Plasma kinetics of lutein, zeaxanthin, and 3'-dehydro-lutein after multiple oral doses of a lutein supplement

Petra A Thürmann, Wolfgang Schalch, Jean-Claude Aebischer, Ute Tenter, and William Cohn

ABSTRACT

Background: Adequate intake of lutein is postulated to reduce the risk of age-related macular degeneration, but kinetic information for developing a dosing regimen is sparse.

Objective: The objective was to characterize lutein plasma kinetics in a multiple dosing design and to assess the effects of lutein intake on concentrations of other plasma carotenoids.

Design: After a run-in period of 7 d, 19 healthy volunteers were assigned to receive daily oral doses of 4.1 mg lutein (n = 8; group 1) or 20.5 mg lutein (n = 8; group 2) for 42 d or no lutein (n = 3; control group). The supplement contained 8.3% zeaxanthin relative to lutein (100%). The time profiles of plasma xanthophyll concentrations were monitored over the dosing phase, and samples were collected frequently on day 42 and for 24 d after dosing.

Results: Average plasma all-E-lutein concentrations increased from 0.14 to 0.52 ± 0.13 and 1.45 ± 0.69 μmol/L in groups 1 and 2, respectively. Dose-normalized lutein bioavailability in group 2 was ≈60% of that in group 1. Kinetic disposition half-life did not differ significantly between groups. On average, dosing for 18 d was required to reach a >90% fraction of the steady state concentration, which is consistent with an effective half-life for accumulation of ≈5.6 d. Plasma kinetics of all-E-lutein were paralleled by those of all-E-3′-dehydro-lutein. Kinetic analysis indicated formation of all-E-3′-dehydro-lutein from lutein. Lutein was well tolerated and did not affect the concentrations of other carotenoids.

Conclusion: Long-term supplementation with 4.1 and 20.5 mg lutein as beadlets increased plasma lutein concentrations ≈3.5- and 10-fold, respectively. Am J Clin Nutr 2005;82:88–97.

KEY WORDS Xanthophylls, carotenoids, lutein, zeaxanthin, all-E-3′-dehydro-lutein, multiple oral dose kinetics, macular pigment, age-related macular degeneration

INTRODUCTION

An adequate supply of the dietary carotenoids lutein and zeaxanthin depends on regular intakes of fruit and green and yellow vegetables, which represent major natural sources of xanthophylls (1). The combined daily dietary intake of lutein plus zeaxanthin ranges, on average, between 2 and 26 mg, for which a lutein-to-zeaxanthin ratio of ≈5:1 is generally assumed (2–4). In the human eye, lutein and zeaxanthin are specifically located in the center of the retina, where they form the “yellow spot,” or macula lutea (5). Because of this specific location and their physicochemical properties, such as the absorption of high-energy blue light and their capability to quench reactive oxygen species, a protective action of the xanthophylls in the retina has been postulated (6). Evidence indicates that a low intake of lutein and zeaxanthin is related to an increased risk of age-related macular degeneration (7, 8). Moreover, an increased intake of lutein and zeaxanthin appears to be associated with a lower risk of cataract (9, 10). Although the supplemental intake of lutein, zeaxanthin, or both has been suggested to be useful in persons at high risk of macular degeneration (11–13), critical comment asks for additional prospective trials before general recommendations should be made (14).

The development of appropriate dosing regimens depends on adequate information on the pharmacokinetic properties of a compound. However, little is known about the kinetics of lutein. Several studies have dealt with the comparative availability of lutein in plasma, providing xanthophyll as lutein or as lutein ester in various foodstuffs or in formulated supplements (15–18). Landrum et al (19) treated 2 volunteers with a 30-mg daily dose for 140 d. Plasma concentrations plateaued after 20–40 d, with a 10-fold increase from baseline, and returned to baseline concentrations 40–50 d after supplementation was discontinued. Depletion studies estimate the terminal half-life of lutein to be ≈15 d in patients with type 1 diabetes (20) and to be ≈76 d in healthy subjects (21).

We recently reported on the pharmacokinetics of zeaxanthin after multiple dosing in healthy subjects and showed that all-E-3′-dehydro-lutein is formed from zeaxanthin (22). The present study aimed to acquire additional kinetic data on lutein in a multiple-dose design study in healthy subjects. The dose was chosen to provide an amount in the range of ≈2- to 4-fold the average daily intake (4.1 mg/d; low dose) and to facilitate the assessment of pharmacokinetic parameters (20.5 mg/d; high dose). The kinetic data to be generated were plasma concentration-dose response at steady state, time to attain steady state, index of accumulation, effective half-life for accumulation, and dose proportionality. Moreover, the plasma kinetics of all-E-3′-dehydro-lutein (3R,6R-3-hydroxy-β,ε-carotene-3′-one) and its

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Subjects and Methods

Subjects

Nineteen healthy men and women gave their written informed consent to participate in this monocentric, randomized, open-label, parallel-group study. All subjects were healthy according to the results of a physical examination that included blood pressure and heart rate measurements, an electrocardiogram, and clinical chemistry, hematology, and serology (hepatitis B and C, HIV-1, and HIV-2) tests. Written informed consent was obtained from each subject after they received an adequate explanation of the aims, methods, objectives, and potential hazards of the study. The study protocol was in accordance with the German drug law and the Declaration of Helsinki and was approved by the local ethics committee of the University of Witten/Herdecke, Germany. Demographic data and information on nutritional habits are provided in Table 1.

