Serum Antioxidant Concentrations and Metabolic Syndrome Are Associated among U.S. Adolescents in Recent National Surveys

May A. Beydoun, J. Atilio Canas, Hind A. Beydoun, Xiaoli Chen, Monal R. Shroff, and Alan B. Zonderman

Abstract

Specific micronutrients, including retinol, retinyl esters, carotenoids [α-carotene, β-carotene (cis-trans), β-cryptoxanthin, lutein+zeaxanthin, and total lycopene], vitamin E, and vitamin C have antiinflammatory and antioxidant effects, properties shown to reduce oxidative stress, a process that accompanies the pathogenesis of many chronic diseases. It is still largely unknown whether they are associated with the occurrence of metabolic syndrome (MetS) in the adolescent U.S. population. MetS was defined by the International Diabetes Federation (IDF) criteria. Other non-MetS outcomes relying on blood measurements were elevated HOMA-IR, C-reactive protein (CRP), and hyperuricemia. We tested associations between serum antioxidants and MetS outcomes among adolescents aged 12–19 y using cross-sectional data from NHANES 2001–2006 (n = 782–4285). IDF MetS prevalence was estimated at 7% among boys and 3% among girls. In adjusted models, adolescents with MetS had consistently lower carotenoid concentrations compared with their counterparts without MetS. Total carotenoids were also inversely related to HOMA-IR and CRP. Vitamin C was inversely related to uric acid level and MetS binary outcome. Retinol+retinyl esters exhibited an inverse relationship with CRP and a positive relationship with uric acid and HOMA-IR as well as MetS binary outcome. Vitamin E had no association with MetS, particularly after controlling for serum cholesterol and TG. In conclusion, among U.S. adolescents, serum carotenoid concentrations were inversely associated with MetS status, HOMA-IR, and CRP, whereas serum vitamin C was inversely related to MetS status and serum uric acid. Vitamin E had no consistent association with MetS, whereas retinol+retinyl esters had a positive relationship with HOMA-IR, uric acid, and MetS, while being inversely related to CRP. These associations need further study.

Introduction

Increased risks of type 2 diabetes, cardiovascular disease (CVD)\(^9\), and all-cause mortality among adults (1–6) and obese children (7,8) is associated with metabolic syndrome (MetS), a clustering of several conditions including abdominal obesity, elevated blood pressure (BP), hyperglycemia, and 2 types of dyslipidemia, high fasting TG and low HDL-cholesterol (HDL-C) concentrations, based on the National Cholesterol Education Program Adult Treatment Panel III criteria (9,10). In fact, the rising prevalence of obesity and MetS among adults (1–5) in the US still constitutes a major public health threat, leading to higher levels of mortality and disability as well as higher health care costs (6–14). In children and adolescents, obesity has also reached epidemic proportions in the US, with an overall prevalence of 16% based on recent surveys (11) and a more pronounced shift in measures of central adiposity observed in minority groups such as non-Hispanic (NH) black girls (12). In addition, a number of long-term cohort studies have shown that MetS in children and adolescents predicted cardiovascular outcomes in adults (13–15).

* To whom correspondence should be addressed. E-mail: baydounm@mail.nih.gov.
of MetS was recently estimated at ~30% in the US (17), whereas a recent study using NHANES 2001–2006 data collected on adolescents estimated MetS prevalence at 6.9% among boys and 3.9% among girls based on National Cholesterol Education Program Adult Treatment Panel III criteria (9,10,18). For both the youth and adults, MetS is often comorbid with inflammatory markers, including a higher serum C-reactive protein (CRP) (19,20), a high HOMA-IR (21,22), hyperuricemia (23), and increased hemostasis (24).

