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CLINICAL RESEARCH

The Effect of Randomized Beta-Carotene Supplementation on CKD in Men

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Introduction: Beta-carotene (BC) protects the body against free radicals that may damage the kidney and lead to the development of acute kidney injury and chronic kidney disease (CKD). Previous studies in animal models have demonstrated a potential protective effect of 30 mg/kg BC supplementation on renal ischemia or reperfusion injury and subsequently improved kidney function. The extension of these findings to humans, however, remains unclear.

Methods: Our study leverages previously collected data from the Physicians' Health Study I (PHS I), a large-scale, long-term, randomized trial of middle-aged and older US male physicians testing 50 mg BC every other day for primary prevention of cardiovascular disease and cancer. We examined the impact of randomized BC supplementation on self-reported incident CKD identified by self-reports stating "yes" to kidney disease from annual follow-up questionnaires from randomization in 1982 through the end of the randomized BC intervention at the end of 1995, and on CKD defined as an estimated glomerular filtration rate (eGFR) $<$ 60 ml/min per 1.73 m^2 at the end of 1995. Analyses compared incident CKD between BC supplementation and placebo using Cox proportional hazards regression models and logistic regression. We also examined whether smoking status (current vs. former or never smoker) or other factors modified the effect of randomized BC supplementation on CKD.

Results: A total of 10,966 participants were randomized to BC, and 10,952 participants were randomized to a placebo group. Baseline characteristics between randomized BC groups were similar. There was no significant benefit between BC supplementation and self-reported incident CKD after adjusting for age and randomized aspirin treatment (hazard ratio [HR] = 0.97, 95% confidence interval [CI]: 0.86–1.08, *P*-value = 0.56). Stratified by smoking status, there was no significant benefit of BC supplementation and self-reported incident CKD either among former or never smokers (HR = 0.95, 95% CI: 0.84–1.07, *P*-value = 0.41) or current smokers (HR = 1.08, 95% CI: 0.78–1.50, *P*-value = 0.64). Smoking status did not modify the association between BC supplementation and incident CKD (*P*-interaction = 0.47). In subgroup analysis among those with available serum creatinine at the study end (5480 with BC and 5496 with placebo), there was no significant benefit between BC supplementation and CKD based on eGFR $<$ 60 ml/min per 1.73 m^2 (odds ratio [OR] = 0.96, 95% CI: 0.85–1.08, *P*-value = 0.49).

Conclusion: Long-term randomized BC supplementation did not affect the risk of incident CKD in middle-aged and older male physicians.

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CKD affects 15% of the US adult population and is commonly underrecognized by both patients and physicians.[1,2] CKD is associated with a higher risk of cardiovascular disease, end-stage kidney disease, infection, malignancy, degenerative brain diseases, and mortality.[3] Therefore, early detection and prevention of CKD are pivotal. The production of free radicals and reactive oxygen species as byproducts from mitochondria can damage kidneys and lead to the development of acute kidney injury and CKD.[4,5] Therefore,

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interventions that can mitigate oxidative stress may help prevent CKD.

BC is one of the most important provitamin A carotenoids that can protect the body against free radicals.[6] Dietary BC is primarily obtained through fruit and vegetable intake.[7] Typically, dietary intake of vitamin A, including BC, is adequate in most countries and does not need to be supplemented.[8] However, about 30% of the general population has reported using individual dietary supplements or more commonly multivitamin-multimineral supplements that contain vitamin A in the form of retinol or BC.[9] Even though BC supplementation was hypothesized to help reduce cardiovascular disease and cancer due in part to its antioxidative effects, completed large-scale clinical trials reported no benefit for BC in preventing cancers, and the meta-analysis of 8 randomized trials showed a significant increase in all-cause and cardiovascular mortality with BC supplementation.[10-12] Previous studies also reported that BC supplementation increased the risk of lung cancer among smokers, suggesting a potential interaction between BC and cigarette smoking.[13,14]

