

Determining the mediation role of CRP and IL-6 for associations between carotenoids, vitamin A, and vitamin E and insulin resistance in elderly

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Abstract

Background: This study aimed to investigate whether C-reactive protein (CRP) and interleukin-6 (IL-6) mediate the relationship between various antioxidants—specifically vitamin A, vitamin E isoforms, and carotenoids—and insulin resistance (IR).

Methods: We conducted a bias-corrected bootstrapping cross-sectional mediation analysis using biomarker data from the Midlife in the United States (MIDUS3) study (2017–2022). Antioxidants evaluated included total lycopene, β -cryptoxanthin, retinol, and α - and γ -tocopherol. IR was assessed using the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR). CRP and IL-6 were included as mediators. Analyses were adjusted for age, sex, waist-hip ratio (WHR), smoking status, physical activity, dyslipidemia (assessed *via* total to high-density lipoprotein [HDL] cholesterol ratio), and dietary quality, with all continuous covariates mean-centered.

Results: The analytic sample comprised 747 participants (56% female; mean age, 66 years; standard deviation [SD] = 9.6). After adjustment, higher total lycopene and β -cryptoxanthin levels were significantly associated with lower HOMA-IR, with no evidence of mediation by CRP or IL-6. In contrast, γ -tocopherol was associated with higher HOMA-IR, an effect partially mediated by elevated CRP (percent change per 10-unit increase: 7.79%; 95% confidence interval [CI]: 2.12–18.53). Retinol showed significant indirect mediating associations through reduced CRP (–31.48%; 95% CI: –50.24 to –13.50) and IL-6 (–15.46%; 95% CI: –33.57 to –1.49), though these effects did not yield a significant total association. However, α -tocopherol did not demonstrate a significant indirect or total association.

Conclusions: Among older adults, γ -tocopherol was positively associated with IR, partially mediated by CRP, whereas β -cryptoxanthin and lycopene were inversely associated with HOMA-IR without a significant mediation for inflammatory markers. Although retinol reduced IR *via* reduced inflammatory markers, its overall association with IR was not significant.

Keywords: Insulin resistance, β -Cryptoxanthin, Lycopene, Retinol, Tocopherol, CRP, IL-6

Introduction

Oxidation is a chemical process that generates free radicals, leading to cellular damage through mechanisms such as lipid peroxidation, DNA mutations, and protein dysfunction.^[1] Antioxidants

are molecules that neutralize reactive oxygen species (ROS) or free radicals, thereby preventing damage to other cellular components.^[2] Vitamins A and E, along with carotenoids, are key antioxidants involved in these protective processes. Several antioxidants within these groups have been extensively studied for their potential clinical benefits. For instance, β -cryptoxanthin, a provitamin A carotenoid found in citrus fruits, exhibits both antioxidant and anti-inflammatory properties.^[3] Lycopene, a non-provitamin A carotenoid, is a potent singlet oxygen scavenger and has been shown in animal models to improve insulin resistance (IR) and reduce inflammation.^[4] Among vitamin E isoforms, α -tocopherol (the most biologically active form) and γ -tocopherol (a structurally distinct isoform) both inhibit nuclear factor kappa B (NF- κ B) activation, thereby attenuating inflammation and potentially influencing IR.^[5]

Due to these proposed anti-inflammatory and antioxidative mechanisms, supplementation with vitamins and carotenoids

The datasets generated during and/or analyzed during the current study are publicly available.

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has demonstrated clinical benefits. When used appropriately, these micronutrients play essential roles in maintaining immune function.^[6,7] Evidence from systematic reviews and meta-analyses supports their capacity to lower serum levels of C-reactive protein (CRP), interleukin (IL)-6, and tumor necrosis factor alpha (TNF- α). However, these effects may vary or even reverse depending on factors such as IR status, dosage, and age group.^[8–13]

In older adults, IR is frequently accompanied by increased antioxidant activity, a compensatory response to oxidative stress that disrupts insulin signaling pathways.^[14] Nevertheless, antioxidant defenses alone may be insufficient to meaningfully alter insulin sensitivity. Studies indicate that reduced oxidative stress and enhanced antioxidant interactions are more pronounced in individuals with IR.^[15] Furthermore, antioxidant-rich diets have been shown to reduce oxidative stress and improve metabolic syndrome parameters in older populations.^[16]

Given these findings, understanding the potential interaction between antioxidant status, inflammation, and IR is essential. This is one of the few studies examining specific antioxidant isoforms in the elderly population with a mediation framework, and this distinguishes it from other cohort studies, as many nutritional studies (including National Health and Nutrition Examination Survey [NHANES] dataset-based studies) did not highlight these pathways in older adults. Exploring this relationship in older adults—a population highly vulnerable to metabolic and inflammatory dysregulation—could provide insight into the mechanisms by which antioxidants influence metabolism. We hypothesize that CRP and IL-6 mediate the relationship between vitamin A, vitamin E isoforms, and carotenoids with IR. This study aims to clarify whether these antioxidants exert protective or adverse effects on IR in this demographic.