Specific micronutrients have anti-inflammatory and antioxidant effects by reducing oxidative stress, a process that accompanies the pathogenesis of many chronic diseases, including type 2 diabetes, CVD, rheumatological conditions, and carcinogenesis (25). The serum concentrations of these antioxidants reflected their dietary intakes in many studies (26–28). Among other antioxidants, epidemiological studies and randomized controlled trials (RCT) have recently focused on carotenoids, particularly in adult populations. Carotenoids’ primary dietary sources are fruits and vegetables (FV), though they can also be obtained from bread, eggs, beverages, fats, and oils (29). In the human diet, there over 40 carotenoids, but only a handful are ubiquitous in human serum, namely β-carotene, α-carotene, β-cryptoxanthin, lycopene, lutein, and zeaxanthin (29).

Inverse associations were detected in observational studies among adults between carotenoids and CVD (30) as well as type 2 diabetes (31–35), though the results were inconsistent in other studies (30,36–39). Importantly, none of those studies included children or adolescents. Two studies used the NHANES III and, more recently, the 2001–2006 data for adults to describe the relationship between antioxidant status and MetS (17,40), while 5 studies overall looked at serum or dietary carotenoids in particular as related to MetS (17,40–43). Although it is now becoming clearer that antioxidants are important for the prevention of MetS and its complications, more research is needed to differentiate the effects of major serum antioxidants on MetS, particularly carotenoids, and its components. Importantly, no nationally representative study to our knowledge has examined this research question among adolescents. Among adults, only one study (17) controlled for serum 25-hydroxyvitamin D [25(OH)D], which was shown to reduce the risk of MetS and its components (44–57). Recent nationally representative data (NHANES 2001–2006) from U.S. adolescents aged 12–19 y were used in our present study to test associations between serum antioxidant concentrations and MetS. We additionally tested the predictive value of socio-demographic and dietary factors in relation to serum antioxidant status.

Materials and Methods

Database and study population

The NHANES are a series of cross-sectional surveys of nationally representative samples conducted on a continuous basis since 1999. NHANES have a stratified, multistage probability cluster sampling design and cover the U.S. noninstitutionalized population. They include in-home interviews that are followed by health examinations in a mobile examination center (MEC). The MEC examination consists of anthropometric, BP, and laboratory measurements among others (58). The procedures complied with ethical standards and approval was obtained from the relevant committee on human subject welfare (59).

NHANES data from adolescents aged 12–19 y from 2001–2006 were analyzed. Of 7078 adolescents with complete basic demographic data (sample 1), 4769 had complete plasma antioxidant status (retinol, retinyl esters, total carotenoids, and vitamin E) and dietary data (total energy intake). Among those, other dietary and supplement consumption data were complete for 4285 participants (sample 2). In sample 2, those with complete data on MetS components, including the IDF (16) and other components (sample 3, a-h) ranged between 2046 and 4285, whereas those with complete data on all IDF components combined (sample 4) had a sample size of 1339. In sample 4, only 782 had complete data on serum vitamin C levels (2003–2004 and 2005–2006 waves). The present study was approved for ethical treatment of participants by the Institutional Review Board of the National Institute on Aging, Intramural Research Program.

Outcome assessment

Anthropometric measures. Among anthropometric measures in the MEC, waist circumference (WC) was measured for all adolescents (60). While not holding his/her breath, a participant’s WC was assessed with a tape measure wrapped around the waist, kept parallel to the floor, and starting from the hip bone in a medium loose fashion.

BP. The mean of 3 BP (systolic and diastolic) measurements taken during MECS examinations was computed. BP was measured with a mercury sphygmomanometer (61).

Laboratory tests. Protocols for the collection and processing of specimens in the NHANES are reported elsewhere (62). Briefly, fasting blood glucose was measured using a reaction between glucose and ATP catalyzed by hexokinase; serum or plasma TG was enzymatically measured via coupled reactions where TG was hydrolyzed to produce glycerol; HDL-C was determined using 2 distinct methods, a heparin-manganese (Mn) precipitation method or a direct immunoassay technique (in case of limited specimen sample). Coupled reactions using cholesterol ester hydrolase, cholesterol oxidase, and peroxidase were used to measure total cholesterol (Roche Hitachi Models 717 and 912). Fasting serum insulin was assessed using an RIA reagent (Pharmacia Diagnostics), the uric acid level was assessed by oxidation with uricase to form allantoin and H₂O₂ (Hitachi Model 737 Multichannel Analyzer, Boehringer Mannheim Diagnostics), and high-sensitivity CRP was quantified by latex-enhanced nephelometry (Behring Diagnostics).

