Complement C3 Is Inversely Associated with Habitual Intake of Provitamin A but Not with Dietary Fat, Fatty Acids, or Vitamin E in Middle-Aged to Older White Adults and Positively Associated with Intake of Retinol in Middle-Aged to Older White Women


Abstract

Complement factor 3 (C3) has been identified as a novel risk factor for obesity-associated cardiometabolic diseases. Data in the literature suggest that C3 concentrations may be influenced by diet. Therefore, we investigated the associations of intake of total fat, specific fatty acids, and fat-soluble vitamin E (and individual tocopherols) and vitamin A (and its dietary precursors) with circulating C3. In a white cohort (Cohort on Diabetes and Atherosclerosis Maastricht [CODAM]; n = 501; 59.4 ± 7.1 y; 61% men), associations of habitual nutrient intake (assessed by a food-frequency questionnaire) with circulating C3 were evaluated by using cross-sectional multiple linear regression analyses. Adjustments were first performed for age, sex, glucose metabolism status (i.e., impaired glucose metabolism or type 2 diabetes), and energy intake and subsequently for BMI, waist circumference, alcohol intake, smoking behavior, and season of blood collection. No associations with C3 were observed for total dietary fat intake or intake of specific fatty acids [saturated, monounsaturated, polyunsaturated, n–6 (ω6), and n–3 (ω3) fatty acids], vitamin E, or individual tocopherols. We observed an inverse association with intake of provitamin A carotenoids α-carotene (in μg/d; regression coefficient β = −0.075; 95% CI: −0.140, −0.010; P = 0.025) and β-carotene (in μg/d; β = −0.021; 95% CI: −0.044, 0.002; P = 0.069) with C3 (in mg/L). In contrast, and only in women, dietary retinol intake (in μg/d) was positively associated with C3 (β = 0.116; 95% CI: 0.014, 0.218; P = 0.026; n = 196). In conclusion, these data suggest that fasting concentrations of C3 may, in a complex manner, be modifiable by variation in dietary provitamin A carotenoids and/or retinol content of the usual diet but most likely not by variations in fat composition and vitamin E content.


Introduction

Cardiometabolic diseases, including cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM), are a major health problem, and the available options for prevention cannot sufficiently reduce their prevalence (1). For this reason, it is important to identify modifiable factors that contribute to these diseases, especially in high-risk individuals. Dietary composition may be such a risk factor (2); the identification of dietary components that may affect cardiometabolic risk is therefore of utmost relevance.

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Cardiometabolic diseases are related to immune dysfunction (3), and diet and nutritional status can influence immune status (4). The complement system is part of the innate immune system and can be activated via 3 pathways—classical, lectin, and alternative—that all converge on complement factor 3 (C3). Complement activation is associated with (systemic) inflammation (5,6). In addition, activation of C3 may be involved in adipose tissue inflammation and insulin resistance (7). Moreover, higher concentrations of circulating C3 have consistently been implicated in the development of CVD and T2DM (8–14). Activated C3 has also been detected in atherosclerotic plaques, suggesting active involvement of C3 in the atherosclerotic process (15,16). For these reasons, C3 can be considered a novel cardiovascular risk factor.

Studies have convincingly shown that systemic C3 concentrations may be manipulated by total energy intake primarily via effects on body weight (as reviewed in (17)), whereas other data suggest that systemic C3 concentrations may also be influenced by dietary composition (18–20). In vitro studies suggested that dietary fat or fat-associated nutrients, including fat-soluble vitamins, may have direct effects on C3 expression. Firstly, C3 expression was found to be regulated by the farnesoid X receptor (21), a target receptor for PUFAs. Secondly, α-tocopherol (the most active tocopherol in vitamin E) was identified as a regulator of C3 production in bovine cells (22). Thirdly, retinoic acid (a vitamin A derivative) was found to stimulate C3 production in human adipocytes (23). There are also data from in vivo human studies; although these data are relatively scarce and not always fully consistent, they suggest that systemic C3 may indeed be affected by dietary composition (i.e., total fat intake, intake of certain types of fatty acids, and/or intakes of fat-soluble vitamins E and A; summarized in Supplemental Table 1). However, most of these studies were short-term (<1 y) dietary interventions, and only a few addressed the long-term effects of these nutrients in a usual diet on the systemic concentration of C3 (20,24,25).

