

# Plasma appearance and distribution of astaxanthin *E/Z* and *R/S* isomers in plasma lipoproteins of men after single dose administration of astaxanthin

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*Appearance, pharmacokinetics, and distribution of astaxanthin E/Z and R/S isomers in plasma and lipoprotein fractions were studied in 3 middle-aged male volunteers (37–43 years) after ingestion of a single meal containing a 100 mg dose of astaxanthin. The astaxanthin source consisted of 74% all-E-, 9% 9Z-, 17% 13Z-astaxanthin (3R,3'R-, 3R,3'S; meso-, and 3S,3'S-astaxanthin in a 1:2:1 ratio). The plasma astaxanthin concentration–time curves were measured during 72 hr. Maximum levels of astaxanthin ( $1.3 \pm 0.1$  mg/L) were reached  $6.7 \pm 1.2$  hr after administration, and the plasma astaxanthin elimination half-life was  $21 \pm 11$  hr. 13Z-Astaxanthin accumulated selectively, whereas the 3 and 3'R/S astaxanthin distribution was similar to that of the experimental meal. Astaxanthin was present mainly in very low-density lipoproteins containing chylomicrons (VLDL/CM; 36–64% of total astaxanthin), whereas low-density lipoprotein (LDL) and high-density lipoprotein (HDL) contained 29% and 24% of total astaxanthin, respectively. The astaxanthin isomer distribution in plasma, VLDL/CM, LDL, and HDL was not affected by time. The results indicate that a selective process increases the relative proportion of astaxanthin Z-isomers compared to the all-E-astaxanthin during blood uptake and that astaxanthin E/Z isomers have similar pharmacokinetics. (J. Nutr. Biochem. 11:482–490, 2000) © Elsevier Science Inc. 2000. All rights reserved.*

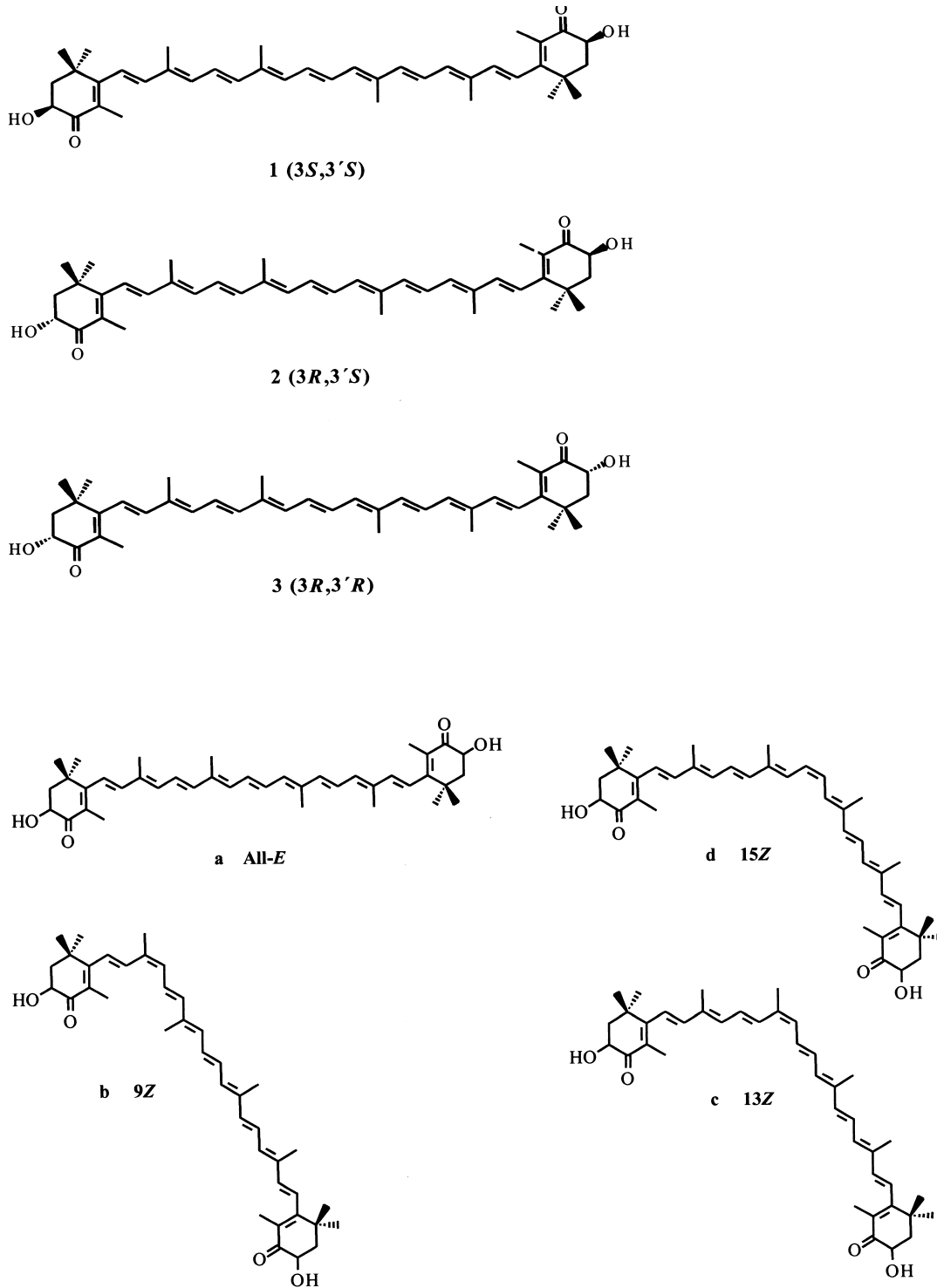
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## Introduction