Animal models indicate that BC pretreatment at 30 mg/kg may protect against renal ischemia or reperfusion injury in rats, leading to an improvement in kidney function[15] perhaps due to its scavenging of excess free radicals. BC reduces oxidative stress that increases the accumulation of extracellular matrix proteins, podocyte injury, mesangial expansion, tubulointerstitial fibrosis, and glomerulosclerosis which leads to CKD.[16] BC serves as a precursor to retinoic acid, which helps regulate inflammation and may reduce renal fibrosis, podocyte injury, and diabetic nephropathy based on mechanistic studies.[17,18] Therefore, BC supplementation may mitigate the development of CKD. To our knowledge, no clinical trials have investigated the potential benefit of BC in preventing kidney damage and CKD. Our hypothesis was that BC supplementation might help prevent CKD without increasing the risk of CKD among smokers.

We, therefore, examined data from PHS I, a randomized clinical trial originally testing BC and aspirin on the primary prevention of cardiovascular disease and cancer, to examine in *post hoc* analyses the effect of long-term BC supplementation on self-reported incident CKD among healthy male adults, and CKD based on eGFR $<$ 60 ml/min per 1.73 m^2. We also explored the role of smoking status and other selected variables for potential effect modification on randomized BC and CKD.

METHODS

Study Population

PHS I was a randomized, double-blinded, placebo-controlled, 2$\times$2 factorial trial of low-dose aspirin (325 mg on alternate days in the form of Bufferin; provided by Bristol-Myers Products, New York, NY), and BC (50 mg on alternate days in the form of Lurotin; supplied by BASF Corporation, Mt Olive, NJ), in the primary prevention of cardiovascular disease and cancer among 22,071 healthy male physicians between 40 and 84 years old. Study participants had no previous history of cardiovascular disease, cancer (except nonmelanoma skin cancer), current liver disease, or other significant illnesses, and were equally and randomly allocated to active aspirin, active BC, both active agents, or both placebos. Furthermore, participants who reported current kidney disease were excluded from our study (Figure 1). Information was collected at baseline in 1981 from self-reports on mailed questionnaires on height, weight, history of cigarette smoking, alcohol use, blood pressure, cholesterol, history of diabetes mellitus, physical activity, and multivitamin use. Participants completed annual follow-up questionnaires regarding compliance with the treatment regimens and the occurrence of study outcomes. Informed consent was obtained from all participants. The research protocol was reviewed and approved by the Institutional Review Board at Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA.

The aspirin arm of PHS I was terminated early, on January 25, 1988, after a mean follow-up of 60.2 months, based on a statistically extreme 44% reduction in risk of a first myocardial infarction among those randomized to active aspirin. The randomized BC arm was continued until completion on December 31, 1995. At that time, 99.2% of the randomized participants were still providing information on morbidity, and mortality follow-up was virtually 100% complete. In both the BC and placebo groups, 80% of participants were still taking the study pills, with mean compliance among pill takers of more than 97%. In the placebo group, 6% reported taking supplemental BC or vitamin A. The validity of reported compliance with the assigned treatment was assessed by measuring plasma BC concentrations in blood obtained at unannounced visits to a small sample of participants in 3 geographic areas. Those assigned to BC group had significantly higher mean plasma BC levels than those receiving placebo (2.24 vs. 0.56 mmol/l, $P < 0.001$).[19]

Ascertainment and Definition of End Points

The primary outcome was self-reported incident CKD identified by self-reports stating "yes" to "Have you been newly diagnosed as having kidney disease" or reporting "chronic kidney disease" or "kidney failure" to "Other new conditions requiring medical treatment" from annual follow-up questionnaires reported from randomization in 1982 through the end of the

