

Dietary total antioxidant capacity and current asthma in Spanish schoolchildren: a case control–control study

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Abstract The aim of this work was to study the relationship between dietary total antioxidant capacity (TAC) and current asthma in a group of Spanish schoolchildren. A total of 78 Spanish schoolchildren (26 asthmatic and 52 healthy controls) were randomly selected from a cohort of 564 children (9–12 years of age). The weight and height of all subjects were recorded. A questionnaire, completed by the subjects' parents, was used to obtain personal and health information. Current asthma was established when children had ever had asthma, they had been diagnosed with asthma by a physician, and they had been treated with medications at some time in the previous 12 months. Food intake was monitored using a 3-day food record. All consumed foods were converted into energy and nutrients. Dietary TAC was evaluated using the ferric reducing antioxidant power (FRAP), radical-trapping antioxidant parameter (TRAP), and Trolox equivalent antioxidant capacity (TEAC) assays. The TAC measured using all the assays was significantly lower in children with asthma than in children without this condition (2.95 (2.10–3.75)

mmol Fe(II)/day vs. 3.70 (3.08–4.49)mmol Fe(II)/day, $p < 0.01$; 1.50 (1.06–2.05)mmol Trolox equivalents/day vs. 2.10 (1.40–2.65)mmol Trolox equivalents/day, $p < 0.05$; and 1.60 (1.08–2.00)mmol Trolox equivalents/day vs. 1.85 (1.50–2.68)mmol Trolox equivalents/day, $p < 0.05$ for FRAP, TEAC, and TRAP, respectively). After adjusting for energy intake, children with FRAP values higher than 3.5 mmol Fe(II)/day (p50) and TEAC values higher than 1.9 mmol Trolox equivalents/day (p50) had 22.6 and 35.0 %, respectively, lower likelihood of suffering asthma episodes than children with lower values. When logistic regression analysis was performed separately for children with nonsmoker and smoker (at least one) parents, the association between dietary TAC and asthma was only observed in the nonsmoker group (OR=0.257, 95 % CI=0.107–0.618, $p=0.002$ for FRAP; OR=0.212, 95 % CI=0.069–0.639, $p=0.006$ for TEAC; and OR=0.264, 95 % CI=0.091–0.769, $p=0.015$ for TRAP assay). **Conclusion:** Dietary TAC may have a favorable role in asthma in children and, specially, in those with nonsmoker parents.

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Abbreviations

TAC	Total antioxidant capacity
BMI	Body mass index
FRAP	Ferric reducing antioxidant power
TRAP	Radical-trapping antioxidant parameter
TEAC	Trolox equivalent antioxidant capacity
SD	Standard deviation
OR	Odds ratio
HR	Hazard ratio
RSD	Relative standard deviation

Introduction

Asthma is a chronic and heterogeneous disease in which airway inflammation, transient obstruction, and bronchial hyperresponsiveness are the major features of the illness [19]. Oxidative stress plays a key role in the pathogenesis of asthma since it may initiate and augment inflammation and may also result from inflammation. Thus, asthmatic patients have high levels of prostanoids, leukotrienes, oxygen reactive species, and nitrous oxide systems [20, 31]. Additionally, asthmatic patients suffer alterations in antioxidant enzyme systems such as superoxide dismutase, glutathione peroxidase, and glutathione reductase [21]. Accordingly, antioxidant intake may improve the antioxidant defenses of the organism and, therefore, decrease the incidence and development of asthma. Nevertheless, in Westernized countries, there has been a change from traditional to modern diet, which is characterized by a decline in omega-3 polyunsaturated fatty acids (PUFAs) and dietary antioxidant intake. It may explain the marked increase in asthma prevalence observed in the past years [1].

Therefore, different studies have investigated the link between asthma and the status of antioxidant vitamins and minerals, as well as the consumption of antioxidant-rich foods [14, 24]. An inverse association between asthma, antioxidants status [1, 2, 20], and the consumption of several antioxidant-rich foods has been reported [4, 35].

As foods are eaten in combination, the evaluation of dietary total antioxidant capacity (TAC) may be a suitable approach to measure their joint antioxidant effects [27]. Hence, dietary TAC sums up the free radical-scavenging ability of antioxidants in foods [6]. Diets with a high TAC have been related with inflammation reductions and an increase in circulating antioxidants in cross-sectional and randomized studies [6, 34]. Based on these observations, we explored the potential relation between asthma and TAC, a comprehensive index that takes into account several different antioxidants and their synergistic interactions.