Materials and methods

Study population

The Midlife in the United States (MIDUS) study is a national longitudinal cohort initiated in 1995, enrolling over 7000 adults aged 25 to 75 years, with oversampling in five metropolitan areas. The primary objective of MIDUS is to investigate psychological, behavioral, and social determinants of age-related physical and mental health outcomes. Follow-up studies were conducted in 2009 (MIDUS2) and 2013 (MIDUS3), both incorporating biomarker collection.

This analysis utilized data from the MIDUS3 Biomarker Project (2017–2022). Participants were invited for a 24-hour inpatient assessment at one of three research facilities: the University of California, Los Angeles, the University of Wisconsin–Madison, or Georgetown University. Trained clinicians collected biological samples and performed comprehensive evaluations of musculoskeletal, neurological, immune, and other physiological systems. The MIDUS3 biomarker sample included 747 participants: 644 from the longitudinal cohort and 103 from a Milwaukee subsample, yielding an adjusted response rate of 64.3% (747/1162).

Study design

This study employed a mediation analysis with a cross-sectional design using variables exclusively derived from the MIDUS3 Biomarker dataset (2017–2022).

Study variables

The antioxidants included in the analysis were β -cryptoxanthin, total lycopene, α -tocopherol, γ -tocopherol, and retinol, all measured in $\mu\text{mol/L}$. Laboratory assays were performed at the Nutrition Evaluation Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University (Boston, MA). Plasma samples were extracted using chloroform/methanol followed by hexane, with echinenone and retinyl acetate in ethanol as internal standards. Antioxidant levels were quantified using reverse-phase gradient high-performance liquid chromatography (HPLC) with ultraviolet-visible (UV/VIS) detection and separated on a C30 carotenoid column (YMC America, Devens, MA, USA). Detailed assay protocols are available in the MIDUS3 biomarker documentation.^[17]

Covariates included sex, age, regular smoking history (yes/no), waist-hip ratio (WHR), total/HDL cholesterol ratio, physical activity (assessed using the total number of metabolic equivalent of task [MET] minutes per week), and dietary quality, which was assessed using the MIDUS Healthy Eating Index (MHEI). The MHEI was derived from a questionnaire evaluating weekly consumption of healthy foods (eg, fruits, vegetables, lean meats, fish, whole grains) and unhealthy foods (eg, fast foods, high-fat meals, sugary beverages). Higher consumption of healthy foods and lower consumption of unhealthy foods yielded higher scores, with total scores ranging from 0 to 11, where higher scores indicated healthier dietary habits. The raw MHEI score was used in the analyses.

The dependent variable, Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), was pre-calculated in the MIDUS3 dataset^[18] and log-transformed in our analysis to reduce skewness. The mediators were CRP and IL-6. CRP levels were measured using the V-PLEX Plus Human CRP Kit (cat# K151STG, Meso Scale Discovery, Rockville, MD, USA), and IL-6 was measured with the V-PLEX Plus Proinflammatory Panel 1 Human Kit (cat# K15049G, Meso Scale Discovery, Rockville, MD, USA). Additional assay details about these inflammatory markers and about lab-based covariates are available in the MIDUS3 biomarker documentation.^[17]

Weighting variables were not provided in the MIDUS3 biomarker dataset; therefore, weighted analyses were not performed.

Statistical analysis

Descriptive statistics were computed to summarize participant characteristics. Continuous variables with normal distributions are presented as mean $\pm$ standard deviation (SD), while skewed variables are summarized as median (interquartile range [IQR]); all variables were reported using both measures to facilitate data interpretation. Categorical variables are expressed as counts and percentages.