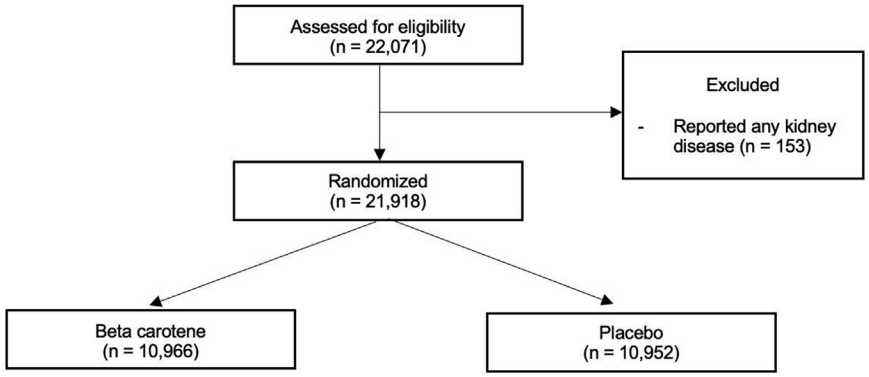

Figure 1. Flow chart of randomized Physicians' Health Study I participants for the primary outcome of self-reported chronic kidney disease.

randomized BC intervention at the end of 1995 for PHS I participants. The average period of follow-up was 12.1 years.

The secondary outcome of interest was CKD, defined as an eGFR $<$ 60 ml/min per 1.73 m^2 at the end of 1995. eGFR was calculated using the CKD-Epidemiology Collaboration creatinine equation 2021[20] based upon blood collected in a large subset of 11,067 PHS I participants at the end of the PHS I randomized BC intervention. There were 4759 participants who had serum creatinine available at baseline.[21] Among those with available serum creatinine at baseline, we excluded 91 participants with eGFR $<$60 ml/min per 1.73 m^2 and 38 participants who opted out of analyses (Figure 2). Serum creatinine was obtained from blood drawn into the vacuum tube containing ethylenediaminetetraacetic acid between December 1995 and October 1997. Each sample was centrifuged, divided into aliquots, and stored at -82 °C. Samples were sent to Oxford University, England, for serum creatinine measurements using an automated Jaffe rate method on a SYNCHRON LX20 autoanalyzer (Beckman Coulter, Fullerton, CA). Split samples were also included for quality control. The difference in means between the study samples and the repeat quality control samples was 0.018 $\pm$ 0.67 mg/dl. Intrabatch coefficients of variation on internal quality control runs were 1.4% to 3.6%.[22]

Statistical Analyses

Descriptive statistics of baseline characteristics were summarized and compared with a *t*-test or χ^2 test. Cox proportional hazard models estimated the HRs and 95% CIs for associations between BC supplementation and self-reported CKD. Logistic regression models estimated ORs and 95% CIs for associations between BC