Methods and materials

Sample recruitment

A total of 564 schoolchildren aged between 9 and 12 years from 14 schools in Madrid (Spain) were recruited to take part in the study. The head of each school was contacted by phone to arrange an interview wherein the characteristics and the importance of the study were explained. An interview with the parents of the children in the age group 9–12 years was then requested. Once permission was given, the details of the study were explained to the parents and all questions about the study

were answered. Signed permission was then sought to include their children in the study. All subjects took part voluntarily.

The exclusion criteria were the following:

- A lack of authorization or the nonacceptance of one or more of the conditions required to take part in the study
- Nonattendance on days when tests or interviews were performed
- Having a pathology that might modify the results, alter food habits (and therefore nutrient intake), or where participation was not recommended

This study was conducted according to the Declaration of Helsinki, and all procedures were approved by the Human Research Ethics Committee of the Faculty of Pharmacy, Complutense University of Madrid. A case-control study was chosen which included 26 asthmatic children as a study group. A control group of 52 nonasthmatic subjects was randomly selected. They were matched for age and gender with the study group.

Health survey

Parents completed a questionnaire designed to provide information about the children's health (past episodes of asthma, other diseases, and medication taken). They also gave information about their smoking habits and whether the children were breast-fed and for how long.

To establish the diagnosis of current asthma, parents were asked if their children had ever had asthma, if their children had been diagnosed with asthma by a physician, and if their children had had an episode of asthma or an asthma attack during the past 12 months. These questions have been used by Sistek et al. [32] to define current asthma in adults. In the present study, the final diagnosis of current asthma was established when a positive response was given to the first two questions [32] and, according to Miyamoto et al. [22], when children had been treated with medications at some time in the previous 12 months. Furthermore, the diagnostic approach was supervised and confirmed by a pulmonologist.

Anthropometric survey

All anthropometric measurements were completed in the morning following the norms set out by the World Health Organization [36] in the facilities assigned by the school for the study. Weight and height were determined using a digital electronic balance (Seca Alpha, GMBH & Co., Igny, France) (range 0.1–150 kg, precision 100 g) and a Harpenden stadiometer (Pfifter, Carlstadt, NJ, USA) (range 70–205 cm, precision 1 mm), respectively. For these measurements, the subjects were barefoot and wore underwear only. The subjects' body mass index (BMI) was calculated as weight (kg) divided by height² (m²).

Dietary survey

Participants recorded their dietary intake using a “3-day dietary record” from Sunday to Tuesday [26]. Parents were asked to weigh the foods ingested if possible or to use household measures (spoonfuls, cups, etc.) to evaluate the dietary intake. Trained personnel visited the school canteen on Monday and Tuesday to record the foods served in the menu, the amount of food served to each child, and the amount of food left on the plate.

Energy and nutrient intakes were calculated using food composition tables [10]. The values obtained were compared

to those recommended [11] to determine the adequacy of the diets. Special attention was paid to the consumption of antioxidant-rich foods. The nutritional analysis software DIAL was used to process all data [12]. Theoretical energy expenditure was established by taking into account the body weight, age, and physical activity of all children, using equations proposed by the Institute of Medicine [18].

To validate the results of the dietetic study, energy intake was compared to the theoretical energy expenditure. The percentage discrepancy between the energy intake and the sum of the measured and declared intakes was determined using the following formula:

$$(\text{Theoretical energy expenditure} - \text{energy intake}) \times 100 / \text{theoretical energy expenditure}$$

A negative value indicated that the declared energy intake was to be greater than the theoretical energy expenditure (probable overestimation), while a positive value indicated it to be lower than the theoretical energy expenditure (probable underestimation) [25].

Different specific assays exist to quantify TAC in foods. The ferric reducing antioxidant power (FRAP) evaluates the reducing power of the sample [5], the radical-trapping antioxidant parameter (TRAP) measures the chain-breaking antioxidant potential [15], and the Trolox equivalent antioxidant capacity (TEAC) assay measures the antioxidants' ability to reduce a radical cation (ABTS⁺) in both lipophilic and hydrophilic condition [30]. In the present study, the individual TAC of foods was added to the Spanish food composition tables [10]. These values were gathered from published databases that provided the antioxidant capacity measured in foods by three different assays: FRAP [7, 16], TRAP, and TEAC [27, 28].

The corresponding value of TAC was assigned per 100 g of the edible portion of foods. If several TAC values for the same food were found, the average value was calculated. If the TAC value for a determined food was not found, a TAC value for a similar food was assigned (according to their qualitative characteristics). If the TAC value for cooked foods were not available, the TAC value of the raw food was used to estimate its value. Finally, the TAC database consisted of 483 foods measured by FRAP (mmol Fe(II)/100 g), 140 by TRAP (mmol Trolox equivalents/100 g), and 158 by TEAC (mmol Trolox equivalents/100 g).