The aim of the present study was to explore the relation between intakes of dietary lipids, vitamin A, and vitamin E with C3 concentrations. Given the available data, we specifically hypothesized a positive association of habitual intake of retinol and/or provitamin A carotenoids with concentrations of C3. We investigated these relations in the Cohort on Diabetes and Atherosclerosis Maastricht (CODAM) study, a cohort of middle-aged to older individuals selected for an increased risk of T2DM and CVD. In such a population, C3 is usually positively associated with cardiometabolic diseases (9–13). Therefore, inverse associations between dietary intake and C3 concentrations in the CODAM study population were considered beneficial and would lend support to further investigations on the possibility that C3 is a modifiable CVD risk factor that can be manipulated by changes in dietary composition.

**Participants and Methods**

**Study population**

The CODAM study includes 574 individuals who were selected from a large population-based study on the basis of a moderately increased risk of T2DM and CVD. Details of the CODAM study have been described elsewhere (26). The present study reports on cross-sectional analyses in 501 individuals (305 men). We determined this sample size by stepwise exclusion of individuals who had missing data on the main outcome variable (serum C3; n = 3), who had missing values of >10% on the FFQ (n = 56), who reported implausible energy intakes [n = 5; <3360 or >17,640 kJ/d (<800 or >4200 kcal/d) for men and <2100 or >14,700 kJ/d (<500 or >3500 kcal/d) for women], and/or who had missing data on any of the other covariates (n = 9). The study was approved by the medical ethics committee of Maastricht University, and all individuals gave written informed consent.

**Dietary assessment**

The main potential determinants in this study were intakes of total fat, specific types of fat, and vitamin E (and individual tocopherols) and vitamin A (and its dietary precursors). The primary instrument to derive these data was a self-administered FFQ to evaluate habitual consumption of 178 food items, which was originally developed for the Dutch cohort of the European Prospective Investigation into Cancer and Nutrition (27). All individuals were asked to report their usual diet of the past year, and nutrients used in the current evaluation were calculated according to the extended version of the 2001 Dutch food-composition table (28). Total energy intake (kJ/d) and alcohol consumption (g/d) were also obtained from this FFQ. The use of supplements for vitamin A (defined as vitamin A, vitamin A/D, or multivitamin preparations) and vitamin E (defined as vitamin E or multivitamin preparations) was also recorded.

The FFQ we used was validated for several, but not all, nutrients that were included in the current evaluations (27). It was considered adequate to rank individuals with respect to intakes of total fat, SFAs, MUFAs, PUFAs, and retinol, and to a lesser extent of vitamin E and β-carotene; other nutrients were not assessed (27). Nevertheless, other investigators have shown significant associations between intakes of both validated and nonvalidated nutrients and health outcomes using the same FFQ in other Dutch populations (29–33).

**Biochemical analyses**

**Main outcome variable.** Serum C3 [in g/L; Roche assay (no. 11875078); Hitachi 912 autoanalyzer], was measured exactly as described (11).

**Covariates.** Obesity was assessed by BMI (kg/m²) and central obesity by waist circumference (cm) as described (34). All individuals were categorized as having normal glucose metabolism (NGM), impaired glucose metabolism (IGM; including both individuals with impaired fasting glucose and with impaired glucose tolerance), or T2DM; prevalent CVD (yes/no) was defined as previously described (34). Smoking behavior was assessed by means of questionnaires and expressed in total pack-years of smoking and as a dichotomous variable (current smoker: yes/no). Physical activity was assessed with the use of a validated questionnaire [SQUASH (35)] and expressed as a dichotomous variable (36).

**Statistical analyses**

Participants were first classified according to tertiles of C3 concentration. To test for differences in the covariates across C3 tertiles, ANOVA was used for continuous variables with normal distribution, Kruskal-Wallis for variables with skewed distribution, and chi-square test for proportions. Variables with a normal distribution are presented as means ± SDs, and variables with a skewed distribution are presented as medians and IQRs. The energy-consuming nutrients—total fat and specific dietary fatty acids—were evaluated as nutrient density (g/1000 kcal) adjusted for total energy intake (kJ). The non-energy-containing nutrients—vitamin E (and individual tocopherols) and vitamin A (and its dietary precursors)—were evaluated as absolute intakes (mg/d or µg/d, as indicated) adjusted for total energy intake (37). A 2-sided P value <0.05 was considered significant. All analyses were performed with the Predictive Analytics Software (PASW), version 18.0 (SPSS IBM Corporation).