Study design

Lutein, extracted from marigold, was formulated in beadlets (5% lutein, tablet grade; DSM Nutritional Products, Ltd), which were incorporated in hard gelatin capsules containing 4.1 mg lutein (of which 93% was all-E-lutein) and 0.34 mg was zeaxanthin (of which 97% was all-E-zeaxanthin).

Eight subjects (4 women, 4 men) were randomly assigned to receive 4.1 mg lutein/d (one capsule; group 1), 8 (4 women, 4 men) were randomly assigned to receive 20.5 mg lutein/d (5 capsules; group 2), and 3 (2 women, 1 man) were randomly assigned to served as control subjects to estimate baseline fluctuations of plasma lutein and zeaxanthin concentrations. For the entire study, subjects were asked to avoid lutein- and zeaxanthin-rich vegetables and fruit such as kale, Brassica oleracea, spinach, carrots, corn, tomatoes, nectarines, and peaches. Daily fat intake was restricted to 100 g. The subjects received nutritional advice and were required to complete a 1-d dietary record thrice a week. The capsules were ingested with 150 mL water and a light breakfast. Compliance was controlled by counting the remaining capsules at each visit.

On day 67 a final physical examination and laboratory tests were performed following the same protocol used at the inclusion visit. After a 1-wk run-in period, blood samples for the measurement of baseline plasma lutein concentrations were drawn on 3 consecutive d and thereafter at weekly intervals until week 5 (day 35). Additional blood samples were taken on days 38, 39, 40, and 41. Except for the blood specimens collected on day 42, all blood specimens were drawn in the morning, before dosing, after the subjects had fasted overnight. On day 42 blood samples were obtained before dosing and 2, 4, 6, 8, 12, and 24 h after dosing (24-h kinetic profile). On this “pharmacokinetic study day,” the capsules were taken with a standardized breakfast consisting of:

Table 1

Demographic data and nutritional habits of the subjects

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 8)</th>
<th>Group 2 (n = 8)</th>
<th>Control group (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>28.6 ± 7.9 (26.5)</td>
<td>28.6 ± 4.8 (28.0)</td>
<td>38.7 ± 6.7 (37.0)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76.9 ± 14.3 (78.8)</td>
<td>72.3 ± 7.4 (74.5)</td>
<td>61.0 ± 5.5 (60.0)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>172.5 ± 6.9 (173.5)</td>
<td>175.8 ± 5.5 (176.0)</td>
<td>168.3 ± 6.7 (170.0)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.7 ± 3.5 (25.5)</td>
<td>23.4 ± 2.3 (23.2)</td>
<td>21.5 ± 0.7 (21.6)</td>
</tr>
<tr>
<td>Energy intake (kcal/d)</td>
<td>1862 ± 616 (1835)</td>
<td>2051 ± 851 (1894)</td>
<td>1716 ± 567 (2040)</td>
</tr>
<tr>
<td>Fat intake (g/d)</td>
<td>77 ± 33 (71)</td>
<td>79 ± 39 (75)</td>
<td>74 ± 25 (71)</td>
</tr>
<tr>
<td>Dietary lutein intake (mg/d)</td>
<td>0.552 ± 0.726 (0.300)</td>
<td>0.753 ± 2.025 (0.240)</td>
<td>1.295 ± 2.208 (0.487)</td>
</tr>
</tbody>
</table>

1 All values are ± SD; medians in parentheses. There were no significant differences between groups, P > 0.05.
2 Low-dose group: 4.1 mg lutein/d.
3 High-dose group: 20.5 mg lutein/d.
one roll of bread with cheese and coffee or tea. In the postdosing period, additional blood samples were drawn in the morning on days 43, 44, 48, 53, 58, 62, and 67. For the control subjects, blood sampling was performed only at the end of the run-in period (3 samples) and on days 28, 48, and 67.

Blood samples of 7.5 mL each were drawn into precooled monovettes (Sarstedt, Nuembrecht, Germany) containing EDTA and immediately centrifuged for 10 min at 4 °C and 2500 g. Plasma was separated under light protection, immediately transferred into polypropylene tubes, and stored at −35 °C.

**Analytic methods**

Plasma samples were analyzed for the E and Z isomers of lutein, all-E-3'-dehydro-lutein, and all-E-zeaxanthin and for the sums of Z-zeaxanthin isomers (typically: 85% 13-Z-zeaxanthin, 9-Z-zeaxanthin, and 15-Z-zeaxanthin) and Z-lutein isomers (9-Z-lutein, 9'-Z-lutein, 13-Z-lutein, 13'-Z-lutein, and 15-Z-lutein isomers) as previously described (22). Recovery rates of lutein and zeaxanthin were 100% and 99%, respectively. Plasma concentrations in the range 0.005–2.8 μmol/L could be measured with intraday CVs of 4.8% (lutein) and 5.6% (zeaxanthin) and interday CVs of 2.6% (lutein) and 6.8% (zeaxanthin), respectively. The lower limit of detection was 0.002 μmol/L; the lower limit of quantification was 0.007 ± 0.002 μmol/L.

β-Carotene, lycopene, β-cryptoxanthin, α-carotene, and retinol concentrations were measured according to Aebischer et al (25). Cholesterol and triacylglycerol concentrations in plasma were measured according to Richmond (26), Fossati and Prencipe (27), and McGowan et al (28) by using the CHOD-PAP method (Merck AG, Dietikon, Switzerland), adapted to a centrifugal analyzer (Cobas-Bio, Roche Diagnostics, Basel, Switzerland).