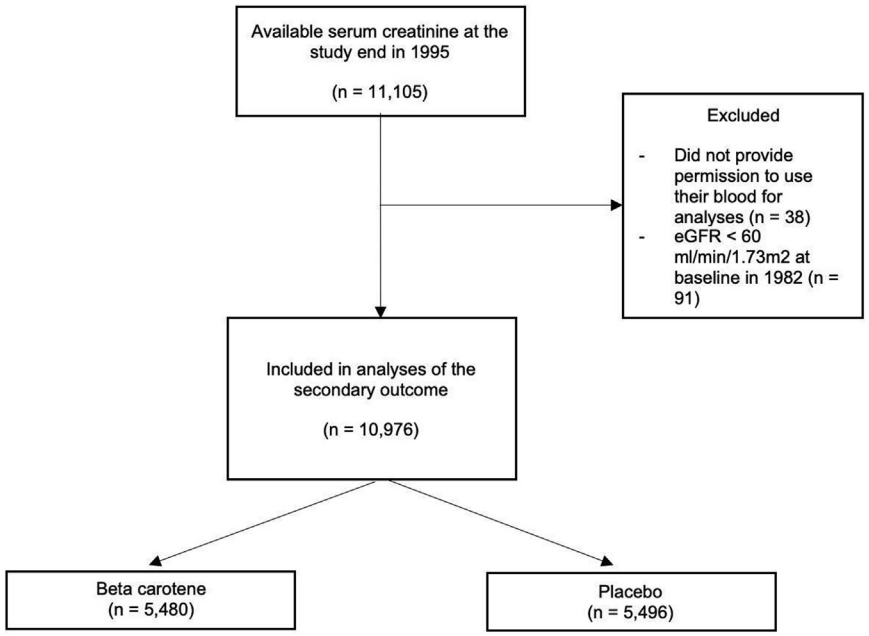

Figure 2. Flow chart of Physicians' Health Study I participants with serum creatinine available at the study end included in the analysis of the secondary outcome of eGFR $<$ 60 ml/min per 1.73 m^2. eGFR, estimated glomerular filtration rate.

supplementation and CKD after excluding 91 participants with eGFR < 60 ml/min per 1.73 m^2 at baseline out of 4759 participants with serum creatinine available at baseline. Cox proportional hazard and logistic regression models were adjusted for age and random assignment to aspirin or placebo group in the aspirin component of the trial. Sensitivity analyses of these 2 models were performed by adjusting for age; race; random assignment to aspirin or placebo group in the aspirin component of the trial; health behaviors, including smoking status, physical activity, and alcohol status; body mass index; diabetes mellitus; hypertension; and high cholesterol.

We assessed subgroup analyses by smoking status to explore whether there were any differential effects of BC supplementation on self-reported incidental CKD among smokers and nonsmokers using Cox proportional hazard models, including product term between BC supplementation and smoking status (current smokers vs. never/former smokers).

Furthermore, we also examined subgroup analyses by baseline serum creatinine availability, dietary BC intake at baseline, incident diabetes, and incident hypertension to explore whether there were any differential effects of BC supplementation on self-reported incidental CKD or CKD based on eGFR.

All analyses were performed in SAS 9.4 (SAS Institute, Cary, NC). Results were considered statistically significant if a 2-sided $\alpha < 0.05$ threshold was reached.

RESULTS

Among 22,071 healthy male physicians, there were additional 153 participants reporting having kidney disease at baseline, which were excluded from our study. A total of 10,966 participants were randomized to BC, and 10,952 participants were randomized to placebo. Baseline characteristics between randomized BC groups were similar (Table 1). Baseline serum creatinine was 1.0 (0.9–1.1) in both groups. There were 91 participants with baseline eGFR < 60 ml/min per 1.73 m^2. Serum creatinine was available at the study end among 11,067 participants (5520 taking BC and 5547 taking placebo).

Table 1. Baseline characteristics comparing the randomized beta-carotene supplementation group and placebo

Baseline characteristics	Active beta-carotene (n = 10,966)	Placebo (n = 10,952)	P-value
Age, mean (SD), yr	53.8 (9.5)	53.8 (9.6)	0.93
BMI, mean (SD), kg/m^2	24.8 (2.8)	24.8 (2.8)	0.33
Serum creatinine in 1982, mean (SD), mg/dl	1.02 (0.15)	1.02 (0.15)	0.37
Randomized to aspirin, n (%)			0.98
Placebo	5490 (50)	5481 (50)	
Active	5476 (50)	5471 (50)	
Race, n (%)			0.88
White	9267 (91.7)	9231 (91.7)	
non-White	837 (8.3)	840 (8.3)	
Smoking status, n (%)			0.88
Current	1217 (11.1)	1202 (11.0)	
Past	4288 (39.2)	4315 (39.5)	
Never	5445 (49.7)	5412 (49.5)	
Physical activity, n (%)			0.18
≥5 times/wk	1814 (16.7)	1721 (15.9)	
1 time/mo to 4 times/wk	7579 (69.9)	7619 (70.3)	
None	1450 (13.4)	1499 (13.8)	
Hypertension, n (%)			0.58
Yes	2560 (23.6)	2593 (23.9)	
No	8298 (76.4)	8259 (76.1)	
High Cholesterol, n (%)			0.30
Yes	1123 (11.7)	1173 (12.2)	
No	8471 (88.3)	8446 (87.8)	
Alcohol consumption, n (%)			0.63
≥1 drink/mo	9258 (85.2)	9225 (85.0)	
Rarely/never	1609 (14.8)	1633 (15.0)	
Dietary beta-carotene intake, mean (SD), mg/d	9.10 (6.51)	9.15 (6.56)	0.58

BMI, body mass index.