Mediation analysis was conducted to assess the indirect and total effects of antioxidants on HOMA-IR *via* CRP and IL-6. Bias-corrected bootstrapping with 5000 replications was used to calculate 95% confidence intervals (CIs), providing robust effect estimation. Prior to the analysis, CRP values above 20 were excluded and considered outliers, and physical activity was ranked into four quartiles to meet the analysis assumptions. A different sensitivity analysis with a more conservative cutoff (CRP >10 being excluded) was also conducted to ensure accurate results. Assumptions of mediation analysis, such as linearity and homoskedasticity, were verified, and full information

maximum likelihood (FIML) estimation was applied to handle missing data. Continuous predictors were mean-centered to avoid collinearity. For clinical interpretability, coefficients for predictors of HOMA-IR were reported as percentage change per 10-unit increase in the predictor (per 0.1-unit increase for WHR, 5-unit increase in MIDUS HEI, and 2-unit increase in total/HDL ratio).

A two-sided *P* value of <0.05 was considered statistically significant. CIs not containing zero were interpreted as significant. Analyses were performed using Jamovi software, version 2.6.13 (The Jamovi Project, 2024).

Ethical approval

This investigation was a secondary analysis of publicly available MIDUS data. All MIDUS study protocols were approved by the Institutional Review Board of the University of Wisconsin–Madison.

Results

The total number of participants in our study was 747, with the majority (56%) being females and a mean age of 66 years (SD = 9.6 years). Basic features are displayed in Table 1.

The mediation analysis revealed that total lycopene and β -cryptoxanthin were both significantly associated with lower HOMA-IR levels. In contrast, γ -tocopherol was significantly associated with higher HOMA-IR. Although these total effects were significant, no evidence of mediation by CRP or IL-6 was found for the associations between lycopene and β -cryptoxanthin and HOMA-IR.

For γ -tocopherol, the relationship with HOMA-IR was partially mediated by elevated inflammatory markers, with significant indirect effects through CRP (percent change per 10-unit

increase: 7.79%; 95% CI: 2.12–18.53), but not IL-6 (Table 2). Simple mediation follow-up analysis revealed that the percentage mediated by CRP was 21.10%.

Although the total effects of retinol on HOMA-IR were not significant, it demonstrated significant indirect associations, indicating indirect-only mediation through CRP and IL-6. Specifically, significant negative indirect effects were observed through CRP (–31.48%; 95% CI: –50.24 to –13.50) and IL-6 (–15.46%; 95% CI: –33.57 to –1.49), though these did not result in a significant overall effect. Simple mediation follow-up analysis revealed that the percentage mediated by CRP was 39.89%, and by IL-6 was 38.13%.

Notably, α -tocopherol showed no significant total or indirect association with HOMA-IR (Figure 1).

Additionally, several covariates demonstrated significant indirect associations with HOMA-IR through CRP and IL-6 pathways. The absence of regular smoking and higher levels of physical activity were inversely associated with HOMA-IR *via* an indirect-only mediation of lower IL-6 and CRP. In contrast, higher total/HDL cholesterol and WHRs were positively associated with HOMA-IR, as partially mediated by elevated IL-6 and CRP levels (see Table 2 and component paths in Table 3).

A sensitivity analysis excluding CRP levels >10 revealed similar results to these, with no change in the significance of any of the paths [Supplementary Table S1, <https://links.lww.com/PN9/A88>].

Notably, antihyperlipidemic and antidiabetic medications were excluded from the final model to mitigate the risk of confounding by indication and collider bias. In such instances, medication use can be positively associated with IR—not as a causal effect of the pharmacological agent, but as a reflection of the severity of the underlying pathology. The inclusion of these variables has

Table 1
Baseline characteristics of participants.

Sex	Counts		% of Total		
Male	330		44%		
Female	417		56%		
History of regular smoking (yes/no)					
Yes	329		44%		
No	418		56%		
Descriptives of continuous variables					
	Mean	SD	Median	Percentiles	
				Q1	Q3
HOMA-IR*	4.28	6.81	2.48	1.43	4.3
HOMA-IR (log-transformed)	0.98	0.88	0.91	0.36	1.46
Age (years)	65.96	9.64	66	58	72
MIDUS healthy eating index score	5.46	1.5	5.5	4.5	6.5
Total/HDL cholesterol ratio*	3.54	1.16	3.33	2.73	4.08
Total number of MET (min/week)*	1223.5	1657.7	666	0	1620
WHR	0.91	0.1	0.91	0.84	0.98
IL-6 (pg/mL)*	3.64	3.07	2.77	1.75	4.43
CRP (μ g/mL)*	4.05	5.81	1.77	0.77	4.53
Beta cryptoxanthine (μ mol/L)*	0.14	0.14	0.11	0.06	0.16
Total lycopene (μ mol/L)	1.1	0.54	1.03	0.71	1.4
γ -Tocopherol (μ mol/L)	4.47	2.27	4.13	2.75	5.76
α -Tocopherol (μ mol/L)	30.62	11.76	28.66	22.91	35.76
Retinol (μ mol/L)	2.01	0.57	1.96	1.63	2.33