**Main analyses.** We used multiple linear regression analysis to evaluate the associations between dietary intake of fat-associated nutrients and serum C3 concentrations. All regression models were adjusted for total energy intake. To control for confounding due to the selection procedures of the CODAM study (the population-based cohorts from whom the CODAM study population was selected were stratified for age and sex, and CODAM was oversampled for IGM and T2DM), all analyses were adjusted for sex, age (in y), and glucose metabolism status (NGM/IGM/T2DM as dummy variables with NGM as reference category). The following covariates were evaluated as potential confounders in the
linear regression models described above: BMI (kg/m²), waist circumference (cm), alcohol consumption (g/d), smoking status (current smoker: yes/no; pack-years), physical activity (does the person meet the Dutch guidelines of 30 min/day: yes/no), and season of participant’s evaluation (winter, spring, summer, and fall as dummy variables with winter as the reference category). Current smoker and physical activity did not act as confounders in any of the associations (i.e., adjustment for this variable did not change the strength of the crude association by >10% in any model) and were not included in the multiple regression analyses presented herein. All models complied with the assumptions of linearity, normal distribution of residuals, and homoscedasticity.

Additional analyses. In these cross-sectional evaluations, it can be reasoned that prevalent CVD (yes/no) may act as either a confounder (i.e., CVD is a determinant of both dietary composition and of systemic C3) or as a collider (i.e., CVD may be the outcome of both dietary composition and systemic C3). If CVD is a confounder then failure to adjust for this covariate may result in residual confounding, whereas if CVD is a collider then adjustment for CVD may actually introduce a biased association between dietary composition and systemic C3 (38). To evaluate the effect of prevalent CVD, if any, on the strength of the associations under study in our current analyses, we performed the multiple regression analyses without (main analyses) and with (additional analyses) CVD included as a covariate. Also, the associations of vitamin E (and individual tocopherols) and vitamin A (and its dietary precursors) with serum C3 concentrations were additionally evaluated in regression analyses with the exclusion of individuals who reported the use of vitamin supplements; and because C3 concentration may be affected by an immediate past history of infection or injury, we also repeated the analyses with the exclusion of individuals with C-reactive protein (CRP) concentrations >10 mg/L (39). Because FFQs may be primarily used for ranking individuals with respect to dietary exposure, we also performed the analyses with the individuals categorized into quintiles of nutrient intake. Last, data in the literature suggest that the effects of dietary vitamin intake may differ between men and women. This is most convincingly the case for retinol and its metabolite retinoic acid (40–43) but potentially also for provitamin A carotenoids, vitamin E, and/or tocopherols (44–46). An additional, nonbiologic argument to perform analyses stratified by sex specifically for retinol intake was the observation that for intake of retinol, the correlation of FFQ data with intakes derived from 12 monthly 24-h dietary records (adjusted for total energy intake) differed substantially between sexes [Pearson’s $r$ was 0.29 for men and 0.62 for women (27)]. This difference was not observed before energy adjustment (Pearson’s $r$ was 0.61 for men and 0.63 for women). For the above reasons, interaction between (pro)vitamin intake and sex on serum concentrations of C3 was evaluated by the addition of sex by vitamin cross-products in the fully adjusted main models. Stratified analyses were conducted when $P$-interaction <0.1.

Results

Table 1 shows the basic characteristics of the study population according to tertiles of C3 concentration. In summary, individuals in the higher tertiles of C3 concentration were more obese and had higher prevalences of CVD, IGM, and T2DM.

**Total fat intake and composition of dietary fat.** Median fat intake was 83.8 (IQR: 65.6–108) g/d, which corresponded to 35.0% (IQR: 32.4–38.4%) of energy. Table 2; $n = 5$ shows intakes of individual fatty acids as well as the associations of intakes of total dietary fat and specific fatty acids with C3 concentrations. No associations in the models adjusted for energy intake, age, sex, and glucose metabolism status or in the fully adjusted models were observed for total fat intake or for intake of any of the individual dietary fatty acids.

