

# Carotenoids in biological emulsions: solubility, surface-to-core distribution, and release from lipid droplets

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**Abstract** Data on the physico-chemical properties of carotenoids in biological emulsions are essential to our knowledge of carotenoid metabolism. Therefore, we determined the behavior of carotenoids in phospholipid-stabilized triglyceride emulsions, a model for biological emulsions such as dietary emulsions, triglyceride-rich lipoproteins, and intracellular storage droplets. The solubility of  $\beta$ -carotene (a model for apolar carotenoids, carotenes) in pure bulk triglycerides (0.112 to 0.141 wt % according to triglycerides) was significantly higher than zeaxanthin (a model for polar carotenoids, xanthophylls) (0.022 to 0.088 wt %). The solubility of both carotenoids increased when the chain-length of the triglycerides' fatty acids decreased. The amount of zeaxanthin associated with lipid droplet dramatically increased in phospholipid-triglyceride droplets as compared to the pure corresponding triglyceride droplets, whereas the amount of  $\beta$ -carotene associated with lipid droplets increased only slightly.  $\beta$ -Carotene distributed almost exclusively in the core of triolein-lecithin-carotenoid droplets, while zeaxanthin distributed preferentially at the droplet's surface. A significant percentage (8.3%) of zeaxanthin was spontaneously transferred from lipid droplets to aqueous phase and the remaining part was transferred during triglyceride hydrolysis catalysed by pancreatic lipase, while  $\beta$ -carotene absolutely required triglyceride lipolysis to be transferred to the aqueous phase. **Our results show that polar and apolar carotenoids behave differently in biological emulsions. They further our understanding of the bioavailability of polar and apolar carotenoids and of their distribution between lipoprotein particles.**—Borel, P., P. Grolier, M. Armand, A. Partier, H. Lafont, D. Lairon, and V. Azais-Braesco. Carotenoids in biological emulsions: solubility, surface-to-core distribution, and release from lipid droplets. *J. Lipid Res.* 1996. **37**: 250–261.

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Although numerous studies have suggested that carotenoids (CARs) may protect against some human

diseases, they are not yet considered as essential micronutrients. Among the 620 CARs (1) that have already been identified in nature, about 10% are nutritionally active as precursors of vitamin A (2), but it is widely assumed that CARs have biological properties unrelated to their provitamin A status (3–6). The probable protective effect of CARs against some human cancers (7–11) and cardiovascular diseases (12–14) is mainly attributed to CARs' well-established antioxidant properties (3, 15, 16), but some CARs seem to have particular biological properties (6). Surprisingly, very few data are available concerning the intestinal absorption and the lipoprotein metabolism of CARs in human (17–19).

The CAR family can be divided in two main groups: polar CARs (xanthophylls) and apolar CARs (carotenes). Given their hydrophobicity, a major proportion of CARs is probably solubilized in dietary lipid emulsions in the gastrointestinal tract, before being transported by lipoproteins in the blood (18, 19) and then stored in intracellular lipid droplets. All these lipid structures are basically biological emulsions (BIO-EM) showing a comparable basic structure: a triglyceride (TG) core surrounded by a monomolecular film of amphipatic lipids, especially phospholipids (PL). Unfortunately, no data are available concerning CAR solubility in BIO-EM.

Like other lipid molecules, CARs would distribute between the TG core and the PL surface phases of PL-stabilized TG droplets, a model for BIO-EM (20, 21). Lipids found at the lipoprotein surface (i.e., PL, free fatty acids, free cholesterol) exchange spontaneously

Abbreviations: CAR, carotenoid;  $\beta$ -CAR,  $\beta$ -carotene; ZEA, zeaxanthin; TG, triglyceride; PL, phospholipid; BIO-EM, biological emulsion; TRL, triglyceride-rich lipoproteins.

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between lipoproteins, while lipids recovered in the lipoprotein core (TG, esterified cholesterol) require a lipid transfer protein (22). Thus, data on the distribution of polar and apolar CARs between the core and the surface of BIO-EM can further our knowledge of the potential transfer of CARs between biological lipid-structures, i.e., *i*) from dietary emulsions to mixed micelles in the gastrointestinal tract, *ii*) between lipoprotein classes, or *iii*) between lipoproteins and cell membranes.