Outcome variables: classification of MetS and its components.

Based on the IDF criteria for children and adolescents (16), MetS was positive (MetS⁺) when ≥3 of the following conditions simultaneously occurred, including criterion 1 (16): 1) abdominal obesity defined by WC cutoffs for the US that are specific to each age, sex, and race/ethnicity group as detailed in the manual (16); 2) dyslipidemia: TG ≥1.70 mmol/L (150 mg/dL) (16); 3) dyslipidemia: HDL-C <40 mg/dL (<1.04 mmol/L) (16); 4) BP ≥130/85 mm Hg (16); and 5) fasting plasma glucose ≥100 mg/dL (≥5.55 mmol/L) (16). The number of metabolic disturbances was also examined in its continuous form (MetS count score ranging between 0 and 5). Additionally, we examined: 6) elevated HOMA-IR (>2.61) reflecting insulin resistance (63,64); 7) hyperuricemia (>7 mg/dL or >416.36 μmol/L in boys and >6 mg/dL or >356.85 μmol/L in girls) (65); and 8) elevated CRP defined as ≥2.11 mg/L (66).

Exposure assessment: serum concentrations of key antioxidants

Serum concentrations of selected antioxidants were determined using HPLC with photodiode array detection. For the present study, we were interested in retinol and retinyl esters (sum of retinol palmitate and retinyl stearate). Carotenoids were organized as α-carotene, β-carotene (cis+trans), β-cryptoxanthin, lutein+zeaxanthin, lycopene, or as total carotenoids. Vitamin E was defined as the sum of α- and γ-tocopherol. Although carotenoids, retinol-retinyl esters, and vitamin E were available for all 3 NHANES waves (2001–2002, 2003–2004, and 2005–2006), vitamin C was assessed only for 2003–2004 and 2005–2006. Accordingly, a second set of analyses were performed that included vitamin C as an exposure variable.

Covariates

Socio-demographic characteristics. Socio-demographic covariates included age, sex, race/ethnicity (NH whites, NH blacks, Mexican Americans, and other ethnicities) and family income [poverty income ratio (PIR)]; <100, 100–200, and ≥200%].
**Dietary and supplement intakes.** Study participants were eligible for one 24-h recall in the 2001–2002 wave and two 24-h recalls in the 2003–2004 and 2005–2006 waves of NHANES. The first 24-h recall was administered during MEC exams and the second 3–10 d later via telephone interview. The mean of the two 24-h recalls was determined from which nutrient intakes were estimated using a revised nutrient database (67). Total dietary intake of energy (1 kcal = 4.187 kJ), fiber, alcohol, caffeine, and selected antioxidants (β-carotene, vitamin C, and vitamin E) were considered as potential confounders (continuous variables) because of their putative associations with MetS (36,37,43,68–70). Dietary supplement use in the past 30 d was summarized as 0, nonusers; 1, using one supplement; or 2, using 2 supplements or more.

**Other nutritional biomarkers.** The Bio-Rad Laboratories Quanta-phase II Folate radiolysis kit was used to measure serum folate and vitamin B-12 (62); [1 μg/ L of folate = 2.266 nmol/L; 1 pg/mL of vitamin B-12 = 0.738 pmol/L). Serum 25(OH)D was measured by RIA (Diasorin) [1 μg/L of 25(OH)D = 2.496 nmol/L] (62). These nutritional biomarkers were considered as potential confounders and entered into models in their continuous forms.