Dietary carotenoids from fruits and vegetables, in particular  $\beta,\beta$ -carotene, have been associated with a decreased risk of certain cancer forms and cardiovascular diseases.<sup>2,3</sup> Numerous carotenoids may be present in human plasma. The major carotenoids reported are lutein ( $\beta,\epsilon$ -carotene-3,3'-diol), zeaxanthin ( $\beta,\beta$ -carotene-3,3'-diol),  $\alpha$ -cryptoxanthin ( $\beta,\epsilon$ -carotene-3-ol),  $\beta$ -cryptoxanthin ( $\beta,\beta$ -carotene-3-ol), lycopene ( $\psi,\psi$ -carotene),  $\beta,\epsilon$ -carotene, and  $\beta,\beta$ -carotene.<sup>4</sup> In

breast milk and serum of lactating mothers, 34 carotenoids, including 13 different Z-isomers have been identified.<sup>5</sup> Carotenoids are absorbed from the diet by passive diffusion into the intestinal mucosal cells, and transport is associated with plasma lipoproteins and apparently not with specific carotenoid-binding proteins.<sup>6–8</sup> Carotenoids have individual patterns of absorption, plasma transport, and metabolism affected by structural differences including geometrical isomerization.<sup>9</sup> Intact carotenoids are incorporated in chylomicrons (CM) and transported via lymph and blood to the liver where they are partly resecreted with lipoproteins. The polar xanthophylls (oxygen-containing carotenoids) are distributed differently among the lipoprotein fractions than are the non-polar carotenes.<sup>10</sup> Carotenes are mainly carried in low-density lipoproteins (LDL; approximately 76%), whereas xanthophylls are equally distributed between LDL and high-density lipoproteins (HDL).<sup>11–13</sup> This may be rationalized by a preferential solubilization of polar carote-

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**Figure 1** Structures of astaxanthin optical isomers all-*E*-(3*S*,3'*S*)- (**1**), all-*E*-(3*R*,3'*S*; *meso*)- (**2**), and all-*E*-(3*R*,3'*R*)-astaxanthin (**3**), and geometrical isomers all-*E*-(**1a**, **2a**, **3a**), 9*Z*-(**1b**, **2b**, **3b**), 13*Z*-(**1c**, **2c**, **3c**), and 15*Z*-astaxanthin (**1d**, **2d**, **3d**).

noids in the phospholipid surface and of carotenes in the triglyceride core.<sup>14</sup> In humans, a considerable number of studies have focused on the absorption, transport, and metabolism of carotenes, whereas little information is available on xanthophylls.<sup>6–8</sup>

Astaxanthin (3,3'-dihydroxy- $\beta,\beta$ -carotene-4,4'-dione) is

widely distributed in the animal kingdom (in particular, marine seafood such as salmonid fishes and shrimps) and, as such, constitute part of the human diet. Carotenoids with chiral centers or axes may have different optical *R/S* isomers (*Figure 1*). *E/Z*-Configuration of geometrical isomers has a larger influence on the overall molecular shape. The all-*E*-

isomer of astaxanthin predominates in nature, but the 9Z-, 13Z-, and 15Z-isomers are also encountered (Figure 1). In recent years, the bioavailability of carotenoid Z-isomers has been in focus.<sup>6–8</sup> Astaxanthin is effective in promoting secondary immune responses against pathogens and transformed cells<sup>15,16</sup> and acts as an antioxidant.<sup>17</sup> Dietary intake of the astaxanthin-producing red yeast (*Phaffia rhodozyma*) or synthetic astaxanthin improves pigmentation and health status of rainbow trout (*Oncorhynchus mykiss*).<sup>18,19</sup> In rainbow trout, the retention of all-*E*-astaxanthin was much higher than that of the astaxanthin Z-isomers.<sup>20</sup> The higher absorption of all-*E*-astaxanthin compared to Z-astaxanthins may be explained by the sterically more bulky Z-isomers being less permeable to the lipid membrane of the enterocytes. 13Z-astaxanthin accumulates in the liver of rainbow trout when compared to the all-*E*- and 9Z-isomers, indicating a selective metabolism of the *E/Z*-isomers.<sup>21</sup> In a recent study, Elmadfa and Majchrzak<sup>22</sup> failed to detect astaxanthin, but not canthaxanthin, in the plasma of humans fed a salmon meal containing canthaxanthin and astaxanthin. So far, no reports on human absorption and plasma lipoprotein distribution of astaxanthin *E/Z* or *R/S* isomers are available.

The objective of this study was to examine the appearance of astaxanthin *E/Z* or *R/S* isomers in the plasma of 3 healthy middle-aged males given a single meal containing a total of 100 mg of a mixture of all-*E*-, 9Z-, and 13Z-astaxanthin (3*R*,3'*R*:3*R*,3'*S*:3*S*,3'*S*; ratio 1:2:1). Total astaxanthin, optical isomer distribution after derivatization to the corresponding camphanates,<sup>23</sup> and geometrical isomers of astaxanthin in the plasma were quantified by high-performance liquid chromatography (HPLC).<sup>20,24</sup> Distribution of *E/Z*-isomers in different plasma lipoprotein fractions were determined after ultracentrifugation in an iodixanol self-generating density gradient.

## Materials and methods

### Chemicals, instruments, and equipment

Crystalline standard of all-*E*-astaxanthin was a gift from Hoffman-La Roche (Basel, Switzerland). Carophyll Pink, consisting of water-dispersible astaxanthin containing beadlets (8% astaxanthin; Hoffman-La Roche, Basel, Switzerland) was used as a source of astaxanthin, and consisted of 74% all-*E*-, 9% 9Z-, and 17% 13Z-astaxanthin (3*R*,3'*R*-), (3*R*,3'*S*; *meso*-), and (3*S*,3'*S*)-astaxanthin in a 1:2:1 ratio. All HPLC-grade solvents for chromatography and solvents for extraction (pro analysis) were purchased from Merck (Darmstadt, Germany). Solvents for extraction were added 500 mg/L 2,6-di-*t*-butyl-*p*-cresol (BHT, Sigma, St. Louis, MO USA) as an antioxidant. The density gradient medium for ultracentrifugation, iodixanol (5,5'-(2-hydroxy-1-3-propanediyl)-bis(acetylamino)] bis [N,N'-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenecarboxamide]), 60% w/v in water), was obtained from Nycomed Pharma AS (Optiprep, Oslo, Norway). Assay kits (Cholesterol kit no. 236691, Total protein kit no. 124281, Triglyceride kit no. 701912) from Boehringer Mannheim GmbH, (Mannheim, Germany) were applied to determine the content of triglycerides, total protein, and cholesterol in fractions collected from ultracentrifuge tubes.