**Vitamin E and tocopherols.** Table 3 shows median dietary intakes of vitamin E and tocopherols. No significant associations of dietary intake of vitamin E and tocopherols with C3 concentrations were observed in the models adjusted for energy intake, age, sex, and glucose metabolism status or in the fully adjusted models, although we did observe a trend toward an inverse association between β-tocopherol intake and C3 ($P = 0.08$; Table 3). When we additionally excluded the individuals who reported taking any supplements containing vitamin E (i.e., use of supplements that contain vitamin E or use of multivitamin supplements; $n = 59$), the results did not materially change (data not shown). Also, no interaction with sex was observed for vitamin E or tocopherols ($P$-interaction > 0.19).

**Retinol, retinol equivalents, and provitamin A carotenoids.** Table 4 shows median dietary intakes of retinol, retinol equivalents, and provitamin A carotenoids and their associations with C3 concentrations. In contrast to our hypothesis, we did not observe a clear positive association of dietary intake of retinol or retinol equivalents with concentrations of C3 (Table 4).

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**Table 1** Characteristics of the CODAM study population

<table>
<thead>
<tr>
<th></th>
<th>Tertiles of circulating C3 concentrations</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>All participants</td>
<td>1 1010–1630 mg/L ($n = 168$)</td>
<td>2 1640–1940 mg/L ($n = 165$)</td>
<td>3 1950–2830 mg/L ($n = 168$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 1010–1630 mg/L ($n = 168$)</td>
<td>2 1640–1940 mg/L ($n = 165$)</td>
<td>3 1950–2830 mg/L ($n = 168$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>59.4 ± 7.0</td>
<td>59.1 ± 7.0</td>
<td>59.6 ± 7.1</td>
<td>59.4 ± 6.9</td>
<td>0.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sex, % men</td>
<td>61</td>
<td>65</td>
<td>59</td>
<td>58</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28.4 ± 4.2</td>
<td>26.8 ± 3.3</td>
<td>26.4 ± 3.8</td>
<td>30.2 ± 4.7</td>
<td>&lt;0.001</td>
<td></td>
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</tr>
<tr>
<td>Waist circumference, cm</td>
<td>98.9 ± 11.8</td>
<td>94.1 ± 10.9</td>
<td>99.2 ± 10.1</td>
<td>103.5 ± 12.4</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking (current), % yes</td>
<td>21</td>
<td>27</td>
<td>15</td>
<td>20</td>
<td>0.021</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking (former and current), pack-years</td>
<td>13.5 (0–29.3)</td>
<td>11.0 (0–24.6)</td>
<td>12.0 (0–28.6)</td>
<td>16.8 (0–34.0)</td>
<td>0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol consumption, g/d</td>
<td>8.6 (1.3–22.3)</td>
<td>10.5 (2.7–22.7)</td>
<td>9.1 (1.9–24.3)</td>
<td>4.9 (0.5–18.1)</td>
<td>0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physically active, % yes</td>
<td>63</td>
<td>63</td>
<td>65</td>
<td>61</td>
<td>0.76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prior CVD, % yes</td>
<td>28</td>
<td>24</td>
<td>23</td>
<td>36</td>
<td>0.010</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose metabolism status</td>
<td>54, 22, 24</td>
<td>69, 15, 16</td>
<td>51, 25, 24</td>
<td>42, 27, 31</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(NGM, IGM, T2DM), % yes</td>
<td></td>
<td>8.9 (7.4–10.8)</td>
<td>9.1 (7.3–11.0)</td>
<td>9.0 (7.3–10.7)</td>
<td>8.8 (7.4–10.7)</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>Fat, % of energy</td>
<td>35.0 (32.4–38.4)</td>
<td>34.6 (31.8–37.2)</td>
<td>35.1 (32.2–38.4)</td>
<td>35.6 (32.9–39.3)</td>
<td>0.18</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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1 Values are expressed as means ± SDs, medians (IQRs), or percentages, as applicable. CODAM, Cohort on Diabetes and Atherosclerosis Maastricht; CVD, cardiovascular disease; C3, complement factor 3; IGM, impaired glucose metabolism; NGM, normal glucose metabolism; T2DM, type 2 diabetes mellitus.
TABLE 2 Associations between intakes of dietary fat and specific fatty acids with circulating C3 in the CODAM study population