**Pharmacokinetic analyses**

**Lutein and zeaxanthin**

Baseline plasma xanthophyll concentrations \(C_0\) were calculated individually as the mean value of the 3 predosing samples on days −3 to −1. The approach to plateau was monitored by recording lutein or zeaxanthin trough concentrations over the dosing period, and the corresponding plasma concentration-time profiles were modeled according to an equation of the same form as that describing the kinetics following a constant-rate intravenous infusion (29, 30). The data were fitted according to the following equation:

\[
C(t) = H \times (1 - e^{-k_{eff} \times t}) + C_B
\]

where \(C(t)\) is the predicted xanthophyll concentration at time \(t\), \(H\) is the concentration increase to steady state, and \(k_{eff}\) is the rate constant; the latter 2 variables were estimated by fitting as described below. The half-life associated with \(k_{eff}\) was calculated as \(t_{1/2,\text{eff}} = \ln(2)/k_{eff}\).

Steady state parameters were derived from the time concentration profiles over a dosing interval \(t\) (24 h) recorded at day 42. The primary parameters included the area under the plasma concentration-time curve (AUC) and the corresponding baseline-corrected ΔAUC, both calculated by the linear trapezoidal rule (29, 30). The steady state concentration \(C_{ss}\) is defined as the time-averaged concentration over the dosing interval and was calculated as AUC\(/t\). The maximum baseline-corrected plasma concentrations after a dose (\(D_{C_{max}}\)), the time to reach maximum concentrations \(t_{max}\), and the predose baseline-corrected concentrations \(D_{C_{min}}\) were derived directly from the observed data. Baseline-corrected steady state concentration was defined as the average concentration over the dosing interval \(t\) and calculated as \(D_{C_{ss}} = \Delta \text{AUC}/t\). An additional secondary parameter was the peak-trough fluctuation \([PTF = (D_{C_{max}} - D_{C_{min}})/D_{C_{ss}}]\) (30). For the comparison of the 2 dosing groups, \(D_{C_{ss}}, \Delta \text{AUC}, D_{C_{max}},\) and \(D_{C_{min}}\) were dose-normalized by dividing these parameters by the corresponding daily doses (in μmol).

Postdosing concentration-time profiles were best fitted according to the monoexponential plus constant model represented by the following equation:

\[
C(t) = A \times e^{-k_1 \times t} + B
\]

where \(A\) is the preexponential coefficient, \(k_1\) is the apparent disposition rate constant, and \(B\) is a constant. Competing models were the monoeponential and the biexponential model in addition to elimination according to a Michaelis-Menten mechanism. Model selection was based on parameter precision, inspection of weighted residuals, and parsimony criteria by formally testing for nonrandomness of errors as judged by run tests, the \(F\) ratio test (for nested models), the Akaike information criterion, and the Schwarz criterion (31, 32). For the decrease in plasma concentrations of all-E-lutein and -zeaxanthin after day 42, the best fit could be achieved by the monoexponential plus constant model for all subjects in group 2. Because the concentration dose response was much lower for group 1, which affected the sensitivity for model discrimination, kinetic profiles of competing models were not tested for goodness of fit and model order. The half-life associated with \(k_{w}\) was calculated as \(t_{1/2,\text{eff}} = \ln(2)/k_{w}\).

The accumulation index \(R\) was calculated as follows:

\[
R = 1/(1 - e^{-k_{eff} \times t})
\]

For the kinetic analysis, plasma xanthophyll concentrations were fitted to models by using nonlinear least-squares regression analysis (WINSAAM, version 3.03; NIH, Bethesda, MD) (33). Measurement errors were assumed to be independent and normally distributed with a mean of 0 and a fractional SD of 0.05. Weights were chosen equal to the inverse of the variance of the measurement error. The SE of the parameter estimates was determined from the covariance matrix of the least-squares fit and was expressed as the CV.

Attainment of steady state concentrations was detected by monitoring the predose concentration-time data for each subject by linear regression for the interval from day 38 to day 43. Steady state was assumed to be reached, provided that the slopes of individual regression lines were statistically not distinct from 0.

**3'-Dehydro-lutein**

Baseline concentrations of 3'-dehydro-lutein and the baseline-corrected concentrations \(D_{C_{max}}, \Delta \text{AUC},\) and \(D_{C_{ss}}\) were evaluated as described for lutein and zeaxanthin. A model for the formation and elimination of 3'-dehydro-lutein was postulated, assuming that the plasma concentration of this carotenoid was metabolically derived from plasma lutein. To probe for such a precursor-product interrelation, a precursor time course was generated in WINSAAM by using a forcing function for lutein. This functional description of the plasma lutein data was provided by
linear interpolation between sequential pairs of data, recreating the shape of the input system and driving the formation of 3'-dehydro-lutein (22).

The formation and elimination of 3'-dehydro-lutein was modeled by the following differential equation:

\[ \frac{dC_{\text{DHL}}}{dt} = k_t \times \Delta C_{\text{lutein}} - k_e \times (C_{\text{DHL}} - B_{\text{DHL}}) \]  

(4)

where \( d \) is a derivative, \( \Delta C_{\text{lutein}} \) is the baseline-corrected concentrations of lutein, \( C_{\text{DHL}} \) is the plasma concentration of 3'-dehydro-lutein, and \( B_{\text{DHL}} \) is the 3'-dehydro-lutein baseline concentration. The rate constants for 3'-dehydro-lutein formation and elimination are designated as \( k_t \) and \( k_e \), respectively. Data fitting according to this precursor-product model was carried out for the pair all-E-lutein and all-E'-3'-dehydro-lutein; \( k_t, k_e \), and \( B_{\text{DHL}} \) were estimated by nonlinear least-squares regression analysis (WINSAAM, version 3.03) as outlined above.