*Not normally distributed. CRP, C-reactive protein; HDL, high-density lipoprotein; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; IL, interleukin; MET, metabolic equivalent of task; MIDUS, Midlife in the United States; SD, standard deviation; WHR, waist–hip ratio.

Table 2

Mediation effect of CRP and IL-6 in the associations between vitamin A, vitamin E, and carotenoids and insulin resistance in older adults.

Effects type			95% CI				
	Effect	Percentage change in HOMA-IR†	Lower	Upper	β	Significance	
Indirect	Sex (female) ⇒ CRP ⇒ HOMA-IR	5.22	1.85	10.36	0.029	*	
	Sex (female) ⇒ IL-6 ⇒ HOMA-IR	1.29	−0.01	4.10	0.007		
	Smoking (no) ⇒ CRP ⇒ HOMA-IR	−2.26	−5.04	−0.74	−0.013	*	
	Smoking (no) ⇒ IL-6 ⇒ HOMA-IR	−0.99	−2.71	−0.03	−0.006	*	
	MIDUS healthy eating index ⇒ CRP ⇒ HOMA-IR	−0.30	−3.63	2.79	−0.001		
	MIDUS healthy eating index ⇒ IL-6 ⇒ HOMA-IR	−0.30	−4.21	0.30	−0.004		
	Age ⇒ CRP ⇒ HOMA-IR	0.40	−0.30	1.51	0.005		
	Age ⇒ IL-6 ⇒ HOMA-IR	0.40	0.10	3.46	0.019	*	
	Total/HDL cholesterol ratio ⇒ CRP ⇒ HOMA-IR	3.56	1.33	7.06	0.023	*	
	Total/HDL cholesterol ratio ⇒ IL-6 ⇒ HOMA-IR	3.56	0.08	4.21	0.010	*	
	Waist–hip ratio ⇒ CRP ⇒ HOMA-IR	1.82	0.57	3.89	0.020	*	
	Waist–hip ratio ⇒ IL-6 ⇒ HOMA-IR	1.82	0.03	2.37	0.009	*	
	Physical activity ⇒ CRP ⇒ HOMA-IR	−1.02	−2.41	−0.20	−0.013	*	
	Physical activity ⇒ IL-6 ⇒ HOMA-IR	−0.88	−1.84	−0.07	−0.012	*	
	γ-Tocopherol ⇒ CRP ⇒ HOMA-IR	7.79	2.12	18.53	0.019	*	
	γ-Tocopherol ⇒ IL-6 ⇒ HOMA-IR	2.53	−0.10	8.33	0.007		
	α-Tocopherol ⇒ CRP ⇒ HOMA-IR	−0.10	−0.90	0.60	−0.001		
	α-Tocopherol ⇒ IL-6 ⇒ HOMA-IR	−0.40	−1.39	0.10	−0.005		
	Retinol ⇒ CRP ⇒ HOMA-IR	−31.48	−50.24	−13.50	−0.024	*	
	Retinol ⇒ IL-6 ⇒ HOMA-IR	−15.46	−33.57	−1.49	−0.011	*	
	Total lycopene ⇒ CRP ⇒ HOMA-IR	−9.43	−32.83	11.07	−0.004		
	Total lycopene ⇒ IL-6 ⇒ HOMA-IR	−13.15	−37.50	0.10	−0.006		
	β-Cryptoxanthin ⇒ CRP ⇒ HOMA-IR	−16.64	−60.15	59.20	−0.003		
	β-Cryptoxanthin ⇒ IL-6 ⇒ HOMA-IR	−12.28	−50.88	35.26	−0.002		
Direct	Sex (female) ⇒ HOMA-IR	18.98	2.00	38.26	0.098	*	
	Smoking (no) ⇒ HOMA-IR	0.14	−10.44	12.31	0.001		
	MIDUS healthy eating index ⇒ HOMA-IR	−0.85	−20.07	25.29	−0.003		
	Age ⇒ HOMA-IR	−6.29	−12.28	0.50	−0.072		
	Total/HDL cholesterol ratio ⇒ HOMA-IR	32.34	17.14	48.77	0.184	*	
	Waist–hip ratio ⇒ HOMA-IR	31.21	21.18	42.79	0.305	*	
	Physical activity ⇒ HOMA-IR	−3.71	−8.94	2.08	−0.049		
	γ-Tocopherol ⇒ HOMA-IR	63.56	24.23	117.06	0.127	*	
	α-Tocopherol ⇒ HOMA-IR	−2.86	−8.06	2.94	−0.038		
	Retinol ⇒ HOMA-IR	111.28	−42.94	638.91	0.048		
	Total lycopene ⇒ HOMA-IR	−81.58	−97.20	14.45	−0.066		
	β-Cryptoxanthin ⇒ HOMA-IR	−97.65	−99.97	−30.44	−0.061	*	
	Total	Sex (female)⇒ HOMA-IR	26.81	8.95	47.12	0.134	*
		Smoking (no) ⇒ HOMA-IR	−3.09	−13.64	8.62	−0.018	
		MIDUS Healthy Eating Index ⇒ HOMA-IR	−2.32	−21.53	23.18	−0.008	
		Age ⇒ HOMA-IR	−4.30	−10.33	2.53	−0.048	
Total/HDL cholesterol ratio ⇒ HOMA-IR		39.12	23.84	56.33	0.216	*	
Waist–hip ratio ⇒ HOMA-IR		34.69	24.56	46.37	0.334	*	
Physical activity ⇒ HOMA-IR		−5.53	−10.57	0.04	−0.074		
γ-Tocopherol ⇒ HOMA-IR		80.76	36.21	139.89	0.153	*	
α-Tocopherol ⇒ HOMA-IR		−3.34	−8.52	2.33	−0.045		
Retinol ⇒ HOMA-IR		22.38	−64.97	337.98	0.013		
Total lycopene ⇒ HOMA-IR	−85.51	−97.78	−9.43	−0.075	*		
β-Cryptoxanthin ⇒ HOMA-IR	−98.28	−99.98	−47.32	−0.067	*		

