British Journal of Nutrition (2016), **116**, 1256–1264
doi:10.1017/S0007114516003159

Inverse association linking serum levels of potential antioxidant vitamins with C-reactive protein levels using a novel analytical approach

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(Submitted 26 March 2016 – Final revision received 8 July 2016 – Accepted 2 August 2016 – First published online 13 September 2016)

Abstract

Exposures to antioxidants (AO) are associated with levels of C-reactive protein (CRP), but the pattern of evidence is mixed, due in part to studying each potential AO, one at a time, when multiple AO exposures might affect CRP levels. By studying multiple AO via a composite indicator approach, we estimate the degree to which serum CRP level is associated with serum AO level. Standardised field survey protocols for the US National Health and Nutrition Examination Survey (NHANES) 2003–2006 yielded nationally representative cross-sectional samples of adults aged 20 years and older (*n* 8841). NHANES latex-enhanced nephelometry quantified serum CRP levels. Liquid chromatography quantified serum concentrations of vitamins A, E and C and carotenoids. Using structural equations, we regressed CRP level on AO levels, and derived a summary estimate for a composite of these potential antioxidants (CPA), with covariates held constant. The association linking CPA with CRP was inverse, stronger for slightly elevated CRP ($1{\cdot}8 \le$ CRP < 10 mg/l; slope = $-1{\cdot}08$; 95 % CI $-1{\cdot}39$, $-0{\cdot}77$) and weaker for highly elevated CRP ($\ge$10 mg/l; slope = $-0{\cdot}52$; 95 % CI $-0{\cdot}68$, $-0{\cdot}35$), with little change when covariates were added. Vitamins A and C, as well as lutein + zeaxanthin, were prominent contributors to the composite. In these cross-sectional data studied via a composite indicator approach, the CPA level and the CRP level were inversely related. The stage is set for more confirmatory longitudinal or intervention research on multiple vitamins. The composite indicator approach might be most useful in epidemiology when several exposure constructs are too weakly inter-correlated to be studied via formal measurement models for underlying latent dimensions.

Key words: Antioxidants: C-reactive proteins: Inflammation: Epidemiology: National Health and Nutrition Examination Survey

Nutrients with potential antioxidant effects have been postulated to dampen inflammation, which plays a widely appreciated role in the development of cardiometabolic diseases (CMD)[1–3]. Epidemiological evidence of such modulation includes studies of serum C-reactive protein (CRP), an acute-phase reactant protein that can be interpreted as a biomarker of inflammation status and possible intermediate phenotype leading towards CMD[4]; however, studies to date have produced mixed evidence. For example, in some studies, lower levels of vitamin C and carotenoids are inversely linked to CRP levels; in other investigations, the estimates are null[5–8]. Inverse associations of CRP level and vitamin E have been observed, but there are also null estimates, as well as some positive associations[5,7,9–11].

Schwab *et al.*[12] suggested that one potential antioxidant (e.g. vitamin E) might be combined with supplementation of other potential antioxidants in order to dampen serum CRP levels. The popularity of this idea can be seen in a number of studies with mixed estimates, some of which have measured antioxidant levels via a simple summation of self-reported dietary intake (i.e., assigning equal weights to all antioxidants studied)[6,13–17].

The idea that summary scores might not serve well for self-reported diet intake measurements was introduced in nutritional epidemiology more than 25 years ago, when it was recognised that giving an *equal* and *positive* weight to each dietary compound might be a mistake[18]. In addition to ignoring or disguising the relative importance of individual compounds, some nutrients might act as anti- or pro-oxidants, depending on the local chemical milieu[19]. Therefore, assuming *equal* and *positive* contribution of each nutrient in a simple summation approach might lead to seriously biased estimates[20]. One consequence has been a series of studies that estimate each potential antioxidant's hypothesised effects, one by one, in a suboptimal approach that can compromise statistical precision and validity. Another alternative approach involves devising one or more underlying latent dimensions for which each nutrient is studied as a manifest indicator, and

Abbreviations: CMD, cardiometabolic diseases; CPA, composite of potential antioxidants; CRP, C-reactive protein.

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each nutrient can be given a unique weight, with either a positive or a negative sign[18].

Nevertheless, there are two potential shortcomings when using self-reported diet intake measurements in the latent variable approach. First, there might be too much noise and not enough signal in a set of self-reported diet intake values. Consequences include[1] overly complex measurement model covariance structures and[2] the presence of artifactual estimates due to shared method co-variation (e.g. a participant might systematically 'fake good' or 'fake bad' in diet self-reports[13]). Second, there can be value in substituting a measure of bioavailability (e.g. serum levels of these compounds) in place of a food-specific, self-reported dietary intake variable. For example, in addition to the observed variation in the electron modulation characteristics of individual nutrient compounds, these compounds possess a variety of unique or shared chemical and biological properties before and after biotransformation steps. These variations might give rise to a variety of potential underlying mechanisms along the pathway from food intake to CRP levels. For these reasons, neither the one-by-one approach, the simple summation approach nor the latent variable measurement modelling approach serves well[5,20].