Plasma samples were prepared from blood in a refrigerated (4°C) centrifuge (Sigma Kühlzentrifuge 4kl, Osterode, Germany). Separation of plasma lipoproteins was performed in 15 mL Bell-top Quick-Seal tubes sealed with cordless tube toppers using

a Beckman XL-100 ultracentrifuge equipped with a VTi 50 vertical rotor (Beckman, Palo Alto, CA USA). The Quick-Seal tubes were filled and unloaded by a peristaltic pump (Ismayec sa, MS Reglo, Bennett & Co., N. Somerset, England). Densities of lipoprotein fractions were determined using a digital refractometer (RX 5000, Atago, Tokyo, Japan). Equipment for extraction of carotenoids included a mixer (Ultraturax T25, Janke and Kunkel, IKA Labortechnik, Staufen, Germany) and a heating block (Reacti-therm, Pierce, Holland) equipped with a nitrogen-evaporating unit (Reacti-vap, Pierce, Holland). The HPLC system used was a Hewlett Packard liquid chromatograph (Hewlett Packard, Palo Alto, CA USA) connected to a Hewlett Packard photodiode array UV-VIS detector.

### Subjects and study design

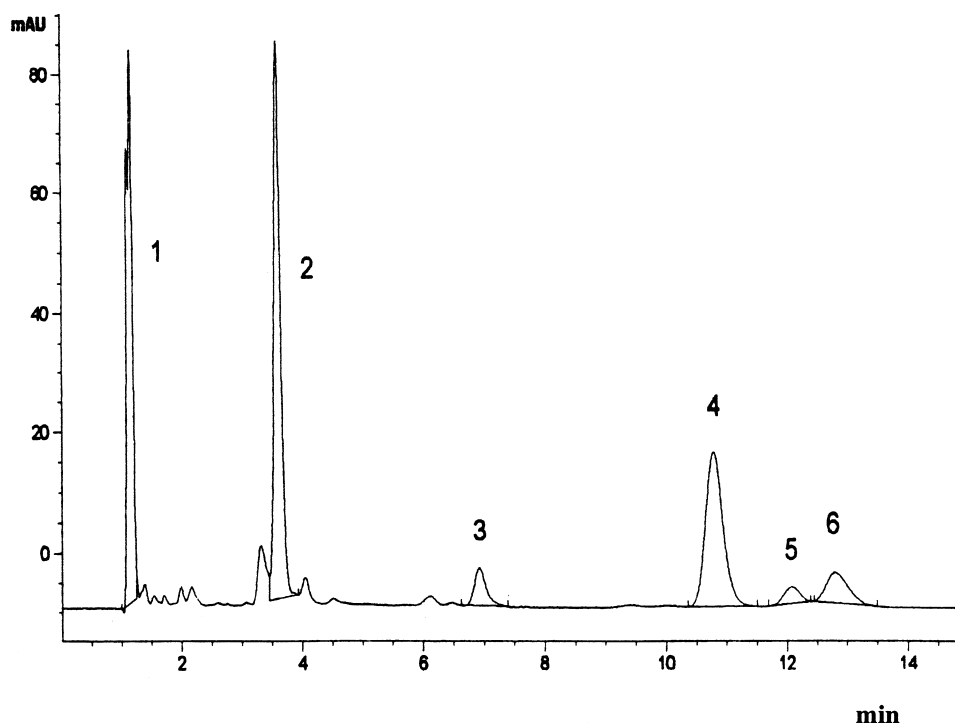
Three healthy male volunteers aged 37–43 years, weight 90–100 kg (individual body-mass indexes, 27.5, 30.5, and 31.7 kg/m<sup>2</sup>, respectively), participated in this study. Written consent was obtained from each participant and each was instructed to abstain from carotenoid-rich food for 3 days before and during the study. A single dose of astaxanthin (100 mg, 168 μmol) was administered. Study subjects were instructed to eat food low in carotenoids during the 72-hr experiment. The astaxanthin dosage was prepared by dispersing the astaxanthin beadlets in warm water (40°C) and mixing with olive oil (50% of meal weight) and cereals.

### Sampling and sample preparation

Blood samples (5 mL) were collected in ethylenediaminetetraacetic acid (EDTA)-containing vacuum tubes (Vacutainer, Terumo, Belgium) equipped with a syringe after venipuncture immediately before and 2, 4, 6, 8, 10, 12, 24, 32, 48, and 72 hr after ingestion of the astaxanthin-containing meal. Samples were stored on ice (up to 30 min) before being centrifuged (4°C, 1,500 × g, 20 min). Aliquots of plasma (0.5 mL) were transferred to small test tubes and stored at –80°C until carotenoids were analyzed. All samples were analyzed for astaxanthin *E/Z* isomer composition in intact plasma. Samples collected at 2, 4, 6, 8, 10, 12, 24, 32, and 48 hr were ultracentrifuged and pooled fractions corresponding to very low-density lipoprotein containing chylomicrons (VLDL/CM), LDL, and HDL were analyzed for astaxanthin *E/Z* isomer composition. Samples collected 72 hr postprandially were used for analyses of astaxanthin *R/S* isomer composition in intact plasma.