**Statistical methods**

Using Stata 11.0 (71), we initially characterized our study sample by sex and MetS status. Differences in means across groups and associations between categorical variables were tested with t- and design-based F-tests, respectively. Subsequently, multiple linear ordinary least square (OLS) regression models were constructed in which potential socio-demographic, lifestyle, and dietary predictors of serum antioxidants (continuous retinol+retinyl esters, total carotenoids, and log-transformed vitamins E and C) were evaluated with the Wald test. Multiple logistic regression models tested associations between antioxidant status (standardized Z-scores obtained through analysis of the NHANES sample aged 12–19 y with known antioxidant levels) and MetS (and its components) binary outcomes, adjusting for confounders and stratifying by sex. In addition to socio-demographic and dietary factors, serum cholesterol and TG were entered into models given their moderately high correlations with many of the antioxidants of interest, particularly vitamin E. These associations were similarly tested with MetS count (range: 0–5) using multiple zero-inflated Poisson (ZIP) regression models. Significance of regression coefficients was assessed using the Wald test. Finally, to examine dose-response relationships, quartiles of main antioxidants were entered into ZIP regression models (when outcome is MetS count) and in OLS regression models for selected non-MetS continuous outcomes (i.e., HOMA-IR, uric acid, and CRP, log-transformed) as ordinal variables and P-trend were computed from the Wald test. In all analyses, we took sampling design complexity into account using Stata survey commands. Two-year fasting, half-sample, MEC exam weights were used for most sample estimations (with the exception of high BP and abdominal obesity, where full MEC exam weights were used) and masked variance units allowed estimating variances using the Taylor series linearization method. Two-sided P values were presented at an α level of 0.05.

**Results**

The prevalence of MetS using IDF criteria among adolescents was estimated at 7% among boys and 3% among girls, with sex differences (P < 0.001, design-based F-test). Sex differences were detected, where boys had higher retinol and retinol+retinyl ester concentrations than girls while having lower β-carotene, vitamin E, and vitamin C concentrations. Boys also had higher total energy, alcohol, caffeine, fiber, vitamin C, and vitamin E intakes and higher 25(OH)D status than girls (Table 1).

Participants with MetS (MetS+) were significantly older, had higher serum retinol+retinyl ester (mainly retinol) and vitamin E concentrations but lower serum total carotenoid and vitamin C concentrations than those without MetS (MetS−) (P < 0.05). Among carotenoids, lower levels in the MetS+ group were noted for β-carotene, β-cryptoxanthin, and lutein+zeaxanthin. Additionally, greater intakes of alcohol and caffeine were found in the MetS+ group, and for other nutritional biomarkers, 25(OH)D and folate status were significantly lower in that group. Boys had worse metabolic profiles than girls on some of the continuous measures of disturbance (e.g., HDL-C, systolic BP, fasting plasma glucose, and uric acid) and on the mean number of IDF conditions (P < 0.05), whereas girls had a worse profile on other measures (e.g., total cholesterol and diastolic BP) and no difference was detected in the remaining measures (e.g., WC, TG, HOMA-IR, and CRP). In both sexes combined, MetS status clearly discriminated between poorer and better metabolic profile on all measures, with the exception of CRP (Supplemental Table 1).

Table 2 indicates that antioxidant status was affected by a number of demographic and dietary factors, though inconsistently across antioxidants. For instance, being older was associated with higher antioxidant status in the case of retinol+retinyl ester and vitamin E but was associated with a lower vitamin C concentration. Compared with boys, girls had higher serum total carotenoids and vitamin C but lower serum retinol+retinyl ester and vitamin E concentrations. Similarly, compared with NH whites, NH blacks had higher serum concentrations of total carotenoids and vitamin C but lower retinol+retinyl ester and vitamin E, whereas Mexican Americans had higher serum carotenoids and lower retinol+retinyl ester concentrations. Independent of other demographic factors, only vitamin C was significantly higher in the uppermost PIR group ≥200% compared with PIR <100%. Supplement use was consistently and positively related to antioxidant status. In addition, dietary intake of β-carotene was specifically related to higher total carotenoid status, whereas vitamin C intake was linked to higher status in all 4 antioxidant groups and vitamin E intake was associated with higher vitamin C status but lower carotenoid status. Alcohol and caffeine intakes were linked to lower carotenoid status, whereas the reverse was true for fiber intake. In addition, caffeine intake was also associated with lower vitamin E and vitamin C concentrations, whereas higher total caloric intake was inversely related to vitamin C status.

