 1.01)	0.0550	0.76 (0.57, 1.03)	0.0749
Plasma vitamin C (umol/l)	56.47	23.58	56.83	24.03	0.95 (0.77, 1.18)	0.6574	0.94 (0.76, 1.17)	0.5592
Serum α-tocopherol (umol/l)	13.66	8.95	16.52	12.09	0.64 (0.42, 0.97)	0.0349	0.64 (0.41, 0.98)	0.0377

Abbreviations: AD, atopic dermatitis; CI, confidence interval; OR, odds ratio.
[a]Adjusted for age and gender.
[b]Adjusted for monthly household income, parental histories of allergic diseases, and the child's age, gender, and body mass index.
[c]Number studied varied by antioxidant (children with and without AD) in models are as follows: serum α-tocopherol, β-carotene, and retinol (141/218); and plasma vitamin C (176/231).
[d]Obtained from trend test (Wald) in logistic regression models.

of the AD children were mild AD characterized by the SCORAD index. Nevertheless, our study found antioxidants such as β-carotene and vitamin E, but not vitamin C, reduced AD risk. Results on these nutrients from diet and blood were consistent.

Similar to ours, the inverse association of maternal vitamin E intake and AD in early childhood was reported (Martindale *et al*., 2005). Conversely, there was no association between atopy and vitamin E intake in UK children (Laitinen *et al*., 2005). Unlike our results, a birth cohort study showed a protective effect of atopic mothers' vitamin C intake during pregnancy on eczema in young children (Hoppu *et al*., 2005). Aside from the dissimilar sample characteristics among the studies, possible explanations for the disagreement may include gene expression, other food compounds associated with AD risk, as well as the intentional dietary change of subjects due to disease status.

Studies have reported significant association between AD and polymorphisms in several different genes including interleukin-13, interleukin-4 receptor, chymase, and serine protease inhibitor, kazal-type 5 (Chien *et al*., 2007). Further, recent studies have identified polymorphisms in genes that are involved in the antioxidant defense system, for example, Mn-SOD, catalase, and glutathione S-transferase (Fabre *et al*., 2008; Islam *et al*., 2009). Thus, gene polymorphisms may confer individual variability in the metabolism of antioxidant nutrients and their potential (protective) role in the pathogenesis of AD.

It has been also reported that barrier disruption, hallmark of AD, was related to the decrease of calcium, magnesium, potassium, and zinc in *in vitro* and animal studies (Denda *et al*., 2000; Denda and Kumazawa, 2002; Makiura *et al*., 2004), although these associations are not clear in human studies (Tricon *et al*., 2006). Like zinc shown in Table 2, when we analyzed our data regarding the intakes of calcium and potassium, there was no statistically significant association between these nutrients and AD in this study (data not shown). The lack of association of these nutrients with AD might partly attribute to the fact that majority of the AD children were mild AD. Furthermore, considering the significant relationships between AD and antioxidant nutrients observed in this study, it suggests that antioxidant nutrients may be more sensitive makers for AD compared with other micronutrients.

With respect to the other food compounds, intercorrelations of nutrients in the diet draw our attention. In addition to antioxidant nutrients, observational studies have shown the association between allergic diseases and other micronutrients including retinol, calcium, vitamin D, zinc, and n3-fatty acids (Tricon *et al*., 2006; Devereux, 2005, 2007). Intervention studies, however, have suggested limited effects of the supplement intakes on allergic disease management (Tricon *et al*., 2006; Devereux, 2005, 2007). In our observational study, the intakes from diet, but not supplements, reduced the risk of AD. Together, it is possible to assume that the protection offered by nutrient intake from diet was partly due to some other component of specific nutrient rich foods. These findings support the nutrient rather than antioxidant hypothesis as a more appropriate model for interaction between diet and AD given that non-antioxidant properties of nutrients may also have a function against AD (Devereux, 2007).

Using antioxidant biomarkers as independent variables, we found lower likelihood of AD with an increase of α-tocopherol or β-carotene concentrations, but not by vitamin C concentrations. This may be due to the fact that the serum vitamin C levels in our study subjects were relatively high compared with the other antioxidant biomarkers examined. Among all subjects, only three children (<1% of subjects) had serum vitamin C concentrations <11.4 μmol/l, an indicative of deficiency. Further, >50% of the children included in this study had serum vitamin C concentrations consistent with tissue saturation (>56.8 μmol/l).

The means of serum α-tocopherol (16.5–18.4 μmol/l) and β-carotene (0.3–0.4 μmol/l) of children reported by other studies (Institute of Medicine, 2000; Cemek *et al*., 2006) were higher than the values of our AD children (13.66 and 0.22 μmol/l, respectively), but comparable to those of the non-AD children (16.52 and 0.30 μmol/l, respectively). It is, thus, plausible to assume that relative nutritional status might have influenced the association between AD and