In this study, we have turned to a composite indicator approach. This approach is theoretically more appropriate, as these potential antioxidant vitamins are not necessarily reflective indicators of common latent constructs. In addition, in line with our conceptualisation, we discovered poor model fit for the conventional latent dimension measurement model, which can be a signal that assumptions required for the latent dimension measurement model were not being met ('Analysis' section shows details of model fit indices). In this study, our starting assumption was that each of the specified potential antioxidants might be inversely associated with CRP levels, even though we have noted some studies in which vitamin E and other supplements might be associated with higher CRP levels[10,11].

Against this background, the main aims of the current study were as follows: (a) to estimate the association that links an aggregated composite of potential antioxidant (CPA) vitamins with serum CRP levels and (b) to illustrate a composite indicator approach that can be used to create a summary for variables when latent variable approaches are inappropriate or misleading[20]. We label this summary variable 'CPA', which does not qualify as a 'latent variable' as it is solely determined by the indicators included in the model and does not have residual variance. We chose the composite approach because it is consistent with our hypothesised specification – that is, various antioxidants represent different aspects of the hypothesised antioxidant network.

At the outset, we appreciate shortcomings in our study. First, CRP is not a specific CMD biomarker or intermediate phenotype; acute infections can yield elevated CRP levels. Second, any study of the CPA–CRP association is just one slice in an array of mechanisms that can lead towards CMD from origins in distal conditions and processes such as inherited predispositions, epigenetic transactions and social deprivation. Nonetheless, we are hopeful that an application of the composite indicator approach in the study of antioxidants and CRP levels can aid future research on CMD pathogenesis and its ultimate prevention.

Methods

Sample

The current study's estimates are from the National Health and Nutrition Examination Surveys (NHANES), 2003–2006, a series of annual cross-sectional surveys in the USA, with stratified multistage probability sampling to draw nationally representative samples of the country's civilian, non-institutionalised population[21]. The aggregate analysis sample for the current study consists of 8411 adults, aged 20 years and older, after exclusion of 482 participants with missing or invalid measurements of potential antioxidants under study. The study protocol was reviewed and approved by the cognizant institutional review boards for protection of human subjects in research.

Assessment

The key response variable was serum CRP level, as quantified by latex-enhanced nephelometry[22], with a non-normal distribution from which we formed three subgroups as follows: low (≤1·8 mg/l), intermediate (>1·8 and <10 mg/l) and high (≥10 mg/l). The value 1·8 was chosen before inspection of antioxidant–CRP associations. In order to optimise variance estimation, we used the median of CRP levels observed in the combined low and intermediate subgroups.

The covariates of central interest included nine potential antioxidant vitamins. Serum concentrations of vitamin A (retinol), carotenoids (*α*-carotene, *cis β*-carotene, *β*-cryptoxanthin, combined lutein + zeaxanthin and total lycopene) and vitamin E (*α*- and *γ*-tocopherol) were measured using HPLC with photodiode array detection. Serum vitamin C (ascorbic acid) was measured using isocratic HPLC with electrochemical detection at 650 mV. Levels of these vitamins were categorised into four groups on the basis of quartiles. *α*-Tocopherol and *γ*-tocopherol are lipophilic compounds that have no specific plasma transport protein, but instead are transported within plasma lipoproteins and their distribution parallels that of total lipids. To avoid underestimating or overestimating true concentrations of tocopherols, we used a tocopherol:cholesterol ratio in place of tocopherol levels[23].

Sex, age, self-identified race/ethnicity, alcohol drinking status (categorised into never drinkers, past drinkers and recently active drinkers) and smoking status (categorised into never smokers, past smokers and recently active smokers) were based on self-report. Recently active drinking was defined as drinking during the past 12 months. An answer 'everyday' or 'somedays' to the question 'do you smoke now' qualifies the respondent as a recently active smoker. BMI was measured as weight in kilograms divided by height in metres squared. The only social deprivation variable in our NHANES data sets was the participant's level of education, controlled here as a covariate.

Analysis

The guiding conceptual model is specified with a multinomial response variable signifying a low serum CRP level, a mid-range

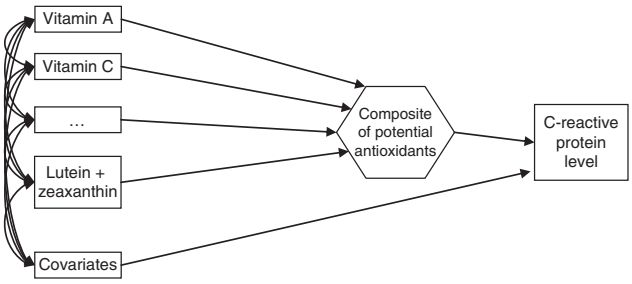

Fig. 1. Cartoon depiction of the conceptual model.