Statistical analysis

Pharmacokinetic parameters of total lutein, all-E-lutein, zeaxanthin, and 3'-dehydro-lutein are presented as means ± 1 SDs. For \( t_{\text{max}} \) and the various half-lives, arithmetic means are given. Geometric means and corresponding SDs were calculated for \( \Delta C_{\text{ss}}, \Delta C_{\text{max}}, \Delta C_{\text{min}} \), AUC, and PTF, assuming a logarithmic normal distribution (as justified in reference 34).

Effects caused by interactions between sex and treatment were tested by ANOVA. In the absence of any significant sex effects, parameters were compared among treatment groups by one-way ANOVA (procedure aov, S-PLUS, version 6; Insightful Corporation, Seattle, WA). Effects were evaluated as differences in arithmetic means (group 1 vs group 2; including baseline concentrations and half-life) or as the ratio of means (group 1/group 2) for natural log-transformed parameters (including dose-normalized \( \Delta C_{\text{ss}} \) and \( \Delta C_{\text{max}}, R \), and PTF). Two-sided 95% CIs were constructed with the \( t \) distribution by using the square root of the residual variance in the analysis of variance table; \( t_{\text{max}} \) was analyzed by applying the Mann-Whitney \( U \) test. To compare effects within the same subject, further differences were evaluated by paired \( t \) tests. Attainment of steady state was tested by examining whether the zero value was contained in the 95% CI of the regression slopes. The level of statistical significance was set to \( P < 0.05 \) for all tests. All additional calculations were computed by using MATHCAD 2000 (MathSoft, Cambridge, MA).

RESULTS

All subjects completed the study according to the trial protocol, and no adverse events occurred that were related to lutein supplementation. Compliance was excellent (97–100%). Although the subjects in this study were required to avoid the intake of lutein-rich vegetables and fruit, the residual lutein intake was determined by differing eating habits. Thus, average dietary lutein consumption was highly variable, but median values were lower in all groups (Table 1) compared with data from nutritional surveys that showed a population mean value of 1–2 mg lutein/d. This finding indicates that the subjects complied with the dietary restrictions of the present study.

Plasma kinetics of all-E-lutein and all-E-zeaxanthin

Mean concentrations of all-E-lutein increased to plateau concentrations in response to administration of multiple oral doses of 4.1 mg lutein (group 1) or 20.5 mg lutein (group 2) and subsequently decreased after the cessation of dosing on day 42 (Figure 2A). all-E-Lutein represented the major fraction of lutein throughout the study, contributing to ≈85% at baseline and during the postdosing period and to ≈90% during dosing (data not shown). The remaining lutein contributions were determined as the sum of the 9-\( Z \), 9'-\( Z \), 13-\( Z \), 13'-\( Z \), and 15-\( Z \) isomers. Concentration-time profiles for all-E-lutein on day 42 are presented in Figure 2B. Control subjects did not ingest lutein supplements; therefore, baseline plasma all-E-lutein concentrations were maintained (Figure 2A).

Concentration-time profiles for all-E-lutein (group 1) and all-E-lutein (group 2) and subsequently decreased after the cessation of dosing on day 42 (Figure 2A). all-E-Lutein represented the major fraction of lutein throughout the study, contributing to ≈85% at baseline and during the postdosing period and to ≈90% during dosing (data not shown). The remaining lutein contributions were determined as the sum of the 9-\( Z \), 9'-\( Z \), 13-\( Z \), 13'-\( Z \), and 15-\( Z \) isomers. Concentration-time profiles for all-E-lutein on day 42 are presented in Figure 2B. Control subjects did not ingest lutein supplements; therefore, baseline plasma all-E-lutein concentrations were maintained (Figure 2A).

Atainment of lutein steady state was tested by evaluating the plasma concentration profile of predose samples (ie, blood specimens collected at the end of a dosing interval and just before ingestion of the next dose) over days 38–43 (Figure 3A) by linear regression. The resulting mean slopes were 0.010 ± 0.031 and 0.003 ± 0.027 for groups 1 and 2, respectively, ie, statistically not distinct from 0. This finding indicated that steady state had been reached at day 38 or before.

In addition to lutein, the dosage form contained a small amount of zeaxanthin (8.3% with respect to lutein). Although the kinetic profiles for all-E-zeaxanthin are not presented, the kinetic parameters for both all-E-xanthophylls are summarized in Table 2. Baseline plasma all-E-lutein and all-E-zeaxanthin concentrations were not significantly different between dosing groups. On day 42 peak xanthophyll plasma concentrations were reached ≈10–11 h after dosing. There was little fluctuation in lutein and zeaxanthin concentration on day 42 (Figure 2B), as evident from...
PTF values (Table 2). Steady state all-E-lutein concentrations exceeded baseline concentrations by \(\approx 3.5\)- and 10-fold for groups 1 and 2, respectively. The increases from baseline to steady state concentrations and the corresponding \(\Delta C_{\text{max}}\) for the ratio of the geometric means (group 1/group 2) for all-E-lutein ranged from 0.19 to 0.45 and from 0.21 to 0.50 for \(\Delta C_{\text{ss}}\) and \(\Delta C_{\text{max}}\) respectively. The corresponding 95% CIs for all-E-zeaxanthin were 0.17–0.42 and 0.30–0.46, respectively. The steady state concentrations of the sums the E- and Z-lutein isomers were 0.59 ± 0.14 and 1.64 ± 0.77 \(\mu\text{mol/L}\) for groups 1 and 2, respectively.