Association Between BC Supplementation and Self-Reported Incident CKD

There were 1184 participants with self-reported incident CKD during the follow-up period (average follow-up was 12 years). The incidence rate of self-reported CKD was 4.47, (95% CI: 4.2–4.7) per 1000 years. There were 1120 participants (10.8%) who were unaware of their CKD status (Supplementary Table S1). There was no benefit of randomized BC supplementation on risk of self-reported incident CKD (HR = 0.97, 95% CI: 0.86–1.08, P-value = 0.54). After adjusting for age and randomized aspirin treatment, there was still no benefit of randomized BC supplementation on risk of self-reported incident CKD (HR = 0.97, 95% CI: 0.86–1.08, P-value = 0.56) (Table 2). After adjusting for all potential confounders, the effect of randomized BC supplementation on risk of self-reported incident CKD remained insignificant (HR = 0.98, 95% CI: 0.87–1.10, P-value = 0.69) (Supplementary Table S2).

Overall, smoking status did not modify the association between BC supplementation and self-reported incident CKD (P-interaction = 0.47) (Table 3). When considering stratified analyses by smoking status, among current smokers, there were 143 participants who developed self-reported incident CKD. The incidence rate of self-reported CKD among current smokers was 5.1 (95% CI: 4.2–5.9) per 1000 years. There was no association between BC supplementation and risk of self-reported incident CKD after adjusting for age and randomized aspirin treatment (HR = 1.08, 95% CI: 0.78–1.50, P-value = 0.64). Among nonsmokers (former or never smokers), there were 1039 participants who developed self-reported incident CKD. The incidence

Table 2. Multivariable Cox proportional hazards model for the effect of randomized beta-carotene supplementation on risk of self-reported chronic kidney disease

Variables	Unadjusted hazard ratio	95% CI	*P*-value	Adjusted hazard ratio[a]	95% CI	*P*-value
Beta-carotene	0.97	0.86–1.08	0.54	0.97	0.86–1.08	0.56
Aspirin				0.95	0.85–1.07	0.40

CI, confidence interval.
[a]Adjusted for age and randomized aspirin assignment.

rate of self-reported CKD was 4.4 (95% CI: 4.1–4.7). There was no association between BC supplementation and risk of self-reported incident CKD after adjusting for age and randomized aspirin treatment (HR = 0.95, 95% CI: 0.84–1.07, *P*-value = 0.41).

Association Between BC Supplementation and CKD Based on Serum Creatinine

For our analyses based on serum creatinine, we excluded 91 participants who had eGFR < 60 ml/min per 1.73 m^2 at baseline. At the end of the study, when a second blood sample was collected and creatinine measured, there were 1263 participants with eGFR < 60 ml/min per 1.73 m^2. There was no association between BC supplementation and odds of CKD defined on eGFR (OR = 0.94, 95% CI: 0.83–1.05, *P*-value = 0.27). After adjusting for age and randomized aspirin treatment, there was still no association between BC supplementation and odds of CKD defined on eGFR (OR = 0.96, 95% CI: 0.85–1.08, *P*-value = 0.49) (Table 4). In sensitivity analysis after adjusting for potential confounders, the effect of randomized BC supplementation on odds of CKD remained insignificant (OR = 0.97, 95% CI: 0.85–1.10, *P*-value = 0.60) (Supplementary Table S3).

Subgroup Analyses

In subgroup analyses, neither baseline serum creatinine availability nor dietary BC intake modified the effect of BC supplementation on self-reported incident CKD or CKD based on eGFR (Supplementary Table S4). In addition, there were 4677 (21.3%) and 979 (4.5%) participants who newly reported hypertension and diabetes, respectively, by the end of the study. We found no differences in the effect of BC supplementation on self-reported incident CKD or CKD based on eGFR among those with or without incident hypertension or diabetes (Supplementary Table S4).