Findings from adjusted models where the main outcome of interest is MetS (binary and count) and main predictors are selected serum antioxidant levels are presented in Table 3. Controlling for socio-demographic and dietary factors (model 1), the odds of MetS more than doubled for each SD of vitamin E [OR = 2.37 (95% CI: 1.62, 3.49)] and each SD of retinol [(OR = 2.08 (95% CI: 1.44, 3.01)] and increased by 78% in the case of retinyl esters [OR = 1.78 (95% CI: 1.11, 2.87)]. In contrast, the β-cryptoxanthin level was strongly and inversely related to MetS [per SD, OR = 0.08 (95% CI: 0.02, 0.31)]. In model 1 with vitamin C included among predictors (2003–2006), an inverse relationship was also found per 1 SD of vitamin C [OR = 0.26 (95% CI: 0.11, 0.62)]. These significant associations were consistently found with MetS count outcome, with the exception of vitamin C and retinyl esters. In addition, serum concentrations of β-carotene and lutein+zeaxanthin were inversely related to the MetS count outcome, particularly in models without vitamin C included.

After inclusion of serum cholesterol and TG into the model (model 2), the positive relationships between MetS binary outcome and some of the antioxidants (vitamin E and retinyl esters) became nonsignificant. However, β-cryptoxanthin and vitamin C concentrations remained inversely related to MetS, whereas retinol was associated with an increased risk of MetS by ~56% in the model without vitamin C [OR = 1.56 (95% CI: 1.06, 2.29)]. In models with count outcome, only serum lutein+zeaxanthin had a significant and inverse relationship with MetS.
In Model 3, there was a clear inverse association between total carotenoids and both binary and count MetS outcomes with and without vitamin C. In contrast, total retinol-retinyl esters and vitamin E were positively related to MetS outcomes, whereas vitamin C was inversely related to only the MetS binary outcome.

In Model 4, when serum cholesterol and TG were entered, the association between total carotenoids and MetS remained significant for most of the models except for MetS binary outcome (2001–2006). Vitamin C’s inverse relationship with binary MetS outcome remained significant, whereas only retinol +retinyl ester’s positive association with binary MetS (2001–2006) remained significant.

We also examined the associations between serum antioxidant status and MetS components adjusting for potentially confounding factors, including serum cholesterol and TG (except for hypertriglyceridemia as outcome where only serum cholesterol was included) (Table 4). Inverse associations between vitamin E and MetS components were noted for abdominal obesity, hyperglycemia, elevated HOMA-IR, and hyperuricemia, although vitamin E was positively associated with hypertriglyceridemia. As for retinol-retinyl esters, their combined serum level was inversely related to low HDL-C but positively associated with hypertriglyceridemia, elevated HOMA-IR, and hyperuricemia. In contrast, carotenoids were consistently inversely related to several