CRP level and a higher CRP level. In multinomial regression, this response variable is regressed on the CPA composite indicator (Fig. 1). Low CRP levels can be in a normal, non-pathological range, and high CRP levels can signal acute infection, whereas intermediate CRP levels can signal chronic inflammation possibly modulated by antioxidants[4,5]. This non-linearity motivated specification of the non-ordered, three-category multinominal response variable, with an advance hypothesis that the CPA–CRP relationship would be inverse in the middle, with null CPA–CRP relationships at high CRP levels.

To try out a latent variable dimensional approach, we examined the dimensionality of the nine potential antioxidants using exploratory factor analysis models, with root mean square error of approximation (RSMEA) as a primary goodness of fit index. In advance, the 'good fit' specification was set as RSMEA < 0·05 (with 90 % CI not entrapping 0·05) and both the Tucker–Lewis index (TLI) and the comparative fit index (CFI) > 0·95[24,25]. Otherwise, the model was considered misfit. Results indicate suboptimal fit for one-, two- and three-factor models (one-factor model: RMSEA = 0·095, 90 % CI 0·092, 0·099, TLI = 0·822, CFI = 0·867; two-factor model: RMSEA = 0·078, 90 % CI 0·074, 0·082, TLI = 0·883, CFI = 0·938; three-factor model: RMSEA = 0·067, 90 % CI 0·062, 0·072, TLI = 0·912, CFI = 0·971). Models with four and more factors were inadmissible. The suboptimal fit of these latent variable models supports our assumption that these antioxidants do not tap a common factor structure.

Next, we turned to the composite approach described by Bollen *et al.*[20] for creation of our CPA indicator. The composite is depicted as follows:

$$C_i = w_0 + w_1x_{1i} + w_2x_{2i} + \ldots + w_kx_{ki},$$

where C_i is the composite variable (CPA in this study) for the ith case. The composite variable is a linear combination of x (vitamins in this study) with estimated weights (regression coefficients) such that the residual variance is 0. In the system of structural equations for this study's multinomial logistic regressions, the weights are derived with the nominal CRP levels regressed on CPA in order to generate initial estimates of the CPA–CRP association. In this approach, the individual weights (w) for CPA indicators are optimised for the association with the outcomes under study (in this case, with slopes denoted for intermediate or high CRP levels relative to a low level). To begin, we chose a high serum level of vitamin A to be the first-listed CPA indicator, with its weight (w_1) fixed to 1 as a scale for all other indicator coefficients. All quartile-based vitamin levels are treated as multinomial variables because previous studies have documented non-linear relationships between these vitamin levels and CRP as well as potential adverse health effects of both low and high levels of vitamins[12,26]. Therefore, three separate weights were generated for each vitamin variable (as shown in Tables 3 and 4). An alternative approach is to standardise the coefficients relative to the variance of the composite variable, as illustrated in the 'Results' section. (Methodologically interested readers will note that because the composite is not a latent variable and the indicators are not intended to be measurements of one underlying construct, any composite indicator's weights (w) should be expected to change when different outcomes are substituted.)

In our primary estimation steps, we introduced sex and age into the model as covariates. The CPA–CRP slope coefficient reflects the change in log odds of being in the high- or intermediate-CRP group as compared with the low-CRP group associated with 1 unit increase of the CPA variable. A quadratic term of age was also included in the model in order to account for potential non-linear relationships between CRP and age. We then estimated potential modulations by including product terms between CPA and sex and age. As a pre-specified rule to guide inclusion of product terms, we set α at 0·10. Thereafter, we included self-identified race/ethnicity, cigarette smoking, alcohol drinking and BMI, as well as levels of education, in our post-estimation exploration, with a focus on whether the CPA–CRP slope coefficients were appreciably different from estimates seen in the primary estimation steps[27].

NHANES analysis weights were used to adjust for differential sampling probabilities as well as post-stratification adjustments required to address non-response and to bring the weighted sample distributions into balance with US Census distributions. Taylor series linearisation was used for variance estimation in this complex survey sample context. Analyses were conducted using Mplus statistical software, version 7.31 (Muthén & Muthén, 2015).

Results

Table 1 provides a description of the study sample and bivariate associations between the covariates and the CRP levels. The mean levels of vitamins are similar to those reported in previous NHANES-based studies[28]. All vitamins were inversely associated with CRP levels except for both forms of vitamin E, for which null and positive associations were found. Despite these unexpected vitamin E associations, we proceeded with our pre-defined model, which included all vitamins.