95% CI calculation: bias-corrected bootstrap (5000 rep). β s are completely standardized effect sizes for log-HOMA-IR.

* $P < 0.05$.

†Reported per 5-unit increase in MIDUS HEI, 2-unit increase in total/HDL ratio, 0.1 increase in WHR, and 10-unit increase in other continuous covariates, only for paths ending in HOMA-IR. CI, confidence interval; CRP, C-reactive protein; HDL, high-density lipoprotein; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; IL, interleukin; MIDUS, Midlife in the United States.

yielded biased estimates for both the medications and the other covariates in the model [Supplementary Table S2, <https://links.lww.com/PN9/A88>]. We addressed these underlying clinical factors independently: dyslipidemia was quantified *via* the total/HDL ratio, and glycemic status was captured by the HOMA-IR outcome itself.

Discussion

Our analysis revealed differential effects of fat-soluble vitamins and carotenoids on IR in older adults, with distinct

roles for inflammatory markers in mediating these associations. Specifically, CRP mediated the relationship between γ -tocopherol and IR, such that γ -tocopherol levels were associated with elevated CRP, resulting in higher HOMA-IR values. A potential physiological mechanism involves dietary intake of γ -tocopherol and omega-6 polyunsaturated fatty acids—commonly found in vegetable oils (eg, corn, soybean, sesame) and nuts (eg, walnuts, pecans, peanuts).^[19] High intake of these unsaturated fats may induce oxidative cellular stress with potential atherogenic effects, disrupting normal vascular endothelial function and activating NF- κ B, an oxidative stress-sensitive