DISCUSSION

To the best of our knowledge, our study is the only large-scale randomized controlled trial to assess the effect of long-term BC supplementation on CKD prevention. Our study did not find any significant benefit of long-term BC supplementation in preventing CKD among middle-aged and older male physicians, either by self-reported incident CKD or CKD based on eGFR < 60 ml/min per 1.73 m^2. In addition, there was no effect modification of the effect of BC on risk of CKD according to smoking status.

Previous studies demonstrated that a higher oxidative balance score was associated with a lower prevalence of CKD[23] and serum carotenoids were significantly lower among CKD patients.[24] Thereby, BC supplementation has been proposed as a preventive strategy to prevent CKD. In an animal model, pretreatment of 30 mg/kg BC appeared to protect renal ischemia or reperfusion injury in rats and subsequently improved kidney function.[15] The study by Pool-Zobel *et al.*[25] showed that the dosage of BC supplementation at 12 to 25 mg/d is enough to diminish oxidative stress and DNA damage. However, PHS I tested BC at 50 mg every other day, and we found no role for BC supplementation in preventing CKD among male physicians. One potential explanation for the lack of effect for BC in PHS I on CKD is that the population included in our study already had adequate serum carotenoid levels prior to randomization to BC treatment. Thus, we did not see any significant difference between the active BC group and placebo, given that additional BC might not help prevent further damage to the kidneys if, in fact, there are any effects on CKD prevention.

An updated systematic review for the US Preventive Services Task Force recommends against the use of BC supplements for the prevention of cardiovascular disease or cancer because BC supplementation increases

Table 3. Multivariable Cox proportional hazards model for the effect of beta-carotene supplementation and risk of self-reported chronic kidney disease stratified by baseline smoking status

Smoking status[a]	Number of events	Unadjusted hazard ratio	95% CI	Adjusted hazard ratio[b]	95% CI
Current smokers	143	1.09	0.78–1.51	1.08	0.78–1.50
Former/never smokers	1039	0.95	0.84–1.07	0.95	0.84–1.07

CI, confidence interval.
[a]*P*-interaction = 0.47.
[b]Adjusted for age and randomized aspirin assignment.

Table 4. Multivariable logistic regression model for the effect of randomized beta-carotene supplementation and risk of eGFR < 60 ml/min/1.73 m^2

Parameters	Unadjusted odds ratio	95% CI	*P*-value	Adjusted odds ratio[a]	95% CI	*P*-value
Beta-carotene	0.94	0.83–1.05	0.27	0.96	0.85–1.08	0.49
Aspirin				0.98	0.87–1.11	0.74

CI, confidence interval.
[a]Adjusted for age and randomized aspirin assignment.

the risk of cardiovascular disease mortality and risk of lung cancer, particularly among smokers or those who were exposed to asbestos.[26] Our study added that BC supplementation also did not help prevent CKD. Therefore, we would not generally recommend BC supplementation among the healthy population who might not have a carotenoid deficiency or require BC supplementation as a part of management for their illness which goes beyond our scope of the study.

Interestingly, our study showed that 10.8% of participants were unaware of their CKD. This speaks to underreporting of incidental self-reported CKD outcome, which should not affect the estimate of the effect of randomized BC on the outcomes. Those who self-reported CKD but had eGFR ≥ 60 ml/min per 1.73 m^2 could be explained by other criteria of CKD defined by Kidney Disease Improving Global Outcomes,[8] such as albuminuria > 30 mg or imaging abnormalities. Participants may have had an eGFR < 60 ml/min between 1982 and 1995, which was not captured only in 1995. Considering that criteria for CKD are typically defined as eGFR < 60 ml/min per 1.73 m^2 for ≥ 3 months,[27] it is difficult to implement clinical definition in epidemiologic research.