Dose proportionality was examined by comparing dose-normalized \(\Delta C_{\text{max}}^{*}\) and \(\Delta C_{\text{ss}}^{*}\) values between group 1 and group 2. Dose-normalized \(\Delta C_{\text{ss}}^{*}\) and \(\Delta C_{\text{max}}^{*}\) values for all-E lutein were significantly higher in the low-dose group (0.054 ± 0.017 and 0.063 ± 0.027 \(\text{L}^{-1}\), respectively) than in the high-dose group (0.036 ± 0.016 and 0.039 ± 0.018 \(\text{L}^{-1}\), respectively). The pertaining two-sided 95% CI for the ratio of geometric means (group 1/group 2) ranged from 1.01 to 2.25 and from 1.02 to 2.52 for \(\Delta C_{\text{ss}}^{*}\) and \(\Delta C_{\text{max}}^{*}\) respectively. For all-E-zeaxanthin, dose-normalized incremental, steady state concentrations did not differ significantly between groups 1 and 2 (\(\Delta C_{\text{ss}}^{*} = 0.045 ± 0.020\) and 0.034 ± 0.016 \(\text{L}^{-1}\), respectively); the 95% CI for the ratio of geometric means (group 1/group 2) ranged from 0.83 to 2.10. Similarly, dose-normalized \(\Delta C_{\text{max}}^{*}\) values for all-E-zeaxanthin concentrations did not differ significantly between groups 1 and 2 (0.058 ± 0.022 and 0.039 ± 0.018 \(\text{L}^{-1}\), respectively). The corresponding 95% CI for the ratio of geometric means (group1/group2) ranged from 0.96 to 2.29.

The cholesterol-normalized concentration-time profiles of lutein resembled those shown in Figure 2A and had similar intersubject variations (data not shown). As described in Subjects and Methods, empirical modeling was used to fit both the time courses of the approach to plateau in response to carotenoid dosing and the decay of plasma concentrations during the postdosing phase (Figure 4). The monoeponential plus constant model was applied to fit the decay curves of all-E-lutein in both groups and for all-E-zeaxanthin in the high-dose group. Model parameters for all-E-zeaxanthin in the low-dose group could not be accurately estimated because the increase in plasma concentrations above baseline was too small. The precision of all-E-lutein disposition half-life estimates for each subject was acceptable, because the CVs were <20% and 12% for groups 1 and 2, respectively. The half-life of apparent lutein disposition \(t_{\text{d}}\) for all-E-lutein and all-E-zeaxanthin ranged between 5 and 7 d (Table 2), and the half-lives for all-E lutein were not significantly different between groups. Again, the precision of the parameters characterizing all-E-lutein plasma accumulation for each subject was acceptable, because the CVs were <16% and 10% for groups 1 and 2, respectively. However, for 2 subjects in group 1 and for 1 subject in Group 2, at least one kinetic parameter resulted in 95% CIs, which included 0, and thus by inference, the

**FIGURE 3.** Attainment of apparent steady state all-E-lutein concentrations for representative women (\(n = 2\)) and men (\(n = 2\)) in group 2. Concentrations in plasma sampled after the subjects fasted overnight and before administration of the daily dose of lutein in the morning are presented. The data for each individual were fitted by linear regression (solid lines). The slopes of individual fits were not significantly different from 0 (\(P < 0.05\)).

**TABLE 2**

Kinetic parameters for all-E-lutein and all-E-zeaxanthin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lutein</th>
<th>Zeaxanthin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1</td>
<td>Group 2</td>
</tr>
<tr>
<td>Dose ((\mu\text{mol/d}))</td>
<td>6.703</td>
<td>33.513</td>
</tr>
<tr>
<td>(C_{\text{ss}}^{*}) ((\mu\text{mol/L}))</td>
<td>0.140 ± 0.031</td>
<td>0.148 ± 0.120</td>
</tr>
<tr>
<td>(\Delta C_{\text{max}}^{*}) ((\mu\text{mol/L}))</td>
<td>0.425 ± 0.179</td>
<td>1.320 ± 0.594 (^{*3})</td>
</tr>
<tr>
<td>(t_{\text{max}}^{*}) (d)</td>
<td>9.8 ± 2.4</td>
<td>11.3 ± 4.6</td>
</tr>
<tr>
<td>(\Delta AUC) ((\mu\text{mol} \cdot \text{h/L}))</td>
<td>9.105 ± 2.904</td>
<td>30.200 ± 13.26 (^{*3})</td>
</tr>
<tr>
<td>(\Delta C_{\text{SS}}^{*}) ((\mu\text{mol/L}))</td>
<td>0.362 ± 0.112</td>
<td>1.200 ± 0.55 (^{*3})</td>
</tr>
<tr>
<td>PTF (^{*})</td>
<td>0.267 ± 0.213</td>
<td>0.220 ± 0.108</td>
</tr>
<tr>
<td>(C_{\text{ss}}^{*}) ((\mu\text{mol/L}))</td>
<td>0.275 ± 0.129</td>
<td>1.452 ± 0.689 (^{*})</td>
</tr>
<tr>
<td>(t_{\text{eff}}^{*}) (d)</td>
<td>4.58 ± 0.38</td>
<td>6.71 ± 1.50 (^{*})</td>
</tr>
<tr>
<td>(t_{\text{d}}^{*}) (d)</td>
<td>5.49 ± 2.12</td>
<td>6.09 ± 0.98</td>
</tr>
<tr>
<td>(R^{2})</td>
<td>7.11 ± 0.55</td>
<td>9.97 ± 2.32 (^{*})</td>
</tr>
</tbody>
</table>

\(^{1}\) \(n = 8\) per group, except for \(t_{\text{eff}}^{*}\) (\(n = 6\) for group 1 and \(n = 7\) for group 2). Data for both sexes were pooled. \(C_{\text{ss}}^{*}\), baseline concentration; \(\Delta C_{\text{max}}^{*}\), baseline-corrected maximum concentration on day 42; \(t_{\text{max}}^{*}\), time to reach maximum concentration on day 42; \(\Delta AUC\), baseline-corrected area under the concentration-time curve over 24 h on day 42; \(\Delta C_{\text{SS}}^{*}\), baseline-corrected steady state concentration on day 42; PTF, peak-trough fluctuation; \(t_{\text{eff}}^{*}\), efficient half-life of accumulation; \(C_{\text{SS}}^{*}\), steady state concentration; \(R^{2}\), accumulation index; ND, not determined.