The mechanisms involved in CAR intestinal absorption are not well understood and, to our knowledge, no study has compared the intestinal absorption of polar and apolar CARs. Numerous dietary and nondietary factors affect CAR absorption (18). Among them, dietary fat is essential for the absorption of CARs.  $\beta$ -Carotene bioavailability is higher when fat is added to the diet (23, 24) and is lower in disease-induced fat malabsorption (25). Some key parameters governing  $\beta$ -carotene luminal absorption have been established such as solubilization of  $\beta$ -carotene in mixed micelles (26, 27) and absorption by passive diffusion (28). But data are lacking concerning the first step of CAR intestinal absorption, i.e., the transfer of CARs from dietary emulsions to mixed micelles. Yet, given the general scheme of fat absorption (29–31), CARs entrapped in TG droplets cannot be directly absorbed by the small intestine mucosa. Thus, it appears essential to have a better knowledge of the transfer of CARs from dietary emulsions to mixed micelles.

It has been shown that various CARs distribute differently among lipoprotein classes (19). CAR distribution might vary for two main reasons: polar CAR solubility in lipoprotein classes might differ from that of apolar CARs and polar and apolar CARs might be differently transferred from one lipoprotein class to another. Thus, data concerning the solubility and the surface-to-core distribution of CARs in BIO-EM and data concerning the transfer of CARs between BIO-EM and other lipid structures should help to explain the distribution and consequently the metabolism of CARs in lipoprotein classes.

The aim of this study was, therefore, to compare the phase behavior of polar and apolar CARs in BIO-EM. We used PL-stabilized TG emulsions as a model for the BIO-EM and, because the CAR family is very large, we used two CAR models. All-*trans*  $\beta$ -carotene ( $\beta$ -CAR) served as a model for the apolar CARs and all-*trans* zeaxanthin (ZEA) as a model for the polar ones. Three aspects were studied: *i*) the solubility of polar and apolar CARs in bulk triglycerides and in lipid-in-water emulsions differing in their TG nature and PL contents, *ii*) the surface-to-core distribution of polar and apolar CARs in lecithin-stabilized triolein droplets, and *iii*) the role of the TG lipolysis catalyzed by pancreatic lipase on

the transfer of polar and apolar CARs from PL-stabilized TG droplets to aqueous soluble lipid structures.

## EXPERIMENTAL PROCEDURES

### Chemicals and enzyme

All-*trans*- $\beta$ -carotene (97% pure) was purchased from Sigma Chimie (La Verpillière, France). All-*trans*- $\beta$ -carotene-3,3' diol (zeaxanthin) and [7,8,7',8'- $^{14}$ C]all-*trans*- $\beta$ -carotene-3,3' diol (2.67 GBq/mmol) were generous gifts from Dr. J. Bausch (Hoffmann-La Roche, Basel, Switzerland). Lycopene,  $\beta$ -cryptoxanthin, and echinenone were gifts from Hoffmann-La Roche. The purity of the  $\beta$ -CAR, the ZEA and the radio-labeled ZEA was verified to be > 95% by reversed-phase HPLC (high performance liquid chromatography).

Tricaprylin (C8:0), triolein (C18:1, n-9), trilinolein (C18:2, n-6), fish oil from menhaden (14.9% eicosapentaenoic acid (C20:5, n-3) and 6.9% docosahexaenoic acid (C22:6, n-3) (32) and L- $\alpha$ -phosphatidylcholine type XI-E from fresh egg yolk were purchased from Sigma Chimie (La Verpillière, France). Carboxy [ $^{14}$ C]triolein (4.07 GBq/mmol) and [9,10- $^3$ H (N)]triolein (991.6 GBq/mmol) were purchased from DuPont de Nemours (Paris, France). The purity of the lipids and the radiolabeled lipids was verified to be > 97% by TLC (thin-layer chromatography). Bile salts were from Calbiochem (San Diego, CA), their purity >95% was given by the company.

Stock solutions were made with chloroform-methanol 2:1 (v/v) and tetrahydrofuran containing 0.025% butylated hydroxytoluene (stabilized tetrahydrofuran) for the lipids and the CARs, respectively. The stock solutions were stored under a blanket of nitrogen at -20°C. The lipid stock solution concentrations were measured gravimetrically; the CAR stock solution concentrations were measured spectrophotometrically at 452 nm ( $E_{1\text{ cm}}^{1\%} = 2560$  for  $\beta$ -CAR,  $E_{1\text{ cm}}^{1\%} = 2348$  for ZEA,  $E_{1\text{ cm}}^{1\%} = 3450$  for lycopene,  $E_{1\text{ cm}}^{1\%} = 2386$  for  $\beta$ -cryptoxanthin,  $E_{1\text{ cm}}^{1\%} = 2158$  for echinenone).