The main estimates of the present study are presented in Table 2. CPA was inversely associated with elevated levels of CRP. The association was approximately 2-fold stronger for slightly elevated CRP (slope = −1·08; 95 % CI −1·39, −0·77) compared with highly elevated CRP (slope = − 0·52; 95 % CI −0·68, −0·35). Standardised coefficients were similar. After adjusting for sex and age, the CPA–CRP relationships did not change appreciably. No evidence of non-linear relationships between CRP and age was found when the quadratic age term was modelled (P > 0·30); therefore, it was excluded from the model.

Table 1. Characteristics of the study sample and initial bivariate associations (unweighted analysis *n* 8411)*
(Logistic regression slope estimates (*b*) and mean values with their standard errors)

Characteristics	Unweighted sample size	Weighted percentages	*b*1† Slope	*b*1† SE	*b*2† Slope	*b*2† SE
Age (years)						
Mean	46·6		0·01		0·01	
SE	0·4		0·002		0·001	
Women (%)	4367	51·9	1·02	0·105	0·39	0·053
Self-identified race/ethnicity (%)						
Non-Hispanic White	4377	72·4				
Non-Hispanic Black	1726	10·9	0·25	0·156	−0·09	0·092
Hispanic	1955	11·3	0·52	0·085	0·24	0·079
All others	353	5·3				
Education categories (%)						
Above high school	3958	56·3	Ref.		Ref.	
High school	2060	25·9	0·36	0·106	0·30	0·069
Below high school	2381	17·7	0·28	0·108	0·17	0·053
Missing	12	0·1	–		–	
Tobacco cigarette smoking (%)						
Never	4304	50·0	Ref.		Ref.	
Former	2228	25·2	0·73	0·472	1·66	0·108
Recently active	1871	24·7	2·02	0·053	3·51	0·001
Missing	8	0·1	–		–	
Alcohol drinking (%)						
Never	1103	10·6	Ref.		Ref.	
Former	1709	16·5	1·82	0·078	1·48	0·149
Recently active	4973	65·9	−2·59	0·015	−2·42	0·022
Missing	626	7·0	–		–	
BMI (%)						
<25 kg/m^2	2547	32·6	Ref.		Ref.	
25–29·9 kg/m^2	2894	33·3	0·56	0·106	0·77	0·069
≥30 kg/m^2	2820	32·7	2·34	0·107	1·84	0·065
Missing	150	1·4	–		–	
	Mean	SE	Mean	SE	Mean	SE
Vitamin A (μmol/l)	2·10	0·02	−0·81	0·114	−0·12	0·052
Vitamin C (μmol/l)	53·93	1·36	−0·02	0·002	−0·01	0·001
α-Tocopherol (μmol/l)	32·32	0·55	−0·004	0·005	0·01	0·002
γ-Tocopherol (μmol/l)	5·60	0·14	0·15	0·017	0·12	0·010
α-Carotene (μmol/l)	0·07	0·01	−5·89	1·748	−2·93	0·306
β-Carotene (μmol/l)	0·34	0·02	−2·20	0·375	−0·63	0·215
β-Cryptoxanthin (μmol/l)	0·15	0·01	−4·63	0·761	−1·92	0·224
Total lycopene (μmol/l)	0·78	0·01	−1·21	0·149	−0·27	0·110
Combined lutein + zeaxanthin (μmol/l)	0·28	0·01	−4·24	0·596	−1·49	0·193
Serum C-reactive protein (mg/l)	4·3	0·1				

Ref., referent values.
* Data from the US National Health and Nutrition Examination Surveys, 2003–2006.
† In order to convey the sign, size and precision of the bivariate association of each covariate with C-reactive protein (CRP) level, we provide analysis-weighted logistic regression slope estimates and their standard errors for each covariate, modelled one by one. We do not provide *P*-values because at this stage we are not drawing inferences. These are results from pre-estimation exploratory data analyses to clarify relationships before fitting more complex statistical models for inference. The first column of slope estimates is for mid-range CRP values *v.* low CRP values. The second column of slope estimates is for higher level CRP values (possibly indicating acute infection) *v.* low CRP values. The specific thresholds for low, mid and higher CRP values are described in the 'Assessment' section.

Estimates from the bivariate and sex- and age-adjusted models are depicted in the online Supplementary Fig. S1 and S2. No evidence was found for a modulation by sex (i.e. $P=0·413$ and 0·399 for product terms for slightly and highly elevated CRP, respectively). Similarly, no age-related modulation was evident for slightly elevated CRP ($P=0·915$). For highly elevated CRP levels, the strength of the CPA–CRP inverse association was larger at older ages (slope = −0·003; 90 % CI −0·005, >−0·001; $P=0·065$). Nonetheless, the CPA–CRP associations remained robust.