<table>
<thead>
<tr>
<th>Dietary intake: CODAM</th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\beta^2 (95% \text{ CI}))</td>
<td>(P) value</td>
</tr>
<tr>
<td>Fat</td>
<td>83.8 (65.6–108)</td>
<td>14.7 (–6.20, 35.6)</td>
</tr>
<tr>
<td>SFA s</td>
<td>32.5 (25.4–40.6)</td>
<td>24.3 (–19.12, 67.8)</td>
</tr>
<tr>
<td>MUFA s</td>
<td>26.7 (20.5–35.1)</td>
<td>19.7 (–33.3, 71.9)</td>
</tr>
<tr>
<td>PUFA s</td>
<td>15.6 (12.0–20.5)</td>
<td>33.6 (–33.4, 101)</td>
</tr>
<tr>
<td>18:2n–6</td>
<td>12.9 (9.8–17.4)</td>
<td>32.6 (–42.2, 107)</td>
</tr>
<tr>
<td>20:5n–3</td>
<td>0.05 (0.02–0.06)</td>
<td>-558 (–4190, 3080)</td>
</tr>
<tr>
<td>22:6n–3</td>
<td>0.11 (0.05–0.14)</td>
<td>89 (–1910, 2090)</td>
</tr>
</tbody>
</table>

1 \(n = 501\). CODAM, Cohort on Diabetes and Atherosclerosis Maastricht; C3, complement factor 3; 18:2n–6, linoleic acid; 20:5n–3, EPA; 22:6n–3, DHA.
2 Values are medians (IQRs). Daily dietary fat intakes are presented as g/d, whereas in the linear regression analyses, these were evaluated as nutrient densities (g/1000 kJ).
3 Analyses were adjusted for total energy intake, age, sex, and glucose metabolism status.
4 Analyses were additionally adjusted for BMI, waist circumference, alcohol consumption, pack-years smoked, and season of blood collection.
5 The regression coefficient \(\beta\) represents the change in serum C3 (in mg/L) per 1 g/1000 kJ increase in dietary fat consumption.

Moreover, in the models adjusted for energy intake, age, sex, and glucose metabolism status and in the fully adjusted models, there was an inverse association of C3 with \(\alpha\)-carotene and a similar trend for \(\beta\)-carotene \((P = 0.07)\), whereas \(\beta\)-cryptoxanthine or retinol equivalents were not associated. When we additionally excluded the individuals who reported taking any supplements containing vitamin A (i.e., use of supplements that contain vitamin A or vitamins A and D or use of multivitamin supplements; \(n = 56\)), the magnitude of the associations did not materially change and the inverse association with \(\beta\)-carotene also reached statistical significance [regression coefficient \(\beta\) per \(\mu g/d\) increase in \(\beta\)-carotene = \(-0.028 \text{ mg/L C3}; 95\% \text{ CI: } -0.052, -0.004; P = 0.022\)]. Excluding 1 individual with an extreme retinol intake of >6000 \(\mu g/d\) and 1 individual with an extreme \(\alpha\)-carotene intake of >3000 \(\mu g/d\) did not materially change these findings (data not shown).

Interaction with sex was observed for retinol (P-interaction = 0.037) but not for retinol equivalents, \(\alpha\)-carotene, \(\beta\)-carotene, or \(\beta\)-cryptoxanthine (P-interaction > 0.17). Therefore, we additionally performed sex-stratified analyses for the association of retinol with C3. In women \((n = 196)\), there was a positive association of retinol with serum C3 concentration \((\beta \text{ per } \mu g/d \text{ increase in retinol in the fully adjusted models } = 0.116 \text{ mg/L C3}; 95\% \text{ CI: } 0.014, 0.218; P = 0.026)\), whereas in men \((n = 305)\) there was no such association \((\beta = -0.023 \text{ mg/L C3}; 95\% \text{ CI: } -0.076, 0.031; P = 0.40)\). Excluding all individuals who reported use of supplements containing vitamin A led to a slight increase in the strength of this association in women \((n = 163)\) but did not appreciably affect it in men \((n = 282)\): \(\beta = 0.144 \text{ mg/L C3 (95\% CI: } 0.033, 0.254; P = 0.011)\) and \(\beta = -0.018 \text{ mg/L C3 (95\% CI: } -0.074, 0.037; P = 0.51)\), respectively. Also, when individuals with extreme retinol (1 man) and \(\alpha\)-carotene (1 woman) intakes were excluded these data basically remained similar (data not shown).