Astaxanthin *E/Z*-isomer composition in the experimental meal was determined by HPLC.<sup>20</sup> Astaxanthin was extracted from plasma and lipoprotein fractions according to Wathne et al.<sup>25</sup> To blood plasma samples (1.0 mL) methanol (1.0 mL; containing 500 ppm butylated hydroxytoluene [BHT]) was added, and the sample mixed (Whirlmixer, Fisons, England) for 20 sec. CHCl<sub>3</sub> (3 mL) was added and the sample mixed again for 20 sec. After settling for 10 min, the sample was mixed and centrifuged (approximately 1,700 g, 10 min). An aliquot (2 mL) of the hypophase containing astaxanthin dissolved in CHCl<sub>3</sub> was pipetted into a test tube and the solvent was evaporated on a heating block (approximately 40°C) using a gentle flow of nitrogen gas (N<sub>2</sub>). After evaporation, the sample was dissolved in 20% acetone in *n*-hexane (1 mL). The solution was filtered (0.45 μm; Minisart SRP15, Sartorius, Germany) directly into the sample vials and immediately sealed. Replicate analyses were performed on all samples. For determination of optical *R/S* isomer composition, astaxanthin was purified by thin-layer chromatography on silica gel plates (Kieselgel 60G, product no. 7731, Merck, Darmstadt, Germany). Purified astaxanthin was converted to the corresponding diesters of (-)-camphanic acid by reacting a dry sample of astaxanthin with (-)-camphanoyl chloride in dry pyridine at 0–4°C.<sup>23</sup>

Self-generating gradients containing Optiprep were used for



**Figure 2** High-performance liquid chromatogram showing *E/Z* isomer composition of plasma astaxanthin 6 hr after a single oral ingestion of a meal containing 100 mg astaxanthin. Chromatographic conditions:  $\text{H}_3\text{PO}_4$  modified silica gel column,<sup>24</sup> flow 1.0 mL/min, detection wavelength 470 nm. Peaks were identified by retention time, co-chromatography with authentic astaxanthin isomers, and VIS spectra. Peaks 1, 2, and 3: unknown; Peak 4: all-*E*-astaxanthin; Peak 5: 9*Z*-astaxanthin; Peak 6: 13*Z*-astaxanthin.

separation of blood fractions, which allowed further analysis without removing the iodixanol.<sup>26</sup> Polyallomer Bell-top Quick-Seal tubes (15 mL) were filled with 1.6 mL buffered saline (0.8% NaCl in water with (n-(2-hydroxyethyl)piperazine-*n'*-(2-ethanesulfonic acid) HEPES buffer, pH 7.4) using a peristaltic pump. First, a layer with 6% Optiprep and thereafter, a layer with Optiprep (1.4 mL) was mixed with plasma and buffered saline added up to a volume of 6.7 mL (corresponding to 12% Optiprep) was pumped underneath. A pillow (up to 0.5 mL) of 60% Optiprep was put at the bottom of the tube to prevent contact between the tube wall and soluble proteins, and the tubes were ultracentrifuged (4 hr, 4°C,  $206,000 \times g_{av}$ ) using a vertical rotor and slow acceleration and deceleration below 160 rpm. Fractions of 0.5 mL were unloaded starting at the bottom of the tube (highest density) and pooled into three fractions—HDL, LDL, and VLDL/CM—according to contents of proteins, triglycerides, and cholesterol.<sup>27</sup> The densities of the fractions were determined using a refractometer, and contents of triglycerides, total proteins, and cholesterol were determined using assay kits from Boehringer Mannheim GmbH (Mannheim, Germany).

### Carotenoid analyses

All-*E*-, 9*Z*-, and 13*Z*-astaxanthin were quantified by HPLC using a  $\text{H}_3\text{PO}_4$  modified silica gel column,<sup>24</sup> using all-*E*-astaxanthin (Hoffmann-La Roche, Basel, Switzerland) as an external standard. The flow was 1.0 mL/min and the pressure was about 22 bar. The mobile phase was renewed daily. Detection wavelength was set at 470 nm. All chromatograms were reintegrated for baseline adjustment. The retention times ( $R_T$ ) for all-*E*-, 9*Z*-, and 13*Z*-astaxanthin were 10.8, 12.0, and 12.8 min, respectively. The employed extinction coefficients,  $E_{1\text{cm},1\%}$ , at 470 nm in hexane containing 4% (v/v)  $\text{CHCl}_3$  were 2,100 for all-*E*-astaxanthin,<sup>28</sup> 1,350 and 1,750 for 13*Z*- and 9*Z*-astaxanthin, respectively. The  $E_{1\text{cm},1\%}$ -values for 13*Z*- and 9*Z*-astaxanthin were estimated from the HPLC response factors of Schüep and Schierle.<sup>29</sup> Spectral fine structures for VIS spectra are expressed as %III/II.<sup>30</sup> Optical isomers of astaxanthin were quantified as dicamphanates after separation by HPLC.<sup>23</sup>