The estimated CPA–CRP associations showed some attenuation when covariates for BMI, alcohol drinking status, smoking status, self-identified race/ethnicity and education were included in the model. However, the statistically robust inverse CPA–CRP associations were still observed (Table 2). The weights for each antioxidant used to form the CPA indicator are presented in Table 3 (with standardised coefficients in Table 4). Vitamin A, vitamin C, *α*-carotene, *β*-carotene, cryptoxanthin, lutein + zeaxanthin had positive weights in the CPA composite indicator. Both forms of vitamin E had negative weights in the CPA composite indicator. Lycopene weights were null.

The observed vitamin E associations were unexpected. Therefore, in a supplementary analysis, we removed vitamin E

Table 2. Estimated associations between composite potential antioxidants (CPA) and serum C-reactive protein levels (CRP)*†

	Bivariate (*n* 8411)		Adjusting for sex and age (*n* 8411)		Adjusting for other covariates‡ (*n* 7669)		Adjusting for sex and age without vitamin E (*n* 8411)	
	Estimate	95 % CI	Estimate	95 % CI	Estimate	95 % CI	Estimate	95 % CI
Y_1 = CRP > 1·8 and <10 mg/l								
CPA (non-standardised)	−1·08	−1·39, −0·77	−0·94	−1·24, −0·65	−1·03	−1·34, −0·73	−1·00	−1·29, −0·70
CPA (standardised§)	−0·98	−1·13, −0·84	−1·24	−1·10, −0·96	−0·85	−1·00, −0·71	−1·09	−1·23, −0·97
Y_2 = CRP ≥ 10 mg/l								
CPA (non-standardised)	−0·52	−0·68, −0·35	−0·47	−0·63, −0·31	−0·42	−0·58, −0·27	−0·43	−0·58, −0·29
CPA (standardised§)	−0·47	−0·56, −0·38	−0·55	−0·63, −0·47	−0·35	−0·45, −0·25	−0·47	−0·56, −0·39

* Data from US National Health and Nutrition Examination Surveys, 2003–2006.
† $Y_1 = Y_2 = 0$ when CRP level ≤1·8 mg/l. CPA summarises serum levels (quartiles) of vitamin C, vitamin A, vitamin E, γ-tocopherol, α-carotene, *trans*-β-carotene, *cis*-carotene, cryptoxanthin, lutein + zeaxanthin and lycopene. A multinomial logistic regression was used for estimation. Therefore, the estimates reflect changes in log odds of being in the high- or intermediate-CRP group as compared with the low-CRP group associated with 1 unit increase of the CPA variable.
‡ Other covariates include BMI, smoking status, alcohol drinking status, self-identified race/ethnicity and level of education.
§ Regression coefficient based on the variance of the composite variable.

Table 3. Weights used to form the composite of potential antioxidants (Regression coefficients and 95 % confidence intervals)†

	Bivariate (*n* 8411)		Adjusting for sex and age (*n* 8411)		Adjusting for other covariates‡ (*n* 7669)	
	β	95 % CI	β	95 % CI	β	95 % CI
Vitamin A						
High	1·00	–	1·00	–	1·00	–
Mid–high	0·92*	0·70, 1·15	0·89*	0·64, 1·13	1·01*	0·72, 1·29
Mid–low	0·63*	0·43, 0·84	0·62*	0·39, 0·86	0·71*	0·47, 0·94
Vitamin C						
High	0·55*	0·28, 0·81	0·74*	0·39, 1·10	0·60*	0·26, 0·94
Mid–high	0·41*	0·23, 0·59	0·40*	0·18, 0·62	0·41*	0·17, 0·64
Mid–low	0·09	−0·12, 0·31	0·01	−0·23, 0·25	0·17	−0·12, 0·47
β-Carotene						
High	0·47*	0·06, 0·88	1·32*	0·65, 1·98	0·90*	0·32, 1·48
Mid–high	0·09	−0·18, 0·36	0·57*	0·18, 0·95	0·37*	0·01, 0·74
Mid–low	0·07	−0·14, 0·27	0·35*	0·07, 0·64	0·20	−0·13, 0·53
α-Carotene						
High	0·36*	0·01, 0·71	0·59*	0·15, 1·03	0·46	−0·03, 0·96
Mid–high	0·14	−0·20, 0·48	0·39*	0·02, 0·75	0·33	−0·11, 0·78
Mid–low	0·05	−0·20, 0·30	0·24	−0·08, 0·56	0·22	−0·13, 0·57
Cryptoxanthin						
High	0·38*	0·04, 0·71	0·10	−0·23, 0·43	0·06	−0·29, 0·40
Mid–high	0·47*	0·22, 0·73	0·31*	0·06, 0·57	0·36*	0·04, 0·69
Mid–low	0·20*	0·02, 0·38	0·12	−0·10, 0·33	0·16	−0·05, 0·38
Lycopene						
High	0·27*	0·06, 0·48	−0·21	−0·50, 0·09	0·14	−0·17, 0·44
Mid–high	0·18	−0·04, 0·39	−0·19	−0·48, 0·11	0·01	−0·26, 0·29
Mid–low	0·18	−0·10, 0·46	−0·01	−0·31, 0·30	0·13	−0·17, 0·42
Lutein + zeaxanthin						
High	0·60*	0·32, 0·88	0·68*	0·35, 1·01	0·40*	0·03, 0·77
Mid–high	0·44*	0·16, 0·73	0·43*	0·10, 0·76	0·19	−0·16, 0·55
Mid–low	0·38*	0·16, 0·61	0·41*	0·14, 0·69	0·32*	0·01, 0·63
α-Tocopherol						
High	−1·14*	−1·45, −0·84	−0·97*	−1·29, −0·66	−0·93*	−1·21, −0·64
Mid–high	−0·78*	−1·11, −0·44	−0·78*	−1·17, −0·39	−0·74*	−1·14, −0·34
Mid–low	−0·25	−0·51, 0·01	−0·23	−0·53, 0·08	−0·31	−0·65, 0·02
γ-Tocopherol						
High	−0·88*	−1·22, −0·55	−1·00*	−1·42, −0·58	−0·29	−0·79, 0·21
Mid–high	−0·40*	−0·71, −0·09	−0·49*	−0·88, −0·09	−0·07	−0·52, 0·37
Mid–low	−0·35*	−0·59, −0·10	−0·51*	−0·84, −0·19	−0·35*	−0·69, −0·002