No material changes were observed in any of the associations presented when the analyses were additionally adjusted for CVD (data not shown). Likewise, excluding individuals with CRP > 10 mg/L \((n = 33)\), to account for an immediate past history of infection or injury, did not change the results. Last, when the main analyses were repeated with individuals categorized into quintiles of nutrient intake, all associations with total fat, fatty acids, and vitamin E and tocopherols remained nonsignificant (P-trend > 0.13), the inverse associations for \(\alpha\) - and \(\beta\)-carotene with C3 were significant (P-trend = 0.041 and 0.050, respectively), and there was a tendency for a positive association with retinol in women (P-trend = 0.08) but not in men (P-trend = 0.25).

Discussion

In this cross-sectional evaluation of the CODAM study, we observed no significant associations of intakes of total dietary fat, specific dietary fatty acids, or vitamin E and tocopherols

TABLE 3 Association between intakes of vitamin E and tocopherols with circulating C3 in the CODAM study population

<table>
<thead>
<tr>
<th>Dietary intake: CODAM</th>
<th>Model 1 (\beta^2 (95% \text{ CI}))</th>
<th>(P) value</th>
<th>Model 2 (\beta^2 (95% \text{ CI}))</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin E</td>
<td>12.9 (10.3–16.9)</td>
<td>2.7 (–5.2, 10.5)</td>
<td>0.50</td>
<td>1.4 (–5.8, 8.8)</td>
</tr>
<tr>
<td>(\alpha)-Tocopherol</td>
<td>6.7 (5.3–8.4)</td>
<td>-4.8 (–16.7, 7.1)</td>
<td>0.43</td>
<td>-5.6 (16.7, 5.5)</td>
</tr>
<tr>
<td>(\beta)-Tocopherol</td>
<td>0.45 (0.36–0.59)</td>
<td>-16 (–337, 105)</td>
<td>0.30</td>
<td>-184 (–390, 22)</td>
</tr>
<tr>
<td>(\gamma)-Tocopherol</td>
<td>8.7 (6.5–11.7)</td>
<td>-6.8 (–14.2, 0.7)</td>
<td>0.08</td>
<td>-4.6 (–11.5, 2.2)</td>
</tr>
<tr>
<td>(\delta)-Tocopherol</td>
<td>1.5 (0.9–2.2)</td>
<td>-23.1 (–47.5, 1.2)</td>
<td>0.06</td>
<td>-16.6 (–39.0, 5.8)</td>
</tr>
</tbody>
</table>

1 \(n = 501\). CODAM, Cohort on Diabetes and Atherosclerosis Maastricht; C3, complement factor 3.
2 Values are medians (IQRs).
3 Analyses were adjusted for total energy intake, age, sex, and glucose metabolism status.
4 Analyses were additionally adjusted for BMI, waist circumference, alcohol consumption, pack-years smoked, and season of blood collection.
5 The regression coefficient \(\beta\) represents the change in serum C3 (in mg/L) per 1 mg/d increase in vitamin E or tocopherol consumption.

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with systemic C3. In contrast, there was a significant inverse relation of intakes of α-carotene and possibly also β-carotene with C3, whereas in women there was a significant positive association between retinol intake and C3, which was absent in men.

In a healthy diet, between 20% and 40% of energy comes from fat (47). In our study population, ~15% of all individuals reported intakes of >40% of energy from fat and 1 individual reported <20% energy from fat. The apparent lack of association of total fat intake and dietary fatty acid composition with C3 is in line with previous reports (as summarized in Supplemental Table 1), implying that such effects, if any, may at best be small.