\(^{2}\) Arithmetic \(\bar{x} \pm \text{SD}\) (including data for both sexes).

\(^{3}\) Geometric \(\bar{x} \pm \text{SD}\).

\(^{4}\) Significantly different from group 1, \(P < 0.05\) (ANOVA).

\(^{5}\) Corresponding dose-normalized data differ for the 2 groups, \(P < 0.05\).
parameter was not established. Data for these subjects were not considered for further data compilation in Table 2. The mean half-lives of accumulation \( t_{\text{eff}} \) for plasma all-E-lutein were, in a formal statistical sense, different between groups 1 and 2, because the 95% CI for the arithmetic mean differences between groups were \(-3.53\) and \(-0.73\) d, respectively. This finding indicated that 15 (group 1) or 22 (group 2) d were required to reach 90% of steady state concentrations. Accordingly, the calculated accumulation factors for all-E-lutein differed between groups. By contrast, the \( t_a \) values for all-E-lutein were not significantly different between groups (95% CI for corresponding mean differences: \(-2.37, 1.17\) d).

As shown by paired \( t \) tests, half-lives determined from lutein accumulation \( (t_{\text{eff}}) \) for plasma all-E-lutein were consistently not different from those from lutein plasma decay \( (t_d) \) for group 1 (95% CI: \(-0.54, 0.38\) d) and group 2 (95% CI: \(-0.57\) to \(1.54\) d) and for the pooled data from both groups (95% CI: \(-0.32, 0.77\) d).

Mean values of the all-E-lutein parameter B, representing the constant of the kinetic disposition model, were 0.158 \( \pm \) 0.062 and 0.226 \( \pm \) 0.223 \( \mu \text{mol/L} \) for groups 1 and 2, respectively, and consistently exceeded baseline concentrations (Table 2). However, the difference between constant term B and basal concentrations accounted to \( \approx 8 \% \) of the incremental dose response for group 2, and these differences were significant (paired \( t \) test) on the basis of the corresponding 95% CIs for the geometric mean ratios (baseline concentration/parameter B) for group 1 (0.60, 1.24) and group 2 (0.49, 0.70), respectively.

Baseline lutein concentrations are, to some extent, a predictor of the increase in lutein from baseline to steady state plasma concentrations, as shown in Figure 5. To emphasize the sigmoid behavior, data were fitted according to a Hill equation (data for group 2 only). There was a significant positive correlation between the dose-normalized increments in plasma all-E-lutein concentrations and the dose-normalized increase in plasma all-E-zeaxanthin concentrations (\( r^2 = 0.68, P < 0.05; \) data not shown).

### Plasma kinetics of all-E-3'-dehydro-lutein

After lutein dosing, lutein concentrations of all-E-3'-dehydro-lutein increased significantly by factors of 1.7 (group 1) and 4.2 (group 2) over mean baseline concentrations, as shown by paired \( t \) tests (Figure 6A). Kinetic parameters of all-E-3'-dehydro-lutein are summarized in Table 3. The plasma concentration time profiles on day 42 were unvarying for both dosing groups (Figure 6B). The dose-normalized \( \Delta C_{\text{max}} \) and \( \Delta \text{AUC} \) were not significantly different between groups 1 and 2.

Because the rise in plasma lutein concentrations was parallelled by an increase in all-E-3'-dehydro-lutein concentrations, a
possible parent-compound metabolite relation was investigated, assuming that the rate of all-E-3’-dehydro-lutein formation is proportional to the lutein plasma concentration. This amounted to postulating first-order kinetics (ie, one step or a series of first-order reactions) for the conversion. For 2 subjects (out of 8) in group 1, at least one kinetic parameter resulted in 95% CIs that included 0, and, thus, by inference, the parameter was not established. The parameters for those 2 subjects were not considered for further data compilation in Table 3. The precision of the parameters for individuals in group 2 was adequate; the CV was 7% for parameter data compilation in Table 3. The precision of the parameters for the 2 lutein-supplemented groups, the observed nonlinearity was established by the highly significant correlation between baseline-corrected steady state plasma concentrations of these compounds on day 42 (Figure 7).

### DISCUSSION

The findings of the present study complement those of a previous trial of the chemically related zeaxanthin (22), ie, the pharmacokinetics of lutein and zeaxanthin showed many similarities. The inclusion of a small fraction of zeaxanthin into the dosing preparation provided an opportunity to study the kinetics of this carotenoid under conditions of lutein excess. In the absence of supplemented lutein, dose-normalized $\Delta C_{all}$ values for all-E-zeaxanthin were 0.086 and 0.050 L/mol for zeaxanthin doses of 1.76 and 17.6 μmol/d (22), which exceeded the dose-normalized concentrations of 0.045 and 0.034 L/mol observed for group 1 (dose = 0.58 μmol) and group 2 (dose = 2.90 μmol) in the present investigation. Therefore, lutein dosing impaired zeaxanthin bioavailability but did not affect plasma concentrations of other carotenoids and retinol (Table 4). Lutein was shown previously to reduce β-carotene absorption in single-dose studies (35–37), but such an effect was not manifested in the present multiple-dose study.