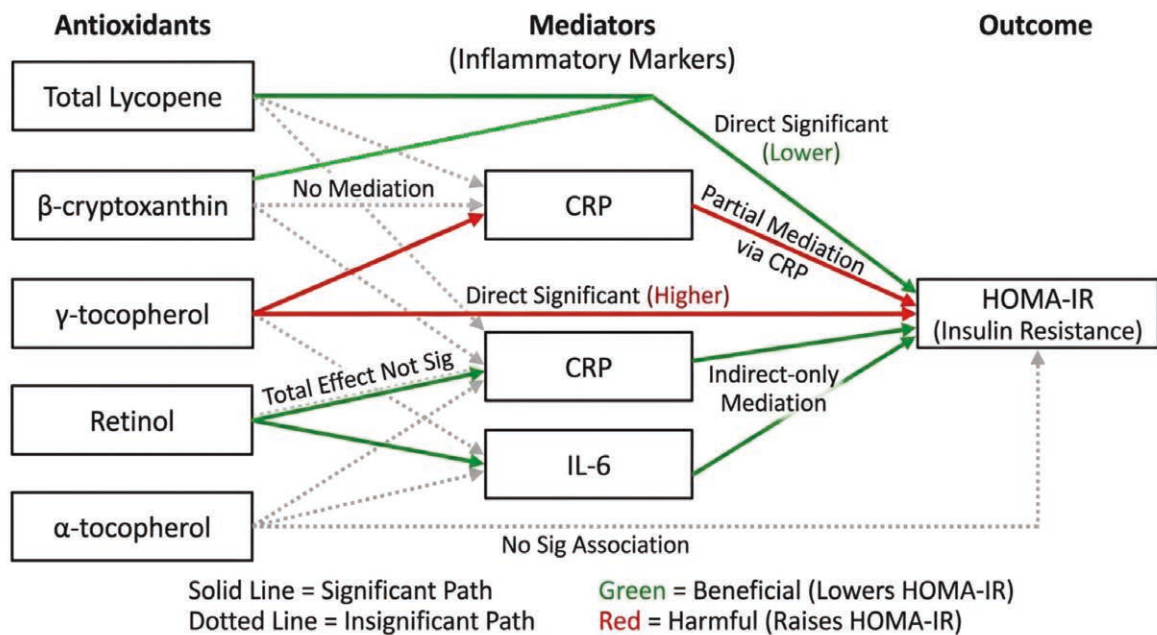


Figure 1: Path diagram showing associations between serum antioxidants and insulin resistance in older adults after controlling for confounding variables. CRP, C-reactive protein; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; IL, interleukin.

Table 3

Component paths showing the associations between the predictor variables and mediators, and between mediators and HOMA-IR.

Effect	Percentage change in HOMA-IR†	Lower	Upper	β	Significance
Sex (female) ⇒ CRP	1.70	0.98	2.41	0.226	
CRP ⇒ HOMA-IR	34.85	11.63	63.89	0.127	*
Sex (female) ⇒ IL-6	0.54	-0.01	1.06	0.094	
IL-6 ⇒ HOMA-IR	26.74	0.50	56.67	0.077	*
Smoking (no) ⇒ CRP	-0.77	-1.33	-0.24	-0.102	*
Smoking (no) ⇒ IL-6	-0.42	-0.82	-0.03	-0.072	*
MIDUS healthy eating index ⇒ CRP	-0.02	-0.21	0.18	-0.009	
MIDUS healthy eating index ⇒ IL-6	-0.10	-0.25	0.04	-0.052	
Age ⇒ CRP	0.02	-0.01	0.04	0.039	
Age ⇒ IL-6	0.07	0.05	0.10	0.244	*
Total/HDL cholesterol ratio ⇒ CRP	0.59	0.32	0.87	0.181	*
Total/HDL cholesterol ratio ⇒ IL-6	0.31	0.09	0.56	0.126	*
Waist-hip ratio ⇒ CRP	6.03	2.41	9.74	0.160	*
Waist-hip ratio ⇒ IL-6	3.41	0.17	6.38	0.118	*
Physical activity ⇒ CRP	-0.34	-0.60	-0.09	-0.105	*
Physical activity ⇒ IL-6	-0.37	-0.55	-0.20	-0.149	*
γ-Tocopherol ⇒ CRP	0.25	0.12	0.40	0.153	*
γ-Tocopherol ⇒ IL-6	0.11	-0.01	0.24	0.085	
α-Tocopherol ⇒ CRP	0.00	-0.03	0.02	-0.011	
α-Tocopherol ⇒ IL-6	-0.02	-0.04	0.01	-0.069	
Retinol ⇒ CRP	-1.26	-1.78	-0.80	-0.190	*
Retinol ⇒ IL-6	-0.71	-1.14	-0.30	-0.140	*
Total lycopene ⇒ CRP	-0.33	-1.04	0.45	-0.030	
Total lycopene ⇒ IL-6	-0.60	-1.25	-0.02	-0.071	*
β-Cryptoxanthin ⇒ CRP	-0.61	-2.36	1.87	-0.023	
β-Cryptoxanthin ⇒ IL-6	-0.55	-2.22	1.51	-0.028	

95% CI calculation: bias-corrected bootstrap (5000 rep). βs are completely standardized effect sizes for Log-HOMA-IR.