The daily advised minimum intakes of vitamin E are 11.8, 19.7, and 9.4 mg/d for men and 9.3, 8.7, and 8.3 mg/d for women at ages 22–50, 50–65, and >65 y, respectively (47). In our study population, 22% of men and 19% of women reported less than the daily advised intake for vitamin A. The scarce data that were available in the literature on possible effects of vitamin E and tocopherols on C3 concentration were equivocal because, on the one hand, α-tocopherol was identified as a positive regulator of C3 production (22), whereas on the other hand, it appeared to decrease C3 in hypercholesterolemic patients (48). Our data do not support a clear effect of dietary intake of vitamin E or tocopherols on circulating C3 in either direction, except for a trend toward an inverse association between β-tocopherol intake and C3. The latter was consistent with the inverse associations we observed for α- and β-carotene, among others, because these 3 compounds all have antioxidant capacities.

The daily advised minimum intake of vitamin A (expressed as retinol equivalents) is 1000 μg/d for men and 800 μg/d for women (47). In our study population, 25% of men and 17% of women reported less than the daily advised intake for vitamin A. We observed a significant inverse association for dietary intake of α-carotene (and a similar trend for β-carotene) with C3 concentrations. The regression coefficient for α-carotene (~0.075) indicates that for each μg/d higher intake of dietary α-carotene there is a 0.075-mg/L lower concentration of C3. The IQR for α-carotene intake in our study population was ~500 μg/d, which corresponds to 35–40-mg/L lower C3 concentrations for individuals in the highest compared with the lowest quartile of α-carotene intake. Likewise, the regression coefficient β of ~0.021 combined with an IQR of ~1500 μg/d for β-carotene corresponds to a difference of ~30 mg/L. The magnitude of these effects is comparable to the effect of selenium on C3 concentration, because a 1-SD higher selenium intake was associated with 0.038–g/L lower C3 concentrations in healthy individuals (20) but was smaller than the 200-mg/L lower C3 concentration reported for a dietary intervention with and without legumes (19). Also, individuals with incident myocardial infarction (12) or T2DM (49) had 0.25-g/L and 0.14-g/L higher baseline C3 concentrations, respectively, than those who did not have these conditions. These data combined with our current results suggest that high versus low intakes of α-carotene and possibly also β-carotene might contribute up to ~15–30% of such clinically relevant differences in C3 concentrations.

For retinol intake, we performed sex-stratified analyses. In women, the IQR for retinol intake was ~400 μg/d, which, given the regression coefficient β of 0.116, corresponds to 40–45-mg/L higher C3 concentrations in the highest compared with the lowest quartile of retinol intake. This effect was thus of a similar, although opposite, magnitude as what we observed for α- and β-carotene. As indicated in Participants and Methods, data in the literature support the concept that the biologic effect of retinol may differ between sexes (40,41,43). In addition, a recent genomewide association study of systemic retinol concentrations showed that the minor allele of a polymorphism in the transthyretin gene was associated with higher plasma retinol concentrations in men but not in women (50), and interestingly, in vitro experiments identified transthyretin as the protein involved in the transport of retinol on chylomicrons and subsequent transfer of retinol to adipocytes, with consequent stimulation of C3 mRNA expression (23). These in vitro and in vivo observations support the potential relevance of our observations.

Thus, in line with our hypothesis, we observed a positive association between dietary retinol intake and C3 concentration but only in women, whereas, in contrast, there was an inverse association with α- and β-carotene. We included α- and

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**TABLE 4** Associations between intakes of retinol, retinol equivalents, and provitamin A carotenoids with circulating C3 in the CODAM study population

<table>
<thead>
<tr>
<th>Dietary intake: CODAM</th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinol</td>
<td>β (95% CI)</td>
<td>P value</td>
</tr>
<tr>
<td>All</td>
<td>0.045 (−0.005, 0.095)</td>
<td>0.08</td>
</tr>
<tr>
<td>Men</td>
<td>0.014 (−0.042, 0.069)</td>
<td>0.63</td>
</tr>
<tr>
<td>Women</td>
<td>0.148 (0.037, 0.259)</td>
<td>0.099</td>
</tr>
<tr>
<td>Retinol equivalents</td>
<td>0.022 (−0.025, 0.070)</td>
<td>0.36</td>
</tr>
<tr>
<td>α-Carotene</td>
<td>−0.088 (−0.159, −0.017)</td>
<td>0.015</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>−0.024 (−0.048, 0.001)</td>
<td>0.06</td>
</tr>
<tr>
<td>β-Cryptoxanthine</td>
<td>−0.195 (−0.597, 0.207)</td>
<td>0.34</td>
</tr>
</tbody>
</table>