According to the literature, interlaboratory comparison and standardization are needed to improve the reproducibility of the methods and to make results more comparable and reliable. At the moment, absolute results in publications have to be used carefully as they are not comparable from one laboratory to the other because each antioxidant assay has a different mechanism, redox potential, reaction media, etc.

For this reason, it is strongly recommended to use at least two different methods (preferably with different mechanisms) within each assay [3]. Thus, the FRAP values used in the present research have been obtained by the same authors [7, 16] in a fully validated assay. The within-day repeatability measured in this assay as relative standard deviation (RSD) in standard solutions ranged from 0.4 to 6 %. The between-day repeatability was <3 %. The variation in the values for replicate food items obtained from the same source was between 3 and 10 % RSD. Similarly, the TEAC and TRAP, but not FRAP, values used in the present work have been obtained by other authors [27, 28], with a variation in the values for replicates between 3 and 10 % RSD.

Statistical analysis

All variables were presented as absolute frequencies, median, and minimum and maximum values (p50 (min–max)). Raw and adjusted values for energy intake dietetic are presented. Student's *t* test and Mann–Whitney test were used to identify significant differences between children with and without current asthma. Dichotomous variables were explored with chi-square tests with Yates' correction for continuity. Fisher's exact test was used when the variables were dichotomous and the numbers were too small to allow usage of the chi-square test. Multiple logistic regression was used to explore the relation between the prevalence of current asthma and dietary TAC and food groups related to them. In order to study this relation, qualitative variables of cereal and fruit consumption and FRAP, TEAC, and TRAP values were created having in mind the p50 (median) value of each variable. All calculations were made using SPSS 19.0 Software. The level of significance was set at $p < 0.05$.

Results

No significant differences were seen between participants with and without asthma in terms of age, anthropometric characteristics, gender, having been breast-fed, parental smoking habits or first-degree family member background of asthma, atopic eczema, and allergic rhinitis (Table 1).

Energy intake, discrepancies between energy intake and energy expenditure, and consumption of antioxidant-rich foods were similar between asthmatics and nonasthmatics. However, the TAC measured using the FRAP, TEAC, and TRAP assays were significantly lower in children with asthma than in children without this condition (Table 2).

The FRAP method was significantly correlated with cereal and fruit consumption, and TEAC and TRAP methods were significantly correlated only with fruit consumption (after adjusting for energy intake and age). The values obtained were $\beta=0.285\pm0.003$ and $\beta=0.454\pm0.001$ ($R^2=0.331$ and $p=0.001$), $\beta=0.110\pm0.003$ and $\beta=0.336\pm0.001$ ($R^2=0.236$ and $p=0.023$), and $\beta=0.112\pm0.002$ and $\beta=0.357\pm0.001$ ($R^2=0.265$ and $p=0.009$) for cereals and fruits in the FRAP, TEAC, and TRAP methods, respectively.

After adjusting for energy intake, children with FRAP values higher than 3.5 mmol Fe(II)/day (p50) and TEAC values higher than 1.9 mmol Trolox equivalents/day (p50) had 22.6 and 35.0 %, respectively, lower likelihood of suffering asthma episodes than children with lower values. TRAP was not associated with the risk of asthma (Table 3).

When logistic regression analysis was performed separately for children with nonsmoker and smoker (at least one) parents, the association between dietary TAC and asthma was only observed in the nonsmoker group (OR=0.257, 95 % CI=0.107–0.618, $p=0.002$ for FRAP; OR=0.212,

95 % CI=0.069–0.639, $p=0.006$ for TEAC assay; and OR=0.264, 95 % CI=0.091–0.769, $p=0.015$ for TRAP assay).

Discussion

The beneficial role of certain foods, including antioxidant nutrients, in asthma has been reported in several studies. However, the present study is the first to investigate the relation between dietary TAC and this pathology.

A number of studies have explored the possible links between antioxidant vitamin and mineral intake and asthma. In a systematic review and meta-analysis of 40 studies, Allen et al. [2] showed that dietary vitamin A intake was significantly lower in people with asthma compared with those without asthma (182 $\mu\text{g/day}$), and in people with severe asthma compared with those with mild asthma. Lower quartile dietary intakes (OR=1.12, 95 % CI=1.04–1.21) and serum levels of vitamin C were also associated with an increased likelihood of suffering asthma. Additionally, vitamin E intake was generally unrelated to asthma status but was significantly lower in severe asthma compared with individuals with mild asthma (1.20 $\mu\text{g/day}$). Similarly, in a more recent review and meta-analysis studying the potential role of diet in the primary prevention of asthma and atopic disease in children aged ≤ 16 years, Nurmatov et al. [24] showed that only serum vitamin A was lower in children with asthma compared with the control group (OR=0.25, 95 % CI=0.10–0.40) with no differences found in other vitamins or minerals.