<table>
<thead>
<tr>
<th>Retinol-retinyl esters</th>
<th>Total carotenoids</th>
<th>Log₆ (vitamin E)</th>
<th>Log₈ (vitamin C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 4285</td>
<td>n = 4285</td>
<td>n = 4285</td>
<td>n = 2675</td>
</tr>
<tr>
<td>Age, y</td>
<td>+0.047 ± 0.004*</td>
<td>+0.003 ± 0.004</td>
<td>+0.158 ± 0.002*</td>
</tr>
<tr>
<td>Sex, girls vs. boys</td>
<td>−0.156 ± 0.019*</td>
<td>+0.047 ± 0.017*</td>
<td>−0.037 ± 0.009*</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH white</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>NH black</td>
<td>−0.277 ± 0.022*</td>
<td>+0.095 ± 0.020*</td>
<td>−0.054 ± 0.012*</td>
</tr>
<tr>
<td>Mexican American</td>
<td>−0.154 ± 0.027*</td>
<td>+0.050 ± 0.017*</td>
<td>+0.004 ± 0.010</td>
</tr>
<tr>
<td>Other ethnicity</td>
<td>−0.119 ± 0.031*</td>
<td>+0.041 ± 0.031</td>
<td>−0.004 ± 0.019</td>
</tr>
<tr>
<td>PIR</td>
<td></td>
<td></td>
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<tr>
<td>&lt;100%</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>100 to &lt;200%</td>
<td>+0.036 ± 0.024</td>
<td>+0.032 ± 0.026</td>
<td>+0.007 ± 0.015</td>
</tr>
<tr>
<td>≥200%</td>
<td>+0.023 ± 0.018</td>
<td>+0.010 ± 0.020</td>
<td>+0.011 ± 0.015</td>
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<td>Dietary intake</td>
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<tr>
<td>Total energy, per 100 kcal/d</td>
<td>−0.000 ± 0.000</td>
<td>−0.002 ± 0.001</td>
<td>−0.001 ± 0.001</td>
</tr>
<tr>
<td>Alcohol, per 10 g/d</td>
<td>+0.015 ± 0.009</td>
<td>−0.013 ± 0.004*</td>
<td>−0.003 ± 0.005</td>
</tr>
<tr>
<td>Caffeine, per 10 mg/d</td>
<td>−0.001 ± 0.001</td>
<td>−0.004 ± 0.001*</td>
<td>−0.002 ± 0.000*</td>
</tr>
<tr>
<td>Fiber, g/d</td>
<td>+0.002 ± 0.001</td>
<td>+0.009 ± 0.003*</td>
<td>+0.000 ± 0.001</td>
</tr>
<tr>
<td>β-Carotene, per 1000 µg/d</td>
<td>+0.000 ± 0.000</td>
<td>+0.024 ± 0.004*</td>
<td>−0.000 ± 0.000</td>
</tr>
<tr>
<td>Vitamin C, per 10 mg/d</td>
<td>+0.002 ± 0.001*</td>
<td>+0.004 ± 0.001*</td>
<td>+0.001 ± 0.001*</td>
</tr>
<tr>
<td>Vitamin E, mg/d</td>
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<td>−0.004 ± 0.002*</td>
<td>+0.002 ± 0.002</td>
</tr>
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<td>Dietary supplement use</td>
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</tr>
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<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>One</td>
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<td>+0.029 ± 0.021</td>
<td>+0.082 ± 0.014*</td>
</tr>
<tr>
<td>≥2</td>
<td>+0.090 ± 0.037*</td>
<td>+0.133 ± 0.035*</td>
<td>+0.186 ± 0.018*</td>
</tr>
</tbody>
</table>

1 Values are $\beta \pm$ SEE. Sampling design complexity is taken into account in all analyses. All models were multivariate-adjusted for all variables included in this table. This analysis was performed on the sample with complete antioxidant status, demographic, lifestyle factors, and dietary data. *P < 0.05 for null hypothesis that $\beta = 0$ based on Wald test. NH, non-Hispanic; PIR, poverty income ratio; OLS, ordinary least square.

2 Vitamin C was available for the only 2003–2004 and 2005–2006 NHANES waves and thus sample size in this analysis was restricted to n = 2675.

MetS components, including abdominal obesity, hypertriglyceridemia, low HDL-C, and elevated HOMA-IR and CRP. Finally, in models with vitamin C, this antioxidant was significantly and inversely related to hyperuricemia, though it was not associated with any of the other components of interest.