Porcine pancreatic lipase (Boehringer Mannheim, Mannheim, Germany) was 95% pure as checked by sodium dodecyl sulfate polyacrylamide gel electrophoresis and its specific activity was 1370 Units/mg protein with tributyrin as the substrate (33). Porcine pancreatic colipase (Boehringer Mannheim, Mannheim, Germany) was 99% pure and its specific activity (34) was 1590 Units/mg protein.

### Determination of the size of the emulsions' droplets

The size of the stable PL-stabilized TG droplets, but not of the pure TG droplets because these droplets are very unstable and fused together very quickly, was determined with a particle-size analyzer (Capa-700,

Horiba, Kyoto, Japan) whose accuracy was checked with calibrated microspheres in the size range 0.2–90  $\mu\text{m}$  (polystyrene size standard-kit, Polyscience INC., Warrington, PA). We measured droplets at 560 nm and used gradient mode analysis at a constant centrifuge acceleration rate (120 rpm/min) to obtain an accurate measurement of large particles (100  $\mu\text{m}$ ) as well as small ones (0.05  $\mu\text{m}$ ). Results are given in the form of a frequency distribution graph. Emulsion median-diameter ( $\mu\text{m}$ ) and emulsion specific-area ( $\text{m}^2/\text{g}$ ) were calculated by the particle-sizer software.

### Lipid and CAR measurements

TGs and TG lipolytic-products (diglycerides, monoglycerides, free fatty acids) were separated by TLC on silica-gel plates (ready plastic sheet F-1500, Schleicher and Schuell, Germany) using two successive development phases: chloroform–methanol–water 65:35:5 (v/v/v) for 5 cm, followed by heptane–diethyl ether–formic acid 90:60:4 (v/v/v) to the top of the plate. Individual lipid classes were visualized by brief exposure to iodine vapors, and after sublimation of the iodine, the spots were scraped into scintillation vials. Note that monoglycerides could have been slightly overestimated as a consequence of their poor separation from the deposited spot on TLC.  $^3\text{H}$  and  $^{14}\text{C}$  radioactivities were measured by scintillation counting using a scintillation spectrometer with an external standard for quench correction (Tri-Carb 1600 TR, Packard, Meriden, CT).

The CARs were quantified by reverse-phase HPLC on a Kontron AG (Zurich, Switzerland) apparatus with visible detection. The column was a C18-Zorbax (250  $\times$  4.6-mm, 5  $\mu\text{m}$ ) and the mobile phase was a mixture of acetonitrile–methylene chloride–methanol 70:20:10 (v/v/v) (35). The CARs were quantified by internal and external calibrations with canthaxanthin (Hoffmann-La Roche, Basel, Switzerland) as the internal standard, and Kontron MT2 software. Overall recovery yields ranged 75–100%.

The PLs were quantified spectrophotometrically by phosphorus measurement (36) or, when phosphate buffer was present, by an enzymatic procedure using a commercial kit (BioMerieux, Marcy l'Etoile, France) and a DU-40 spectrophotometer (Beckman Instruments, Palo Alto, CA).

The bile salts were measured spectrophotometrically by an enzymatic procedure using glycodeoxycholate as the standard (37).

### Measurements of CAR solubility in bulk triglycerides

The solubility of CARs in triglycerides was measured according to Patton et al. (38). To 100 mg TG (tricapryline, triolein, trilinolein, or fish oil) containing 10 dpm/ $\mu\text{g}$  carboxy [ $^{14}\text{C}$ ]triolein, was added 5 mg of crys-

talline CAR. The tubes were then flushed with nitrogen, sealed, and shaken for 2 weeks at room temperature ( $20 \pm 3^\circ\text{C}$ ) in the dark. After the shaking period, an additional 2-day settling period was given. After centrifugation at 3,000  $g$  for 30 min, aliquots of the clear oil were taken and TG and CARs were measured as described above.