* $P < 0·05$.
† Data from US National Health and Nutrition Examination Surveys, 2003–2006.
‡ Weights in the composite indicator approach are very context-specific, particularly with reference to the distribution of the response variable, but also, as shown here, with respect to which covariates are included in the model. For the third set of weights, the model's covariates included sex, age, BMI, smoking status, alcohol drinking status, self-identified race/ethnicity and level of education.

indicators from the composite, and re-specified direct paths from the two forms of vitamin E to CRP, in effect 'holding constant' vitamin E while re-estimating this new CPA–CRP association. As shown in Table 2, this model amendment did not make much difference to the original CPA–CRP estimates adjusted for sex and age (Table 2).

Table 4. Standardised weights used to form the composite of potential antioxidant† (Regression coefficients and 95 % confidence intervals)

	Bivariate (*n* 8411)		Adjusting for sex and age (*n* 8411)		Adjusting for other covariates‡ (*n* 7669)	
	β	95 % CI	*β*	95 % CI	*β*	95 % CI
Vitamin A						
High	1·10*	0·86, 1·33	0·86*	0·63, 1·09	1·21*	0·87, 1·55
Mid–high	1·01*	0·80, 1·22	0·76*	0·52, 1·00	1·22*	0·84, 1·59
Mid–low	0·70*	0·43, 0·96	0·53*	0·28, 0·78	0·86*	0·54, 1·17
Vitamin C						
High	0·60*	0·33, 0·87	0·64*	0·38, 0·89	0·73*	0·35, 1·10
Mid–high	0·45*	0·25, 0·65	0·34*	0·17, 0·52	0·49*	0·23, 0·75
Mid–low	0·10	−0·14, 0·34	0·01	−0·19, 0·21	0·21	−0·16, 0·58
β-Carotene						
High	0·52*	0·12, 0·91	1·13*	0·76, 1·50	1·09*	0·50, 1·68
Mid–high	0·10	−0·19, 0·39	0·49*	0·22, 0·75	0·45*	0·04, 0·86
Mid–low	0·07	−0·15, 0·30	0·30*	0·08, 0·53	0·24	−0·15, 0·63
α-Carotene						
High	0·39*	0·01, 0·79	0·51*	0·12, 0·89	0·56	−0·01, 1·14
Mid–high	0·15	−0·22, 0·53	0·33*	0·02, 0·64	0·40	−0·12, 0·92
Mid–low	0·05	−0·22, 0·32	0·20	−0·06, 0·47	0·27	−0·14, 0·68
Cryptoxanthin						
High	0·41*	0·09, 0·74	0·09	−0·19, 0·36	0·07	−0·34, 0·48
Mid–high	0·52*	0·28, 0·76	0·27*	0·06, 0·47	0·44*	0·07, 0·81
Mid–low	0·22*	0·04, 0·41	0·10	−0·08, 0·27	0·20	−0·05, 0·44
Lycopene						
High	0·30*	0·08, 0·51	−0·18	−0·42, 0·06	0·17	−0·19, 0·52
Mid–high	0·19	−0·04, 0·43	−0·16	−0·41, 0·08	0·02	−0·32, 0·35
Mid–low	0·20	−0·10, 0·49	−0·01	−0·27, 0·26	0·15	−0·18, 0·49
Lutein + zeaxanthin						
High	0·66*	0·37, 0·94	0·58*	0·32, 0·85	0·48*	0·06, 0·91
Mid–high	0·49*	0·21, 0·77	0·37*	0·12, 0·62	0·24	−0·18, 0·65
Mid–low	0·42*	0·21, 0·63	0·36*	0·17, 0·54	0·39*	0·08, 0·71
α-Tocopherol						
High	−1·25*	−1·57, −0·94	−0·84*	−1·12, −0·56	−1·12*	−1·51, −0·73
Mid–high	−0·85*	−1·17, −0·54	−0·67*	−0·97, −0·36	−0·90*	−1·35, −0·45
Mid–low	−0·27	−0·55, 0·01	−0·19	−0·45, 0·06	−0·38	−0·77, 0·02
γ-Tocopherol						
High	−0·97*	−1·28, −0·66	−0·86*	−1·16, −0·56	−0·35	−0·95, 0·25
Mid–high	−0·44*	−0·77, −0·12	−0·42*	−0·74, −0·10	−0·09	−0·63, 0·45
Mid–low	−0·38*	−0·64, −0·12	−0·44*	−0·69, −0·19	−0·42*	−0·82, −0·01