* $P < 0.05$.

†Reported per 5-unit increase in MIDUS HEI, 2-unit increase in total/HDL ratio, 0.1 increase in WHR, and 10-unit increase in other continuous covariates, only for paths ending in HOMA-IR. CI, confidence interval; CRP, C-reactive protein; HDL, high-density lipoprotein; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; IL, interleukin; MIDUS, Midlife in the United States.

nuclear transcription factor.^[20] Activation of NF-κB promotes IL-6 transcription, which in turn induces CRP expression in hepatocytes.^[21,22] Elevated CRP levels subsequently contribute to IR, as established in previous literature.^[23–26]

Supporting these findings, Ford *et al*^[27] analyzed data from the NHANES and found that, among individuals not taking supplements, higher γ-tocopherol levels were associated with poorer glycemic control—higher fasting glucose and HbA1c—in US

adults without diabetes. Additionally, a recent study reported that at vitamin E levels near 10,575 ng/mL—close to the mean α -tocopherol levels and higher than the mean γ -tocopherol levels in our study—vitamin E acts as a risk factor, rather than a protective factor, for IR in diabetic patients.^[28] These clinical observations align with our findings and suggest that elevated γ -tocopherol may negatively affect insulin sensitivity in older adults, consistent with the physiological mechanisms described earlier.

In contrast, in alignment with previous meta-analyses,^[29] α -tocopherol exhibited no significant total or indirect effects on IR. However, given evidence that α -tocopherol supplementation may elevate cholesterol and other lipid parameters,^[28,30] our model adjusted for the total/HDL cholesterol ratio. This adjustment clarified that dyslipidemia—rather than α -tocopherol itself—was the primary driver of IR, operating through a pathway mediated by elevated CRP and IL-6. These findings suggest that α -tocopherol may exert an influence on HOMA-IR indirectly by contributing to dyslipidemia, which in turn exacerbates IR *via* inflammatory pathways.

Another important finding was the inverse association between β -cryptoxanthin and HOMA-IR, which was not mediated by CRP or IL-6. This aligns with previous studies demonstrating the protective effects of β -cryptoxanthin against IR.^[31,32] Mechanistically, β -cryptoxanthin enhances insulin sensitivity by modulating multiple biological pathways: it downregulates NF- κ B and TNF- α in the context of a high-fat diet, activates the nuclear factor erythroid 2-related factor 2/heme oxygenase-1 (Nrf2/HO-1) antioxidant signaling pathway in adipose tissue, upregulates peroxisome proliferator-activated receptor alpha (PPAR- α) to reduce oxidative stress, and improves insulin signaling through increased phosphorylation of insulin receptor substrate (IRS)-1.^[3] Thus, β -cryptoxanthin appears to improve IR in older adults *via* anti-inflammatory and metabolic mechanisms, independent of CRP and IL-6 modulation. These pathways were not directly measured in our study but were inferred based on prior evidence.

Similarly, Total lycopene showed protective associations comparable to those observed for β -cryptoxanthin. A recent meta-analysis indicated that lycopene may ameliorate IR through multiple mechanisms independent of CRP and IL-6. Lycopene acts as a potent antioxidant, neutralizing ROS, protecting pancreatic β -cells from oxidative damage, preserving insulin secretion, regulating lipid metabolism by reducing lipogenesis and enhancing fatty acid oxidation, improving mitochondrial function, and promoting glucose uptake *via* increased adiponectin levels.^[33] These multifaceted effects highlight lycopene's potential to improve insulin sensitivity through mechanisms beyond systemic inflammation.

For retinol, the total effect on HOMA-IR was not significant. However, retinol exhibited an indirect-only mediation effect *via* CRP and IL-6, indicating a potential indirect role in reducing IR by lowering systemic inflammation. The lack of a significant total effect is consistent with the heterogeneous findings in the literature, where retinol's effects on metabolic disease are variable or contradictory.^[34] Some studies even report that elevated retinol levels are associated with increased type 2 diabetes risk mediated by IR.^[35] Our results underscore the importance of chronic, low-grade systemic inflammation, as reflected by CRP and IL-6, as a key driver of IR in older adults.^[36] The rigorous adjustment for multiple antioxidants and relevant confounders in our analysis likely attenuated the direct association between retinol and

HOMA-IR, revealing its indirect inflammation-mediated effect that might be overlooked in less controlled studies.