Generally, the studies that have investigated the effects of antioxidants on health and disease have analyzed the nutrient antioxidant capacity independently of others contained in the same food. However, it is important to consider that all the antioxidant compounds consumed might act synergistically against oxidative stress [33]. Therefore, the evaluation of food TAC is recommended. TAC has been criticized because it is an in vitro parameter, which does not consider all the bioavailability issues related with most of the antioxidant compounds taken in with a varied diet. However, TAC is a very clear and a simple method to describe the total antioxidant capacity of all the different antioxidant molecules contained within foods [9].

Some researchers have demonstrated the beneficial effect of high-TAC diets in human health. A study by Del Rio et al. [9] showed that high-TAC diets were associated with a tendency in the reduction of hazard ratio (HR) for all types of stroke or myocardial infarction ($p=0.054$) in a sample of 41, 620 participants (male and female) never diagnosed with stroke or myocardial infarction. The study also revealed 194 cases of stroke (112 ischemic strokes, 48 hemorrhagic strokes, and 34 of other types) during the follow-up period. When only ischemic strokes were considered, the data showed that dietary TAC was inversely associated with stroke, with a HR=0.41 (95 % CI=0.23–0.74).

Table 1 Personal characteristics of the study subjects with and without asthma

	With asthma	Without asthma
Number	26	52
Age (years) ^a	10.56 (9.43–12.08)	10.57 (9.41–12.08)
Weight (kg) ^a	40.90 (20.40–69.00)	36.85 (27.30–78.30)
Height (cm) ^a	144.1 (125.0–156.0)	143.0 (131.0–163.0)
BMI (kg/m ²) ^a	18.6 (13.10–34.40)	18.1 (14.10–31.10)
Gender		
Boys (<i>n</i>)	16	32
Existence of breastfeeding (<i>n</i>)	19	39
Parents' smoking habit (<i>n</i>)	3	12
Family background (<i>n</i>)	3	2

^aData are given as median and minimum and maximum values (p50 (min–max))

Table 2 Dietetic characteristics of the study subjects with and without asthma

	With asthma	Without asthma
Energy intake (kcal/day)	2,218 (1,567–2,959)	2,179 (1,584–3,223)
Discrepancy intake/expenditure (%)	2.60 (–61.27–35.99)	2.14 (–60.94–49.07)
Cereals (g/day)		
Unadjusted	165.5 (93.8–299)	182.5 (91.7–298.0)
Adjusted by energy	181.0 (105.0–281.2)	179.3 (98.6–266.8)
Pulses (g/day)		
Unadjusted	3.15 (0.0–57.3)	11.1 (0.0–86.8)
Adjusted by energy	5.04 (–3.94–54.88)	12.2 (–2.93–88.35)
Vegetables (g/day)		
Unadjusted	192.0 (50.9–346.0)	169.5 (36.1–462.0)
Adjusted by energy	190.4 (73.1–344.7)	169.1 (17.21–463.6)
Fruits (g/day)		
Unadjusted	225.0 (17.7–533.0)	269.5 (23.3–645.0)
Adjusted by energy	236.0 (–20.54–507.3)	255.7 (9.52–636.8)
Fish (g/day)		
Unadjusted	40.6 (0.0–149.0)	36.95 (0.0–167.0)
Adjusted by energy	39.18 (–5.64–152.58)	40.6 (–19.58–166.84)
Dairy (g/day)		
Unadjusted	514.0 (238.0–867.0)	451.5 (204.0–1047)
Adjusted by energy	493.6 (274.9–832.3)	436.9 (262.5–963.9)
FRAP (mmol Fe(II)/day)		
Unadjusted	2.75 (1.80–6.60)*	3.85 (1.50–7.0)*
Adjusted by energy	2.95 (1.38–5.20)**	3.70 (1.54–6.56)**
TEAC (mmol Trolox equivalents/day)		
Unadjusted	1.50 (0.77–3.40)*	2.10 (0.34–5.70)*
Adjusted by energy	1.53 (0.73–3.70)*	2.06 (0.44–5.50)*
TRAP (mmol Trolox equivalents/day)		
Unadjusted	1.60 (0.84–3.20)*	1.85 (0.28–4.20)*
Adjusted by energy	1.60 (0.72–2.88)*	1.95 (0.36–4.10)*