* $P<0·05$.
† Data from US National Health and Nutrition Examination Surveys, 2003–2006.
‡ Covariates include sex, age, BMI, smoking status, alcohol drinking status, self-identified race/ethnicity and level of education.

Mindful of the suggestion made by Schwab *et al.*[12], we also explored possible modulations of the CPA–CRP relationship by vitamin E. As described in the online Supplementary Table S1, no evidence was found for such modulation. Similarly, we also looked for possible subgroup variations of the vitamin-E–CRP associations across CPA levels (i.e., tertiles of a derived CPA score). No evidence was found for such subgroup variation (online Supplementary Table S2). Before making a statistical inference about the observed inverse CPA–CRP relationship at mid-range CRP levels, one might wish to re-specify the type I error α value as 0·025 or 0·016 rather than the standard 0·05 α. This accommodation might be used to correct for multiple hypothesis tests in contrast to the three discrete categories of CRP levels in that $0·05/2=0·025$ and $0·05/3=0·016$. The observed *P*-value for the estimated inverse CPA–CRP relationship at mid-range CRP levels was <0·001 (i.e., <0·025 as well as <0·016).

To test the sensitivity of our results for slightly elevated CRP, we refit the main sex- and age-adjusted model after excluding the high-CRP group because of the concern that highly elevated CRP reflects acute infection. With exclusion of the high-CRP group, the estimated variance of the outcome variable changed. In consequence, a comparison of results requires a shift to standardised regression slope estimates, based on sample variances of the composite variable, as well as sample variances of the outcome variable. The resulting standardised slope estimates for the CPA–CRP relationship did not vary appreciably with and without the high-CRP group ($S_{with}=-0·89$, 95 % CI −0·94, −0·83; $S_{without}=-0·90$, 95 % CI −0·94, −0·85).

Discussion

In this US nationally representative sample study, we used a composite indicator approach to combine information about nine vitamins with putative antioxidant properties, and used the resulting CPA indicator to discover consistent inverse associations between CPA and CRP. These associations cannot be attributed to imbalances of sex, age or an array of covariates

that previously have been studied as potential influences on CRP levels. In advance, we acknowledge that our study fails 'to contextualise' the CPA–CRP association with respect to hypothesised root causes such as income inequality and social deprivation; our covariate adjustment for the participant's level of education may not serve well as a measure of social deprivation[27,29].

The inverse CPA–CRP association observed in this study should be interpreted with this weakness in mind, among several other limitations. Of central concern is the cross-sectional nature of the current study, which complicates interpretation and inferences based on the observed CPA–CRP associations. With respect to the assessment, the use of a single measurement of serum CRP and serum vitamin levels might raise the possibility of survey errors; however, the prognostic value of CRP has been widely demonstrated in CVD research, especially if CRP elevation is sustained[30,31]. We formed a three-level CRP response variable with the idea that a high CRP level might indicate acute infection states. Nonetheless, we acknowledge that some individuals in our intermediate level also might have had a rising CRP level, not yet in its peak after acute infection, or might have had a falling CRP level in the residual phase of acute infection. We used the conventional 10 mg/l as the cut-off for high CRP. Nonetheless, some individuals may have a CRP level >10 mg/l due to chronic inflammation.

Notwithstanding limitations such as these, the composite indicator approach brings an element of methodological innovation to antioxidant–CRP research. The nationally representative samples, plus the serum measures of CRP levels and antioxidants, combine to enhance the external and internal validity of the study. Built upon previously documented inverse associations linking CRP levels with single antioxidants studied one by one, this study provides supportive evidence of a robust inverse relationship between overall levels of vitamins with potential antioxidant properties and CRP. As mentioned in the first few paragraphs, the composite indicator approach represents a methodological departure from possibly flawed assumptions of the conventional reflective indicator approach (e.g. when summary scores for vitamin indicators are created by assuming *equal* and *positive* weights for each indicator). The composite indicator approach may prove to be especially useful when the functions of various nutrients can vary depending on the local chemical milieu, particularly when multiple nutrients often are thought to interact with each other via a complex network along biotransformation pathways leading towards functional forms[19].