Evidence for the attainment of apparent steady state was 2-fold: 1) predose samples from days 38 to 43 (Figure 3) indicated that plasma lutein concentrations were maintained, and 2) based on $t_{max}$ values (Table 2), the calculated fraction of steady state was >99% for this interval. The time required to attain a >90% fraction of plasma steady state concentration was 15–22 d for lutein. The corresponding time observed for synthetic zeaxanthin was in the same range, ∼17 d (22).

Peak steady state concentrations on day 42 were reached, on average, between 9 and 12 h after dosing; this finding agrees with the $t_{max}$ values observed for other compounds that enter the circulation via the lymphatics incorporated into chylomicrons and eventually get recirculated within hepatic VLDL (38). Mean dose-normalized $\Delta C_{all}$, all-E-lutein concentrations and $\Delta C_{all}$ values for group 2 were 64% of those of group 1. Because the disposition parameter $k_2$ was not significantly different between the 2 lutein-supplemented groups, the observed nonlinearity was not related to dose-dependent disposition kinetics. Therefore, as discussed for other carotenoids, the capacity for intestinal absorption or recirculation within VLDLs may become limiting with increasing dose (39). For all-E-zeaxanthin, which was provided at much lower dosages, the plasma response appeared to be dose proportional, because dose-normalized $C_{max}$ and $\Delta AUC$...
concentrations did not differ significantly. By contrast, at dosages of 1 and 10 mg zeaxanthin (22), nonlinearity was similar to that of lutein in the present trial.

The observation that the increases in plasma lutein concentrations were sigmodially related to baseline concentrations (Figure 5) confirmed similar findings by Olmedilla et al (40). Several factors, including the disposition parameter $k_p$, plasma cholesterol and triacylglycerol concentrations (as indexes of plasma lutein cotransport by lipoproteins), and sex were tested to account for the observed dependency in the present study. None of these parameters was significantly correlated with baseline concentrations or with the increase in $\Delta C_{\text{ss}}$ (data not shown). It appears that the variability in the response of plasma concentrations may be related to individuality in intestinal absorption or other metabolic events.

The half-life of apparent lutein disposition was comparable with effective half-life ($t_{\text{eff}}$), characterizing lutein accumulation during the approach to plateau. Such accumulation is characterized by an effective half-life, which is a weighted average of the absorption half-life and the half-lives describing the disappearance from plasma (41). The effective half-life is always shorter than the terminal half-life. The similarity of $t_{\text{eff}}$ and $t_{\text{eff}}$ values indicates that $t_{\text{eff}}$ represents an estimate of the effective half-life rather than that of the terminal half-life. This is further corroborated by the finding that the constant term B, obtained from data fitting with the monoeponential plus constant term model, consistently exceeded the baseline lutein concentrations. The monoeponential plus constant term model does not represent the general integrated form of a corresponding compartmental model, and the prediction that plasma concentrations would not return to baseline concentrations could point to a biexponential model with a vanishing exponential term. Thus, a monoeponential plus constant term model may be interpreted as an approximation to the integrated form of a 2-compartment model (2 exponential terms) for the extreme case when the second rate constant approaches zero, i.e., the terminal half-life becomes very long and may not be evaluated from data obtained within the selected time window. Such interpretation implies that a very slow plasma disappearance will become indistinguishable from a constant plasma concentration, particularly when the quality of the concentration data are corrupted by measurement errors. In fact, much longer lutein half-lives of $\approx$15 d (20) and 76 d (21) were previously published.

Furthermore, the mean $t_{\text{eff}}$ (5.5 d) was very similar to the $t_{\text{eff}}$ (5.2 d) for zeaxanthin found in our previous study and was distinct from the reported terminal half-life of $\approx$12 d (22). Estimation of the terminal half-life of zeaxanthin in that study was critically dependent on the extended sampling interval to up to 76 d. An alternative explanation to account for the preference of the monoeponential plus constant term model might be the lack of adequate control of the dietary restrictions of lutein ingestion during the trial, which could have resulted in lutein intakes that surpassed prestudy intakes. However, this explanation appears to be highly unlikely on the basis of the subjects’ dietary records and on the basis of the finding that the constant term B exceeded baseline concentrations for all subjects. Moreover, the plasma lutein concentrations of the control subjects did not increase.

The accumulation index, which relates exposure at steady state to that after the first dose, is determined by the $t_{\text{eff}}$. The accumulation index was comparable for all-E-lutein ($=8.5$ on average) and all-E-zeaxanthin ($=8$, assuming that the $t_{\text{eff}}$ reflects $t_{\text{eff}}$), and there was excellent agreement with the accumulation index previously determined for zeaxanthin ($=7.5$) (Table 2) (22).

A considerable accumulation of plasma 3'-dehydro-lutein was observed in response to administration of the lutein preparation. Kinetics of 3'-dehydro-lutein resembled that of lutein, and we postulated that 3'-dehydro-lutein is formed from lutein. Substantiation of this hypothesis followed the same line of argument as described for formation of 3'-dehydro-lutein from zeaxanthin (22). Plasma 3'-dehydro-lutein concentrations were coupled to linearly interpolated all-E-lutein concentrations, which served as input function for driving the metabolite subsystem. This approach allowed for approximation of the kinetics of plasma 3'-dehydro-lutein, assuming first-order kinetics for formation and elimination of the compound, as described by a one-compartment model. The predicted concentrations were not significantly different from experimental plasma concentrations of 3'-dehydro-lutein (Figure 6). Furthermore, the increases in plasma lutein and 3'-dehydro-lutein at steady state were found to be proportional (Figure 7). These findings provide strong evidence that the rise in 3'-dehydro-lutein above baseline concentrations was closely related to plasma lutein concentrations and was, therefore, a consequence of lutein supplementation.