We additionally examined the dose-response relationship between antioxidant status and several outcomes by expressing antioxidant groups (including vitamin C) as quartiles. Models were similar to model 4 in Table 3, with outcomes being MetS count, log₆-transformed HOMA-IR, CRP, and uric acid. Among the 4 antioxidant groups, total carotenoid quartiles had a dose-response inverse association with MetS (count) ($P$-trend = 0.014), HOMA-IR ($P$-trend < 0.001), and CRP ($P$-trend < 0.001). In addition, the retinol-retinyl esters concentration was positively related to HOMA-IR and uric acid but inversely related to CRP. Finally, vitamin C level was inversely related to uric acid ($P$-trend = 0.015) (Fig. 1A–D).

**Discussion**

In this study, we evaluated the relationships of serum antioxidants (retinol, retinyl esters, α-carotene, β-carotene, β-cryptoxanthin, lutein+zeaxanthin, lycopene, and vitamins C and E) with MetS and its components in a nationally representative sample of U.S. adolescents. The estimated prevalence of MetS was 7% among boys and 3% among girls. Several key findings were observed. First, adolescents with MetS had consistently lower serum carotenoid concentrations compared with their counterparts without MetS, even after adjusting for serum cholesterol and TG in most models. Second, total carotenoids were also inversely related to HOMA-IR and CRP. Third, vitamin C was consistently inversely related to serum uric acid level and MetS binary outcome. Fourth, retinol-retinyl esters had an inverse relationship with CRP and a positive relationship with uric acid and HOMA-IR as well as MetS binary outcome. Finally, and unlike other antioxidants, vitamin E had no consistent association with MetS and its components, particularly after controlling for serum cholesterol and TG.

Similar to our first key finding, numerous observational studies conducted among adults have indicated that there are consistent inverse associations of serum carotenoid concentrations with type 2 diabetes and MetS (32,34,40–42,72–74). For instance, a cross-sectional study of Australian adults ($n = 1597$) reported an inverse association between serum carotenoids and type 2 diabetes (32). This finding was replicated elsewhere for the MetS outcome among adults (17,40,42). In terms of associations with specific carotenoids, a case-control study found that lutein+zeaxanthin and cryptoxanthin were lower in coronary artery disease adults compared with controls and had an inverse relationship with several cardiovascular risk factors (72). In a RCT, the baseline serum concentration of β-carotene was inversely associated with MetS incidence after a follow-up period of 7.5 y. This inverse relationship was also noted for vitamin C and MetS incidence (73). In a few studies, our first
key finding was modified by smoking status among adults. The CARDIA study with a 15-y follow-up found lower risks of type 2 diabetes and insulin resistance when baseline serum carotenoids were higher, though this association was restricted to nonsmokers (34). Nevertheless, in 2 separate cross-sectional studies conducted among Japanese adults, serum carotenoids had a significant inverse relationship with MetS in both smokers (n = 1073) (41) and nonsmokers (n = 939) (74). However, in a number of studies conducted among adults, including a nested case-control study (35) and a cohort study of middle-aged women (38), the serum concentrations of antioxidants including vitamin E, β-carotene, and lycopene were not associated with type 2 diabetes, particularly after controlling for cardiovascular risk factors.

Other observational studies conducted among adults found inverse associations between dietary intakes of carotenoids (as well as total antioxidant capacity) and MetS (or its components, including CRP) (43,75–77) as well as type 2 diabetes (31,78). However, large RCT did not find any long-term benefits of combined antioxidant supplements, particularly vitamin E and β-carotene, as a preventive strategy against type 2 diabetes (36,37,79) or MetS (73). Thus, despite epidemiological studies pointing to an inverse association of serum carotenoids with MetS or type 2 diabetes, particularly among adults, RCT have shown that dietary supplementation with β-carotene or α-tocopherol has no impact on type 2 diabetes or MetS incidence rates.