### Data analyses

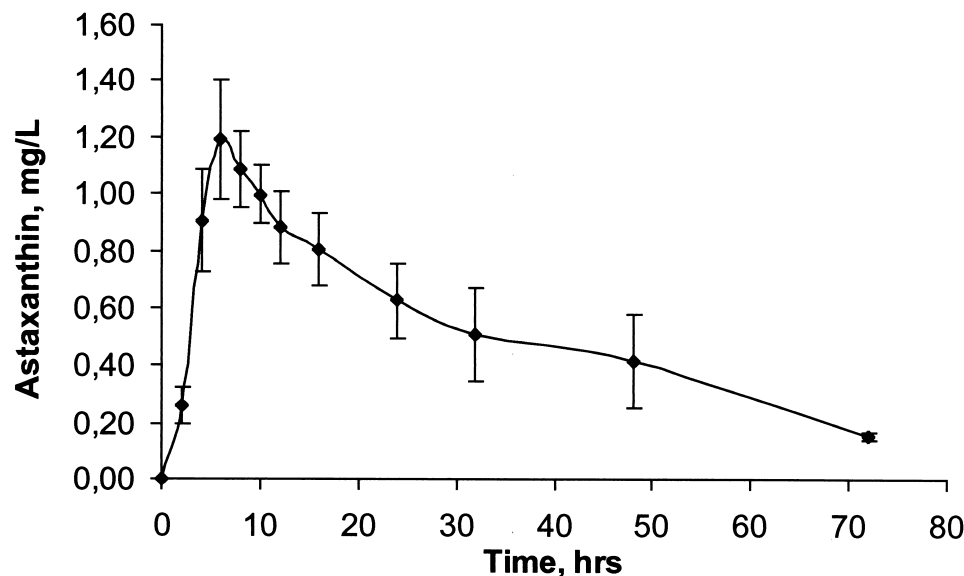
Pharmacokinetic parameters were estimated from plasma astaxanthin concentrations by noncompartmental methods using the PK Solutions 2.0 program (Summit Research Services, Ashland, OH USA). The area under the plasma astaxanthin concentration–time curve ( $\text{AUC}_{0-72}$ ) was calculated by using the linear trapezoidal rule method. The AUC from the last measured time (72 hr) to infinity ( $\text{AUC}_\infty$ ) was calculated on the basis of  $\text{AUC}_{0-72}$  and an extrapolated value. Oral clearance was calculated as dose/ $\text{AUC}_{0-72}$ , assuming an absorbed fraction of astaxanthin of 0.5. We used a higher absorbed fraction than indicated by the 30–40% absorbed amount reported by Elmadfa and Majchrzak<sup>22</sup> because the meal we used contained a high lipid content (50% olive oil). Volume of distribution was estimated as oral clearance/elimination rate. Results are presented as means  $\pm$  SD.

Astaxanthin isomer means for individuals and for HDL, LDL, and VLDL/CM over time were compared by Student *t*-tests using Microsoft Excel 9.0 (Microsoft Corp., Redmond, WA USA). Regression analyses of astaxanthin isomer distributions versus time were performed using the REG procedure in the SAS computer software, Version 6.12 for Windows (SAS Institute Inc., Cary, NC USA).

### Results

The present study shows that oral administration of a mixture of astaxanthin *E/Z* isomers successfully lead to their absorption into plasma without any appreciable metabolic transformation. Astaxanthin was not detected in the initial plasma samples. A typical chromatogram of pooled extracts of plasma illustrates the separation of astaxanthin all-*E*- and *Z*-isomers (see Figure 2). All-*E*-, 9*Z*-, and 13*Z*-astaxanthin were detected at 10.8, 12.0, and 12.8 min, respectively. Peaks 1, 2, and 3 were unidentified components with VIS absorption characteristic for carotenoids:  $\lambda_{\text{max}} = 445, 470, 505$  nm, %III/II = 50 (possibly lycopene),  $\lambda_{\text{max}} = 445$  nm





**Figure 3** Plasma concentration of total astaxanthin (mg/L) in three middle-aged male subjects after oral ingestion of a single meal containing 100 mg astaxanthin (Carophyll Pink). Bars represent standard deviation.

(possibly bilirubin), and  $\lambda_{\max} = 475$  nm (possibly an astaxanthin metabolite), respectively. Peak 3 appeared in the chromatograms 4 hr postprandially and disappeared after 10 hr.  $\beta, \beta$ -Carotene elutes near the solvent front using our HPLC system, but was not detected in the present study.

Means for all plasma samples from all subjects showed a distribution of astaxanthin geometrical isomers different from the dose:  $49.4 \pm 8.8\%$  (range 24.2–73.1%) for all-*E*-astaxanthin,  $13.2 \pm 8.8\%$  (range 8.1–21.3%) for 9*Z*-astaxanthin, and  $37.4 \pm 7.2\%$  (range 18.8–54.5%) for 13*Z*-astaxanthin. The results showed a relative accumulation of the astaxanthin 13*Z*-isomer. Regression analysis revealed no effect of time on astaxanthin isomer distribution, except a tendency ( $P = 0.07$ ) for the percentage of 13*Z*-astaxanthin to increase. No differences were observed among individuals when plasma concentration time data were analyzed by Student *t*-tests. The astaxanthin optical *R/S* isomer distribution in plasma closely resembled that of the dose.

#### Plasma astaxanthin pharmacokinetics

The individual and mean astaxanthin concentrations of plasma are shown in Figure 3 and the pharmacokinetic parameters derived 72 hr post dose are presented in Table 1. Plasma astaxanthin concentrations rapidly reached a peak ( $C_{\max}$ ) after  $6.7 \pm 1.2$  hr, and declined slowly thereafter. After 72 hr, 12% of  $C_{\max}$  (0.15 mg astaxanthin/L) still was present in the plasma. A large individual variation in volume of distribution (range 0.2–0.6 L/kg) was due to large variations in the observed elimination half-life ( $t_{1/2}$ ) (range 11.4–32.1 hr) and elimination rate (range 0.022–0.061 1/hr), not in  $AUC_{0-72}$  (range 34.7–42.5).

#### Appearance of astaxanthin E/Z isomers in plasma lipoprotein fractions

Using a self-generating gradient of iodixanol, a density range from 1.01 to 1.21 g/L was obtained. Distributions of proteins, triglycerides, and cholesterol in a representative gradient are presented in Figure 4. The highest protein

concentration was found in HDL ( $d = 1.21$ – $1.06$  g/L), less in LDL ( $d = 1.21$ – $1.06$  g/L) and small amounts in VLDL/CM ( $d = 1.21$ – $1.06$  g/L; Figure 4a). The highest amount of triglycerides was present in VLDL/CM (Figure 4b), and the highest amount of cholesterol was present in HDL (Figure 4c).