Our study has important strengths worth noting. First, PHS I was a randomized clinical trial in which baseline characteristics were similar in both active and placebo BC groups. Therefore, residual confounding by unknown or unmeasured confounding factors is unlikely to have accounted for the findings. Second, PHS I benefited from long-term follow-up for BC supplementation to examine its effects on CKD, with more than a decade of treatment and follow-up and minimal loss-to-follow-up. This allowed for subgroup analyses on incident hypertension and diabetes by the end of the study because these comorbidities are the major determinants of CKD and could affect the incidence rate of CKD.

There were several limitations in our study that also should be considered in the context of our results. First, our study did not have data on serum carotenoids at baseline, which have been inversely associated with eGFR.[28] Our population consisted of male physicians who were highly unlikely to have BC deficiency at baseline and may have already had adequate levels of BC and other antioxidants to protect them from kidney injury and CKD. Analyses stratified by median baseline dietary BC intake revealed no differences on CKD risk. Second, only 4759 participants had serum creatinine available at baseline. There were 91 participants (2%) with eGFR < 60 ml/min per 1.73 m^2 at baseline that we selectively excluded from our analysis for CKD defined by eGFR. Additional PHS I participants with eGFR $<$ 60 ml/min per 1.73 m^2 at baseline were likely missed and misclassified among those not providing baseline blood to allow for eGFR measurements. However, given the randomized design and higher likelihood of missing data at random for serum creatinine at baseline, we would expect any effects on the point estimates for randomized BC supplementation and CKD to be biased toward the null. In subgroup analyses, availability of baseline serum creatinine did not modify the effect of BC supplementation on our outcomes. Third, our study did not have data on proteinuria as a marker for CKD. Fourth, no specific data were available on the type of blood pressure medications, including angiotensin-converting-enzyme inhibitors, which could diminish the risk of CKD. However, given the randomized controlled trial study design and no difference in the incidence of hypertension between the BC group and the placebo, these medications were less likely to confound the effect of BC supplementation on the risk of CKD. Fifth, with regard to effect modification analysis on smoking status, there were 143 incidents of self-reported CKD, which may not provide sufficient power to detect the interaction between smoking and BC supplementation on incident self-reported CKD. Moreover, the majority of PHS I participants were White male physicians. Therefore, our results cannot be generalized to women, non-Whites, and other population groups. Finally, self-reported incident CKD was based on annual follow-up questionnaires "Have you been newly diagnosed as having kidney disease." Although this question may not accurately reflect CKD, the majority of kidney disease is CKD or at least is concurrently happening along with CKD or will imminently lead to CKD. However, this questionnaire might not accurately capture all CKD events.

In conclusion, long-term randomized BC supplementation did not affect the risk of incident CKD, assessed either by self-reported CKD or eGFR, in middle-aged and older male physicians. In addition,

there was no effect modification by smoking status on the association between BC supplementation and incident CKD.

DISCLOSURE

All the authors declared no competing interests.

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AUTHOR CONTRIBUTIONS

Research idea and study design was by AC and HDS. Data acquisition was done by HDS. Data analysis and interpretation was done by AC, PC, KMR, RJG, JEB, JMG, and HDS. Statistical analysis was done by AC, PC, and HDS. Supervision or mentorship was by HDS. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Table S1. Incident self-reported CKD split by eGFR $\geq$ or $<$60 ml/min per 1.73 m^2.

Table S2. Complete multivariable Cox proportional hazards model for the effect of randomized beta-carotene supplementation and risk of self-reported chronic kidney disease.

Table S3. Complete multivariable logistic regression model for the effect of randomized beta-carotene supplementation and risk of eGFR $<$ 60 ml/min per 1.73 m^2.

Table S4. Subgroup analysis for the effect of randomized beta-carotene supplementation and risk of self-reported chronic kidney disease and risk of eGFR $<$ 60 ml/min per 1.73m^2 on baseline serum creatinine availability, dietary beta-carotene intake at baseline, incidence of diabetes, and incidence of hypertension.

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