Data are given as median and minimum and maximum values (p50 (min–max))

* $p < 0.05$; ** $p < 0.01$

A similar study with 551 men and 467 women showed inverse correlations between FRAP and the concentrations of several diabetes markers (age- and energy-adjusted): log-glucose ($r = -0.149$, $p = 0.001$), log-insulin ($r = -0.221$,

$p = 0.001$), and log-HOMA-IR ($r = -0.186$, $p = 0.001$) [29]. The protective role of dietary TAC on oxidative stress in the lung was assessed in a sample of healthy Italian individuals (5,824 women and 5,848 men), revealing a favorable role of

Table 3 Results from logistic regression analysis that evaluates the association between total dietary TAC and antioxidant-rich foods with the risk of having asthma

	With asthma (n)	Without asthma (n)	OR1 (95 % CI), <i>p</i> value	OR2 (95 % CI), <i>p</i> value
Cereals <p50 (178 g/day)	15	24	0.629 (0.243–1.625), 0.338	0.609 (0.205–1.809), 0.372
≥p50 (178 g/day)	11	28		
Fruits <p50 (263 g/day)	14	25	0.794 (0.309–2.039), 0.631	0.821 (0.307–2.195), 0.694
≥p50 (263 g/day)	12	27		
FRAP <p50 (3.5 mmol Fe(II)/day)	19	22	0.270 (0.097–0.754), 0.012	0.226 (0.073–0.705), 0.010
≥p50 (3.5 mmol Fe(II)/day)	7	30		
TEAC <p50 (1.9 mmol Trolox equivalents/day)	17	21	0.359 (0.135–0.955), 0.040	0.350 (0.127–0.967), 0.043
≥p50 (1.9 mmol Trolox equivalents/day)	9	31		
TRAP <p50 (1.8 mmol Trolox equivalents/day)	19	28	0.430 (0.154–1.197), 0.106	0.431 (0.151–1.225), 0.114
≥p50 (1.8 mmol Trolox equivalents/day)	7	24		

OR1 raw values of antioxidant capacity markers, OR2 antioxidant capacity markers adjusted for energy intake

dietary TAC, measured by the FRAP method, on respiratory health in premenopausal/never-smoker women [13]. Similarly, the present study showed that children with current asthma had diets with low TAC (measured by the all the methods) compared with children without asthma (Table 2). Furthermore, asthma was less likely in children with higher TAC (measured by FRAP and TEAC methods) than in those with lower TAC (Table 3).

Interestingly, the current study showed an association between dietary TAC and asthma only in children with non-smoking parents. A similar protective effect has been observed only in nonsmokers when compared with the smoker counterparts [13]. These results support the deleterious pro-oxidant effect of smoking, which is more potent than the total antioxidant network on lung function [13, 23].

Finally, although in several studies a link between fruit and vegetable consumption and asthma has been showed [8, 24, 35], in the present study, such effect was not found. The reason for this lack of results could be that although fruits and vegetables are foods rich in antioxidants [17], they are not consumed in sufficient quantities by the studied students and, therefore, do not provide sufficient amount of antioxidants to avoid the oxidative stress that occurs in asthma. In fact, none of the students took the minimum five servings of fruits and vegetables recommended. In addition, these foods did not account in any case for more than 30 % of dietary TAC measured by FRAP (20.5 %), TEAC (12.8 %), and TRAP (14.9 %), respectively. Thus, it appears that dietary TAC is a good index to estimate the antioxidant capacity of the diet, and in addition to including enough foods with high TAC, such as fruits and vegetables, it would be convenient choosing high-TAC food in every single food class in order to increase daily dietary TAC [9]. Specifically, having in mind the significant correlation found between fruit consumption and TEAC and TRAP methods, it would be recommended to increase the consumption of this food group. Particularly, it would be desirable to increase the consumption of those fruits with a higher content of antioxidants, which are berries and citrus fruits according with the literature [7, 16, 27, 28].

Some limitations were found within the study, for example, the cross-sectional nature of the study did not enable the determination of causality. Therefore, a prospective cohort study will provide a better description of the effects of dietary TAC on asthma. Secondly, the small size of the study limits the generalizability of the results, and studies with larger samples should be performed. Finally, TAC is an *in vitro* analysis; therefore, some of the antioxidants contributing to the total antioxidant capacity of the nutrient may be poorly absorbed in *in vitro* methods. In conclusion, our results show that dietary TAC may have a favorable role in asthma in children and, specially, in those with nonsmoker parents. Thus, future studies should evaluate the influence of the diet

TAC as a whole rather than analyze the individual antioxidant effects of single nutrients.

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