Astaxanthin was present in all lipoprotein fractions in varying amounts at different times post dosing (Figure 5a), although mainly in VLDL/CM (36–64%). On average, the astaxanthin content in LDL was  $29.0 \pm 7.7\%$  and that of HDL was  $23.5 \pm 4.5\%$ . The astaxanthin concentration–time curve of VLDL/CM exhibited two peaks: one 6–8 hr after dose ingestion, coinciding with the mean plasma astaxanthin concentration–time curve, and one peak approximately 16 hr after ingestion. VLDL/CM astaxanthin and triglyceride concentrations both had maxima at 7 and 16 hr postprandially (Figure 5b). In LDL, the concentration of astaxanthin increased until 5 hr postprandially, then decreased until 9 hr, followed by a small increase before declining. Regression analysis did not reveal any significant relationship with astaxanthin isomer distribution with time for

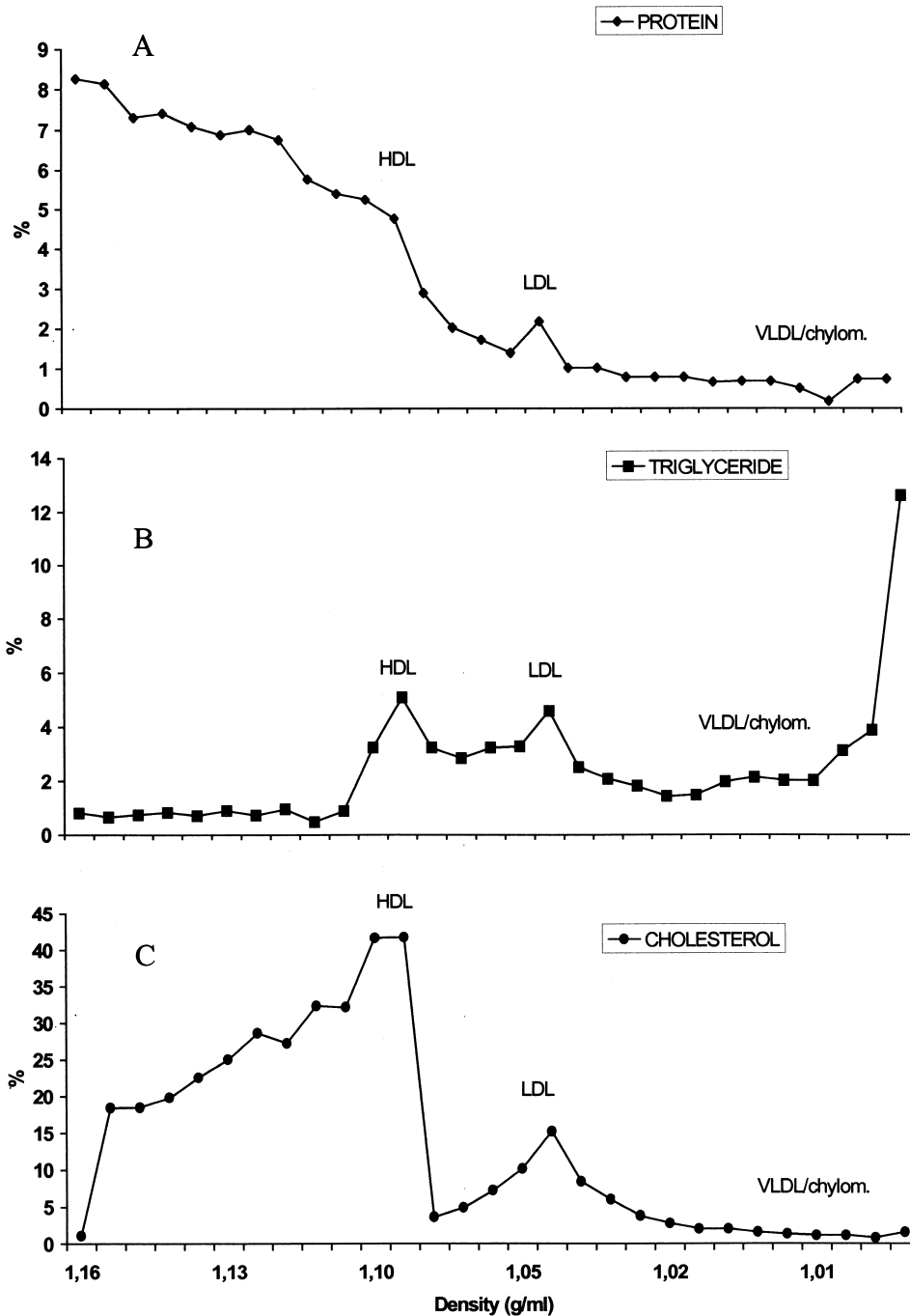
**Table 1** Pharmacokinetic parameters of astaxanthin after administration of a single oral dose of 100 mg astaxanthin (approximately 1.1 mg/kg) to middle-aged men. An absorbed dose of 50% was assumed during calculations

Parameter	Means $\pm$ SD
* $C_{\max}$ (mg/mL)	1.3 $\pm$ 0.1
** $t_{\max}$ (hr)	6.7 $\pm$ 1.2
Elimination $t_{1/2}$ (hr)	21 $\pm$ 11
Elimination rate (1/hr)	0.042 $\pm$ 0.035
*** $AUC_{(0-72)}$ (mg hr/L)	38 $\pm$ 4
*** $AUC_{\infty}$ (mg hr/L)	42 $\pm$ 3
Oral clearance (L/hr)	0.013 $\pm$ 0.01
Volume of distribution (L/kg)	0.40 $\pm$ 0.2

\*Maximal concentration

\*\*Time at maximum concentration

\*\*\*Area under the curve



**Figure 4** Distribution of proteins, triglycerides, and cholesterol in high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein containing chylomicrons (VLDL/chylom.) of a representative density-separated plasma fraction using a self-generating gradient of iodixanol. A: protein; B: triglyceride; C: cholesterol.