Because the lutein preparation also contained a small fraction of zeaxanthin, 3'-dehydro-lutein formation was probably in part derived from zeaxanthin (22). Therefore, the probable contribution of 3'-dehydro-lutein production from zeaxanthin to total metabolite was assessed by referring to the rate constants for formation of 3'-dehydro-lutein from zeaxanthin and eventual elimination (22) and by using the average linearly interpolated plasma all-E-zeaxanthin concentrations of group 2 (present

### Table 4

<table>
<thead>
<tr>
<th>Group</th>
<th>Lycopene</th>
<th>$\beta$-Carotene</th>
<th>$\alpha$-Carotene</th>
<th>$\beta$-Cryptoxanthin</th>
<th>Retinol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_B$</td>
<td>0.830 ± 0.471</td>
<td>0.510 ± 0.231</td>
<td>0.085 ± 0.048</td>
<td>0.337 ± 0.170</td>
<td>1.740 ± 0.403</td>
</tr>
<tr>
<td>$C_{SS}$</td>
<td>0.654 ± 0.226</td>
<td>0.719 ± 0.322</td>
<td>0.098 ± 0.071</td>
<td>0.290 ± 0.144</td>
<td>1.736 ± 0.387</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_B$</td>
<td>0.452 ± 0.223</td>
<td>0.503 ± 0.635</td>
<td>0.133 ± 0.189</td>
<td>0.155 ± 0.106$^2$</td>
<td>1.717 ± 0.284</td>
</tr>
<tr>
<td>$C_{SS}$</td>
<td>0.537 ± 0.336</td>
<td>0.493 ± 0.328</td>
<td>0.104 ± 0.080</td>
<td>0.196 ± 0.093</td>
<td>1.718 ± 0.303</td>
</tr>
</tbody>
</table>

$^1$ All values are $\bar{x}$ ± SD; $n = 8$ per group. Data for both sexes were pooled and represent the sum of E and Z isomers. $C_B$, baseline concentration; $C_{SS}$, time-averaged concentration calculated as area under the curve/24 on day 42. There were no significant differences between baseline and steady state concentrations.

$^2$ Significantly different from group 1, $P < 0.05$.
study) as input function for driving the conversion to all-E-3'-dehydro-lutein. The simulation showed that \( \approx 18\% \) of the increase in 3'-dehydro-lutein concentrations may be related to the added zeaxanthin. The contribution at steady state was calculated from the \( \Delta \text{AUC} \) for all-E-zeaxanthin of the present study and from the slope and intercept of Figure 5 of reference 22. Again, \( \approx 18\% \) of the all-E-3'-dehydro-lutein was estimated to be formed from all-E-zeaxanthin. This confirms that lutein represented the major source of 3'-dehydro-lutein.

The model parameter characterizing the formation of 3'-dehydro-lutein, \( k_6 \), is associated with a half-life of \( \approx 46 \) d, which is much longer than the apparent half-life for lutein disposition (5.3–6 d). This implies that lutein is not exclusively eliminated via formation of 3'-dehydro-lutein, but also by other, more efficient, pathways, which may include metabolic reactions as suggested by Khachik et al (23). Furthermore, this is consistent with the hypothesis that lutein is also eliminated by a comparatively slow process (with a half-life of 46 d) as postulated above.

The 3'-dehydro-lutein formation rate constant from zeaxanthin (\( k_7 \approx 0.055 \, \text{d}^{-1} \)) significantly exceeded that from lutein (\( k_5 \approx 0.017 \, \text{d}^{-1} \)) (Table 3). By contrast, the disposition constants of 3'-dehydro-lutein were not significantly different: \( k_e \approx 0.28 \, \text{d}^{-1} \) (22) and \( k_e \approx 0.24 \, \text{d}^{-1} \) (present study) when zeaxanthin or lutein were supplemented, respectively. Therefore, plasma disposition does not depend on the parent compound of the metabolite. The half-life associated with \( k_e \) is \( \approx 2.8 \) d and, accordingly, the formation of 3'-dehydro-lutein was limited by the rate of formation.

In conclusion, the present study showed that plasma lutein concentrations increased 3.5-fold and 10-fold on average, respectively, after the long-term intake of 4.1 and 20.5 mg lutein. Plasma concentrations of all-E-3'-dehydro-lutein increased in parallel with those of all-E-lutein, and the increase was clearly related to lutein intake. Except for zeaxanthin, which was also present to a small portion in the preparation administered, other carotenoids remained unaffected by lutein administration.

PAT was responsible for the clinical conduct of the study, was involved in the design of the protocol (principal investigator according to GCP guidelines), and wrote the first draft of the manuscript. J-CA was responsible for the carotenoid and lipid analyses. WS initiated and supervised the project. UT performed the clinical investigations and supervised the dietary instructions (clinical coinvestigator). WC designed the study, was responsible for both the kinetic and statistical data analyses, and prepared the final manuscript. PAT received research grants for the conduct of the clinical part of the study from DSM Nutritional Products Ltd (formerly Roche Vitamins Ltd). J-CA, WS, and WC are employees of DSM Nutritional Products Ltd. UT had no conflict of interest.

REFERENCES