1 n = 501. CODAM, Cohort on Diabetes and Atherosclerosis Maastricht; C3, complement factor 3.
2 Values are medians (IQRs).
3 Analyses were adjusted for total energy intake, age, sex, and glucose metabolism status.
4 Analyses were additionally adjusted for BMI, waist circumference, alcohol consumption, pack-years smoked, and season of blood collection.
5 The regression coefficient β represents the change in serum C3 (in mg/L) per 1-μg/d increase in vitamin A or provitamin A carotenoid consumption.
6 For the association with retinol, separate regression coefficients are provided for men (n = 305) and women (n = 198) because the interaction coefficient for retinol × sex was P = 0.036.
β-carotene in our evaluations because of their known capacities as vitamin A precursors. However, bioconversion of provitamin A carotenoids to retinol is not very efficient and varies according to dietary source (51). Also, α- and β-carotene have non-vitamin A-related effects, such as antioxidant capacity [reviewed in (52)]. The observation that higher dietary total antioxidant status was associated with lower systemic C3 concentrations (25) is therefore of particular interest. Moreover, retinol and provitamin A carotenoids are derived from different food substances. The main sources of retinol in the Dutch diet are meats, meat products, and added fats, together comprising ~70% of total retinol intake, whereas the main sources of β-carotene (the most abundant provitamin A carotenoid in food) are vegetables and soups/bouillons, together comprising around 77% of total β-carotene intake, and dairy products add to both retinol and β-carotene intake, ~15% and 5%, respectively (53). We cannot currently exclude that at least part of the observed effects of retinol and α- and β-carotene should be attributed to other, yet to be identified, aspects of the retinol-rich versus α- and β-carotene-rich diets, respectively.

Our current study has several limitations. First, we used cross-sectional analyses to explore an etiologic research question. For this reason, we can obtain clues only on possible relations, but these need corroborations by future longitudinal studies and dietary interventions. Second, the method that was used to assess dietary intake of fatty acids and fat-soluble vitamins was semiquantitative, which may have led to random misclassification of individuals and therefore limited the power to detect an association that was actually present. Also, the particular characteristics of our study population may limit our power to detect an association because CODAM participants may be relatively similar with respect to their dietary habits, thus limiting the ranges of dietary intakes of fat, fatty acids, and vitamins E and A in our data set; and given the relatively small sample size, our power to detect associations in the subgroups was limited. Another potential limitation is that we measured C3 concentrations in the fasting state. If dietary effects on C3 are most pronounced in the postprandial state, this may to some extent reduce the power to detect those effects. However, given the relatively long half-life (2–5 d) of C3 in the circulation (54), it can be anticipated that any postprandial changes will be still reflected in the fasting samples. Finally, given the criteria for inclusion of participants in the CODAM study, extrapolation of the data to the general population should be done with caution. Nonetheless, our study population represents a large group of individuals with a relatively high cardiometabolic risk in whom C3 may be a risk factor and in whom identification of possible ways to lower systemic C3 may be most relevant.

In conclusion, in this cross-sectional evaluation of the CODAM study, we did not observe any significant associations of intakes of total dietary fat, dietary fatty acids, or vitamin E and tocopherols with concentrations of C3. There was, however, a significant inverse association of α-carotene and possibly also β-carotene intake with C3. In contrast, and in women only, there was a significant positive association between retinol intake and C3. These observations suggest that fasting concentrations of C3 may be modifiable by dietary manipulation of provitamin A carotenoids and/or retinol intake but most likely not by variations in fat composition and vitamin E content. Further research is needed to determine whether such manipulation of dietary composition actually has the suggested effects on systemic C3 concentrations and, if so, whether this will affect the development of cardiometabolic disease.

Acknowledgments

Literature Cited