In children, a recent, small, case-control study proposed a similar inverse association between antioxidant status and MetS (81). In particular, plasma β-carotene and vitamin E concentrations adjusted for plasma lipids (cholesterol + TG) were lower (P < 0.05) in obese children with MetS (n = 15) compared with obese children without MetS (n = 17) and nonobese children in the control group (n = 16). The same pattern was observed for plasma total antioxidant status values (80). Cross-sectional studies conducted among obese or overweight children also implicate lower lipophili
nutrients, including \(\beta\)-carotene (20,28,81–85) and \(\alpha\)-tocopherol concentrations, possibly as a result of a reduced intake of FV or entrapment in adipose tissue (86,87). Moreover, a recent RCT in children demonstrated significant inverse correlations between \(\beta\)-carotene and BMI, HOMA-IR, the leptin:adiponectin ratio, and abdominal fat mass (88). Considerable improvements in HOMA-IR and abdominal fat mass were seen when children were supplemented with a FV concentrate for 6 mo, which improved \(\beta\)-carotene and reduced retinol (88).

The proposed mechanisms include a direct effect of \(\beta\)-carotene on adipocyte function via the action of its intracellular metabolites, retinaldehyde and all-\(\alpha\)-trans retinoic acid (89). Both of these retinoids...
function as ligands for nuclear receptors such as RAR, RXR, and PPARγ, which can repress adipogenesis (90,91). Retinol and retinol binding protein 4 (RBP4), on the other hand, mediate cell surface signaling of their receptor STRA6 via JAK2/STAT5, inducing the expression of STAT target genes, which include suppressor of cytokine signaling 3 (SOCS3). SOCS3 in turn inhibits insulin signaling and PPARγ, thereby enhancing lipid accumulation (92). This mechanism aids in understanding how RBP and retinol may influence both insulin signaling and energy balance (92,93).

Emerging evidence of cardioprotective effects of nonprovitamin A carotenoids, especially lycopene and lutein as well as oxygenated carotenoids such as β-cryptoxanthin, suggests that there is more to be learned from carotenoids and their specific associations with CVD and its risk factors, particularly among adults (94). Our study uncovered some of those research gaps and
found that decreased total carotenoids rather than β-carotene by itself and increased retinol and retinyl esters associate with the number of cardio-metabolic risk factors among adolescents. A previous study also showed that total antioxidant capacity was an independent predictor of β-carotene concentration, suggesting that several antioxidants may interact to increase the concentration of each of the carotenoids in serum. This may partly explain the failure of β-carotene supplements by themselves to reduce the risk of MetS (95) and the successful reduction of HOMA-IR with a dehydrated fruit and vegetable concentrate (88).

Finally, recent studies also implicate a link between RBP4, which carries all-trans-retinol to its target tissues, and obesity-related insulin resistance in adults and children (96–99), whereas other studies have reported conflicting results (100,101). Reasons for this dichotomy may involve the failure of most studies to measure vitamin A status (retinol), which is the major determinant of hepatic release of circulating RBP4 (89,96). A limited number of studies have also shown that RBP4 increases in type 2 diabetes patients (96,102,103) and may contribute to renal dysfunction and hyperuricemia among type 2 diabetes patients (104,105). The latter finding of a high concentration of retinol and retinyl esters among hyperuricemic adolescents was replicated in the current study. Our finding of retinol’s negative relationship with CRP was also found in previous research (105).

As suggested by prior research, individuals with MetS have higher marker levels of oxidative stress, including those of singleton oxygen and peroxyl molecules (106,107). Thus, adolescents with MetS potentially have higher dietary requirements for antioxidants such as carotenoids, given that serum carotenoids in our present study were found to be inversely related to MetS and its components, an association also previously found among adults (16). Vitamin C was shown to spare and increase concentrations of antioxidants. Given that serum carotenoids in our present study were found to be inversely related to MetS status and serum uric acid. Vitamin E had no consistent association with MetS, whereas retinol+retinyl esters had a positive relationship with HOMA-IR, uric acid and MetS, while being inversely related to CRP. These associations need further study. Preventive efforts aiming at modifiable risk factors for MetS and its related comorbidities are warranted. The associations of MetS and its components with low serum antioxidant concentrations suggest that future nutritional interventions with recommendation for appropriate dietary intakes should be conducted to assess the utility of modifying serum concentrations of antioxidants.

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Literature Cited
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