Another result is the partial mediation of CRP for the association between female sex and HOMA-IR. This finding was similarly seen in younger females in a prior study, where females, but not males, had a significant association between CRP and HOMA-IR.^[37] The study attributed this finding to lower altered hepatic insulin clearance in females, which makes them more vulnerable to IR and subsequently atherosclerotic disease. At the postmenopausal level, this finding is more important considering the lower levels of protective hormones like estrogen, highlighting another reason for considering the beneficial use of supplemental antioxidants in older adults.

Clinical implications

Our findings support the consumption of carotenoid-rich foods—particularly β -cryptoxanthin and lycopene—for preventing and managing IR in elderly populations. β -Cryptoxanthin is abundant in orange and red fruits such as tangerines, mangoes, oranges, and peaches, as well as in herbs like spearmint and cilantro.^[38–40] Lycopene is concentrated in red fruits and vegetables, including papaya, pink grapefruit, and especially tomatoes.^[41] Encouraging regular intake of diverse carotenoid-rich foods represents a practical strategy to mitigate IR and promote metabolic health.^[42] Policy measures and financial support may be necessary to ensure that older adults with limited resources can access these foods. Additionally, primary care providers should integrate dietary counseling on carotenoid-rich foods into metabolic health management for the elderly.

While vitamin E isoforms possess antioxidant and anti-inflammatory properties, γ -tocopherol supplementation or high dietary intake should be approached with caution. Unlike α -tocopherol—the dominant form in supplements— γ -tocopherol is the primary dietary form but has been less studied independently. Emerging evidence suggests elevated γ -tocopherol may have long-term cardiometabolic effects, including higher systolic blood pressure and increased risk for diabetes-related cardiovascular complications,^[43] and cohort studies link high serum γ -tocopherol with all-cause mortality in older adults.^[44] Although γ -tocopherol can reduce inflammatory signaling in adipocyte-macrophage models,^[45] its safety and efficacy in diabetic or elderly populations remain uncertain. Based on our findings and existing literature, we do not recommend γ -tocopherol supplementation or γ -tocopherol-rich diets for IR management in elderly populations until further evaluation is conducted.

Retinol exhibited modest beneficial effects on IR, consistent with findings that rosiglitazone and fenretinide, a synthetic retinoid, improve insulin sensitivity.^[46] However, excessive retinol (>80 μ g/dL) in older adults is associated with increased all-cause and cardiovascular mortality.^[47] Therefore, while retinol may confer metabolic benefits, its supplemental use in elderly populations should be approached with caution.

Limitations

One of the limitations of our study is the cross-sectional design. Ideally, mediation analysis is best suited for temporal events. Nevertheless, physiologically, the presence of antioxidants and metabolic alterations in serum precedes the rise of inflammatory markers and subsequent IR, making our model justified.

Another limitation in our study is that no weighting variables were available in the dataset, and thus, analyses were unweighted, limiting the ability of our findings to be representative of the whole population (ie, American older adults). Also, although we have used bootstrapping to obtain robust confidence intervals, there is still a probability of inflation for type I error due to multiple mediation paths. Due to data constraints in the MIDUS 3 biomarkers dataset, we could not adjust for supplemental antioxidant intake.

Conclusion

Our study demonstrates that CRP and IL-6 mediate the associations between specific antioxidants and IR in older adults. These findings elucidate important pathophysiological mechanisms and highlight potential therapeutic and dietary strategies for managing IR. Carotenoid-rich diets, particularly those including β -cryptoxanthin and lycopene, appear beneficial, whereas γ -tocopherol intake should be carefully monitored. Further longitudinal research is warranted to evaluate the long-term metabolic and clinical outcomes of these micronutrients in elderly populations.

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Author Contributions

L. Ashour contributed to conceptualization, data acquisition, formal analysis, project administration, and supervision. ZA, AA, AAA, YA contributed to literature review, gap identification, critical input on objectives/concept, and drafting related sections. SB, KAZ, MM, TK, NFA-N, TA, L. Akileh contributed to interpretation of results, determining significance/implication sections, and drafting the related sections. All the authors validated results, reviewed and approved the final manuscript, and are accountable for all aspects of the work. The author sequence reflects scientific contribution, approved by all.

Conflicts of Interest

None.

Generative AI Use Statement

AI tool (ChatGPT) utilized solely for language enhancement and readability. To display a summary of the main results, Figure 1

was constructed with assistance from an AI tool (Gemini 3 Pro). Authors reviewed, edited, and retain full responsibility for the final content.

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