Building from the novel discovery of an inverse association at these mid-range values, future investigations might either apply functional analyses appropriate for the observed non-linearity of relationships, or possibly exclude participants with the lowest and highest CRP values. In addition, the explanatory mechanisms and functional significance of the observed inverse relationships in the CRP mid-range must also be worked out in future studies.

In light of suggestions offered by Schwab *et al.*[12], in relation to the health benefits of combinations of vitamin E and other antioxidants[32], this study's observed positive association between vitamin E and higher CRP levels is noteworthy. Other small-scale clinical studies have disclosed positive vitamin-E–CRP associations[10,11], and there is evidence from randomised controlled trials indicating that supplementation of vitamin E may provide no benefit with respect to prevention of cardiovascular events[32]. Indeed, long-term vitamin E supplementation might be predictive of slightly higher risk of heart failure[33]. Mechanisms include potential pro-oxidant properties of vitamin E as shown in the *in vitro* context[34,35]. A study in humans found that high levels of vitamin E can inhibit effects of other antioxidants[36]. As such, it may be time to reconsider the idea that CRP levels will drop and other health benefits will follow when vitamin E is taken in combination with antioxidants.

We are aware of a previous study showing no association between 'overall antioxidant capacity' and CRP[6], whereas our study has found a robust inverse CPA–CRP association. We are interested to see what might be found when the composite CPA approach is substituted in new analyses of the already gathered data from the previous study. Our finding shows a stronger inverse CPA–CRP association for slightly elevated CRP, which is usually considered an indication of chronic inflammation, compared with highly elevated CRP, which is an indication of acute infection. This finding may be of particular interest for researchers who are interested in diseases that have been linked to chronic inflammation. According to our results, vitamin A, vitamin C and lutein + zeaxanthin are the most prominent vitamins that are inversely associated with CRP. If the causal significance of these observed associations can be substantiated, future studies will be needed to delineate the mechanisms that might account for this inverse and possibly protective association, including potential infra-additive and other interaction pathways.

As noted in the first few paragraphs, the composite approach is a method to summarise various indicators when they cannot be used to measure one underlying factor or latent dimension[20]. The resulting composite variable is a simple summary index for the indicators, which has 0 residual variance. To make the best use of this approach, we can build from previous specifications[20], and offer several rules of thumb: (a) be guided by a theory, including a measurement model theory; (b) consider the composite indicator approach in complement with common factor structure approaches, except when the indicators have deficient signal:noise ratios or otherwise do not yield adequate fit indices in common factor models; and (c) always display the weight for each composite indicator because the coefficients are dependent on the outcome variable[20]. Readers who are interested in more background information and technical details of the composite indicator approach can refer to previous publications by Bollen *et al.*[20].

As for directions for future studies that can build from findings such as these, an important consideration is reproducibility. We encourage researchers with data from randomised trials to use the composite indicator approach to analyse their data in order to remove uncertainties about temporal sequencing of CRP and antioxidant levels as well as potential unobserved heterogeneity, which might be introduced by economic deprivation[29]. Finally, causal significance of observed CPA–CRP associations can be investigated using instrumental variable

approaches, as demonstrated in previous lines of CRP research[37,38] and via randomised-controlled trials in the general population.

Acknowledgements

The authors thank Dr Jean Kerver for her valuable advice. The authors acknowledge the project's funding sources. Michigan State University's (MSU) support is also acknowledged by all authors, including M. D. C.'s participation in the MSU Summer Research Opportunity Program. We thank the NHANES study for making the data publicly available.

This work was supported by the National Institute of Health (K05DA015799 to J. C. A., T32DA021129 to H. G. C. and O. A. and K99AT009156 to O. A.) and MSU (to J. C. A., M. D. C., H. C. and O. A.). The content of this study is the sole responsibility of the authors and does not necessarily represent the official views of MSU, the National Institute on Drug Abuse or the National Institutes of Health.

J. C. A., H. G. C., M. D. C. and O. A. conceptualised and designed the study. H. G. C., O. A. and M. D. C. managed the literature review. H. G. C., O. A. and M. D. C. conducted the data analysis and wrote the first draft. J. C. A. revised the manuscript critically for important intellectual content. All the authors read and approved the final version of the manuscript.

The authors declare that there are no conflicts of interest.

Supplementary material

For supplementary material/s referred to in this article, please visit http://dx.doi.org/10.1017/S0007114516003159

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