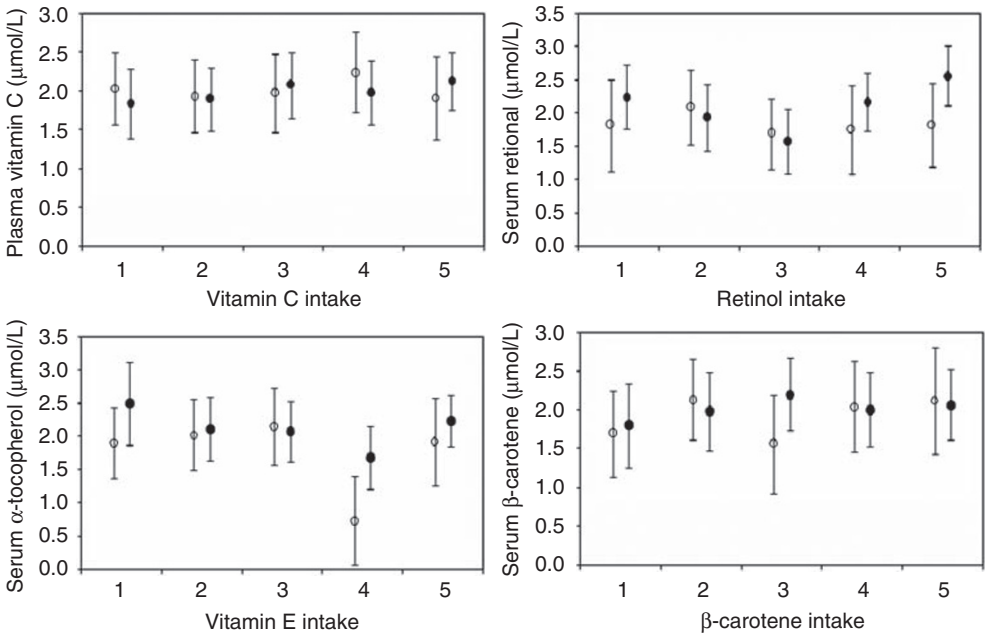

Figure 2 Means and 95% confidence intervals of blood antioxidant nutrient levels according to the quintiles of corresponding energy adjusted antioxidant nutrient intakes among children with (○) and without (●) atopic dermatitis.

Table 4 The association of atopic dermatitis with 1 s.d. increases of antioxidant nutrient intake and corresponding biomarker[a]

Nutrient	*OR (95% CI)*	P *for trend*[b]
Retinol		
Intake	0.92 (0.68, 1.25)	0.6088
Serum	0.78 (0.60, 1.02)	0.0741
β-carotene		
Intake	0.69 (0.50, 0.93)	0.0166
Serum	0.82 (0.60, 1.13)	0.2301
Vitamin C		
Intake	0.96 (0.76, 1.22)	0.7423
Plasma	0.95 (0.75, 1.19)	0.6274
Vitamin E		
Intake	0.68 (0.49, 0.95)	0.0226
Serum[c]	0.84 (0.62, 1.13)	0.2372

[a]Adjusted for monthly household income, parental histories of allergic diseases, and the child's age, gender, body mass index, supplement intake (yes/no), and total energy intake.
[b]Obtained from trend test (Wald) in logistic regression models.
[c]α-tocopherol.

antioxidant biomarkers, although this explanation has limitations in the case of serum retinol.

Serum retinol concentrations were associated with the likelihood of AD, yet most of our subjects had adequate retinol concentrations. Low serum retinol (<1.05 μmol/l) existed in ~11 and 8% of the children with and without AD, respectively. Serum retinol concentrations of our subjects (1.58–1.71 μmol/l) were somewhat higher than those reported in NHANES III (1.22 μmol/l) (Institute of Medicine, 2000) and in Turkish children (1.15 μmol/l) (Cemek *et al*., 2006), but close to the means of children in Brazil with age 7–9.9 years (1.74 μmol/l) (Souza Valente da Silva *et al*., 2007). Considering our results and earlier studies (Tricon *et al*., 2006), the association between serum vitamin A and allergy seems to be inclusive.

The independent role of antioxidant nutrient intake and corresponding biomarker in AD suggest that intake was a marker for AD risk than serum concentration with respect to β-carotene. Similarly, vitamin E intake, but not serum α-tocopherol, was a predictor of AD risk. We assessed antioxidant nutrient intakes by FFQ with a reference period of previous 1 year, which provided long-term usual intake. Antioxidant nutrients in blood, in contrast, are indices of short-term nutritional status (Gibson, 2005). These findings suggest that long-term antioxidant nutritional status measures would be more sensitive markers for AD than the recent ones, although both indicators could be important indices for AD.

This study has several strengths. To our knowledge, this study is the first report on AD and antioxidant nutrition considering both intakes and corresponding biomarkers in the same subjects. None of the earlier studies have reported blood antioxidant nutrient concentrations in relatively large samples of preschoolers. This study considered separate nutrient intakes from diet and supplements. Moreover, the use of population controls and the exclusion of children whose diet was affected by disease status might minimize some of the biases associated with hospital-based case–control studies and reverse causation in the observational study like ours.

Several potential limitations of this study are as follows. There are several ways to diagnose AD using a combination of subjective and objective indices, and each method has its pros and cons. AD was defined based on subjective assessment, yet the possibility of different findings due to different classification of subjects cannot be completely

excluded. Unavailability of supplement intake for all vitamins and minerals may have underestimated true association between some nutrients and AD. Inter-individual differences in bioavailability and utilization of nutrients as well as differences between or synergistic benefits among nutrients may also be considered in the interpretation of the results.

In conclusion, the results of this study suggesting that higher antioxidant nutritional status depending on nutrient type reduces the risk of AD have important implications for nutrition intervention in AD among children.

Conflict of interest

The authors declare no conflict of interest.

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