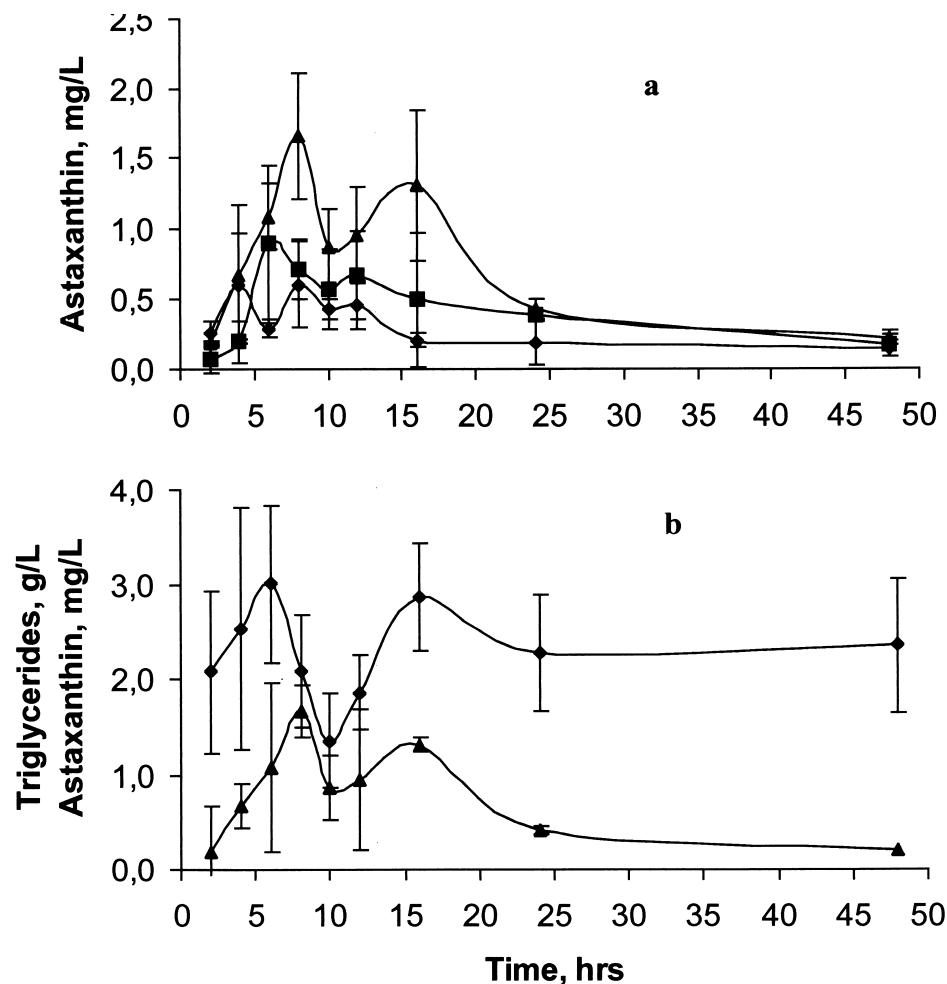
HDL, LDL, or VLDL/CM, nor did Student *t*-tests show any significant difference in astaxanthin isomer distribution between HDL, LDL, and VLDL/CM. The astaxanthin isomers in HDL, LDL, and VLDL/CM ranged from 62.1–66.9% for all-*E*-astaxanthin, 13.2–14.5% for 9*Z*-astaxanthin, and 20.4–24.3% for 13*Z*-astaxanthin, respectively.

## Discussion

### *Astaxanthin isomers in the diet, plasma, and lipoproteins*

A selective accumulation of 13*Z*-astaxanthin was apparent in human plasma. In line with our results with rainbow

trout,<sup>21</sup> no selectivity for astaxanthin *R/S* isomers was detected in human plasma. The astaxanthin isomer distribution pattern in plasma is the combined result of possible isomerization reactions after ingestion and isomer discrimination during absorption and translocation in enterocytes and subsequent incorporation in blood particles. Thus, in vivo *E/Z*-isomerization of carotenoids during intestinal passage was recognized approximately 50 years ago<sup>31</sup> and results in a near absence of 9*Z*- $\beta,\beta$ -carotene in postprandial plasma in humans orally administered <sup>13</sup>C-9*Z*- $\beta,\beta$ -carotene;<sup>32</sup> this is in agreement with data from other human studies using large doses of equimolar mixtures of all-*E*- and 9*Z*- $\beta,\beta$ -carotene.<sup>33–35</sup> Our data were not suitable to



**Figure 5** Distribution of astaxanthin and triglycerides in plasma lipoprotein fractions. A: distribution of astaxanthin (mg/L) in high-density lipoprotein,  $\blacklozenge$ ; low-density lipoprotein,  $\blacksquare$ ; and very low-density lipoprotein containing chylomicrons,  $\blacktriangle$ . B: distribution of astaxanthin (mg/L;  $\blacktriangle$ ) and triglycerides (g/L;  $\blacklozenge$ ) in very low-density lipoprotein containing chylomicrons. Bars represent standard deviation.

quantify the relative contribution from the different processes. The only similar study with accumulation of astaxanthin isomers is with rainbow trout in which all-*E*-astaxanthin selectively accumulated in plasma, muscle, and intestinal tissues, and 13*Z*-astaxanthin accumulated in the liver.<sup>20,21</sup> However, in salmonids,  $t_{\max}$  values for astaxanthin have been reported in the range 18<sup>36</sup> to 30 hr,<sup>37</sup> as compared with 7 hr in the present study. These results clearly indicate that different species-specific mechanisms govern the uptake of astaxanthin *E/Z* isomers in plasma. Although no information is available on plasma, species differences have previously been reported for hepatic accumulation of 9*Z*- and 13*Z*- $\beta,\beta$ -carotene in human liver compared to all-*E*- $\beta,\beta$ -carotene<sup>38</sup> versus the reported 9*Z*-/all-*E*- $\beta,\beta$ -carotene ratios of approximately 0.5 and 2.9 in rat and chicken liver, respectively.<sup>39</sup> However, liver data is not necessarily reflecting selectivity in carotenoid absorption, because complicating factors such as selective isomer metabolism may account for the observations. In conclusion, our study shows that the usefulness of data on carotenoid utilization in one species for comparative use in another is limited.

#### *Astaxanthin plasma appearance and pharmacokinetics*

The plasma concentration–time curve showed monophasic kinetics of plasma appearance and disappearance of astax-

anthin.  $C_{\max}$  was attained after about 7 hr ( $t_{\max}$ ). Reported values of  $t_{\max}$  for other xanthophylls are 6 hr for 5,6-epoxy- $\beta,\beta$ -carotene<sup>40</sup>, 6 hr for  $\beta$ -cryptoxanthin<sup>41</sup>, 7,<sup>42</sup> 8,<sup>43</sup> 10,<sup>22</sup> and 12 hr,<sup>44</sup> respectively, for canthaxanthin, 9<sup>45</sup> and 16 hr,<sup>46</sup> respectively, for lutein, 8 hr for capsanthin (3,3'-dihydroxy- $\beta,\kappa$ -carotene-6'-one),<sup>13</sup> and 2 and 4 hr, respectively, for the acidic apocarotenoids bixin and norbixin.<sup>47</sup> These data may indicate that  $t_{\max}$  is influenced by carotenoid polarity and, thus, gastrointestinal solubility. The acidity of bixin and norbixin may also account for the relatively rapid blood clearance of these carotenoids.<sup>47</sup> Absorption of carotenoids is facilitated by dietary fat concentration<sup>48,49</sup> and higher solubility may imply easier incorporation in gastrointestinal mixed micelles. The plasma elimination half-time reported for astaxanthin ( $t_{1/2} = 20.8$  hr) closely resembled that reported for capsanthin ( $t_{1/2} = 20.1$  hr),<sup>13</sup> which is considerably less than that for lycopene ( $t_{1/2} = 222$  hr).<sup>10</sup> Thus, the former carotenoids appear to have a more rapid metabolism and/or tissue deposition, as also indicated by AUCs and oral clearance data. Astaxanthin is virtually insoluble in water. The relatively high volume of distribution therefore indicates a fairly extensive uptake, binding, or metabolism of astaxanthin by extravascular tissues. The different astaxanthin isomers appeared to have similar pharmacokinetics, as judged from the regression analyses and the similar temporal distribution.

### Astaxanthin in lipoproteins

The main part of plasma astaxanthin was present in the VLDL/CM fraction (36–64% of total astaxanthin). The remainder was nearly equally distributed between HDL and LDL. Our findings are in line with that for canthaxanthin, of which  $23.4 \pm 2.9\%$  was associated with LDL 6 hr after a single ingestion.<sup>44</sup> In our experiment, the time-course of appearance of triglycerides and astaxanthin in the VLDL/CM fraction coincided (*Figure 5b*), as reported by Paetau et al.<sup>44</sup> The carotenoid concentration curve in the triglyceride-rich fraction after ingestion of 150 mg canthaxanthin showed three peaks at 3, 7, and 12 hr, respectively; the two earliest peaks probably resulting from variations of the speed of the lymphatic flow.<sup>43</sup> The kinetics of astaxanthin in VLDL/CM and plasma are not similar, explained by the fact that plasma responses do not allow distinction between newly absorbed carotenoids and those mobilized from endogenous sources. The observed kinetics of astaxanthin in lipoprotein fractions and the rapid increase of astaxanthin content in both VLDL/CM and LDL are similar to plasma appearance of canthaxanthin for 12 hr<sup>44</sup> and capsanthin after a single ingestion of paprika juice.<sup>13</sup> A preferential uptake of the xanthophylls lutein and zeaxanthin, as compared with that of all-*E*- $\beta$ , $\beta$ -carotene in CM, was shown after Betatene ingestion, with maximum uptake at 9 hr and distinct decline from 9 to 12 hr.<sup>45</sup> In another in vitro study modeling biological emulsions, more polar carotenoids such as astaxanthin were solubilized in the phospholipid surface of lipid droplets, and the apolar carotenoids were associated with the triglyceride core.<sup>14</sup> The transfer of the polar carotenoids between bioemulsion-like lipoproteins was easier than that of the apolar carotenoids, which required triglyceride lipolysis by lipase. The similar distribution of the different astaxanthin *E/Z* isomers in the plasma protein fractions during the trial may indicate that isomer selection is associated with the digestive process, although isomerization may also occur. Analysis of blood of individuals given doses of purified astaxanthin *E/Z* isomers is required to obtain more information on isomerization during digestion.

### Conclusion

As reported for other xanthophylls cited in this article, dietary astaxanthin was readily absorbed and incorporated in human plasma lipoproteins at a considerable degree. Compared to rainbow trout,<sup>20,21</sup> different *E/Z* isomers were selectively accumulated in human plasma, indicating different uptake mechanisms. The bioavailability of carotenoids is influenced by a number of factors, termed SLAMENGI (Species of carotenoids, molecular Linkage, Amount of carotenoids consumed in a meal, Matrix, Effectors of absorption and bioconversion, Nutrient status of the host, Genetic factors, Host-related factors, and mathematical Interactions), as reviewed by Castenmiller and West.<sup>50</sup> Apparently, none of these factors are able to explain differences in uptake between species. Future studies should seek to establish the biochemical basis for species differences in carotenoid utilization.

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