### Measurements of CAR associated with pure TG droplets and with PL-stabilized TG droplets

Aliquots of the stock solutions giving 2.5 mg TG (tricaprylin, triolein, trilinolein, or fish oil TG), 20 dpm/ $\mu\text{g}$  carboxy [ $^{14}\text{C}$ ]triolein, 1 mg CAR, and 62.5  $\mu\text{g}$  egg PL (to make PL-stabilized TG droplets only) were mixed in a polyallomer tube. Preliminary experiments during which we had measured the amount of CARs associated with lipid droplets as a function of the CAR to TG mass ratio (data not shown) had indicated that this ratio was necessary to saturate lipids with CARs. The lipid mixture was dried under a stream of nitrogen, then 1 mL of a 10 mmol/L sodium phosphate buffer, pH 7.50, at  $25^\circ\text{C}$  was added. The aqueous-lipid mixture obtained was incubated at  $25^\circ\text{C}$  for 30 min under a blanket of nitrogen in the polyallomer tube. The tube was then put in ice and the mixture was sonicated for three periods of 15 sec, with a 100 watt MSE sonifier (London, U.K.) set to maximum power and a titanium probe (0.25 cm diameter). We checked by TLC and HPLC that neither the lipids nor the CARs had been degraded during the preparation of the lipid-droplet suspension. We added 10.9 ml of a 10 mmol/L phosphate buffer, pH 7.50, to the lipid-droplet suspension, and an aliquot was immediately taken to measure the lipid-droplet size. Control experiments had showed that the lipid-droplet median diameter was not affected by storage at  $5^\circ\text{C}$  for up to 8 days. The lipid-droplet suspension was ultracentrifuged at 125,000  $g$  for 1 h at  $25^\circ\text{C}$  with a Beckman SW 40 Ti swinging bucket rotor at  $20^\circ\text{C}$  in a L2-65B ultracentrifuge (Beckman Instruments, Palo Alto, CA) to concentrate the lipid droplets and to remove remaining CAR macrocrystals (38). The ultracentrifuge was operated without braking to minimize perturbation of the floating concentrated lipid-droplets which were collected by slicing the polyallomer tubes. The droplets' TG and CARs were measured as described above.

As the methodology used to prepare lipid-in-water emulsions could lead to the incorporation of CAR microcrystals in the lipid droplets, the absorption spectra of the CARs associated with the TG droplets were measured to specify whether the CARs were solubilized as monomeric molecules or as a dispersion of microcrystals.

## Measurements of lipid and CAR surface-to-core distributions in triolein-PL-CAR droplets

Aliquots of the stock solutions of lipids and CARs containing: 0.61% triolein, 2000 dpm/ $\mu\text{g}$  [ $9,10\text{-}^3\text{H}$  (N)]triolein, 0.14% egg PL, 150 mmol/L NaCl, 0.00061%  $\beta$ -CAR (triolein-PL- $\beta$ -CAR emulsion), or 0.00061% ZEA and 30,000 dpm/ $\mu\text{g}$  ( $7,8,7',8'\text{-}^{14}\text{C}$ )all-trans- $\beta$ -carotene-3,3' diol (triolein-PL-ZEA emulsion) were mixed in a polyallomer tube and dried under a stream of nitrogen. Then, 33 mL of a 10 mmol/L sodium phosphate buffer, pH 7.0, was added. The mixtures were stirred with a magnetic bar at a rotational speed of 500 rpm for 18 h at 37°C in the dark, under a blanket of nitrogen. The coarse emulsions obtained were sonicated, with a titanium probe (1.9 cm diameter), at the maximum power of the sonifier, for three periods of 5 min each, in a 100-mL glass vial placed in a cold bath under a stream of nitrogen. The fine emulsions obtained were ultracentrifuged for 3 h at 50,000  $g$  with the SW 40 Ti swinging-bucket rotor at 20°C. The floating concentrated emulsions were collected by slicing the polyallomer tubes. The compositions of the concentrated emulsions obtained were TG-PL-CAR 1:0.0561:0.000496 (w/w/w) and TG-PL-CAR 1:0.0715:0.000176 (w/w/w) for the  $\beta$ -carotene and the zeaxanthin concentrated emulsion, respectively. Note that there was an important loss of lipids and carotenoids during the preparation of the concentrated emulsions, but neither the lipids nor the CARs were altered during the preparation of the emulsions, as checked by TLC and by HPLC. The concentrated emulsions were transferred to pyrex disposable micro-sampling pipets (i.d. = 1.1–1.2 mm  $\times$  L = 75 mm), which had been flame-sealed at one end. The pipets were centrifuged inside plastic adaptors that had been machined to fit into the buckets of the SW 40 Ti rotor. The core and surface phases were separated by two successive ultracentrifugations at 50,000  $g$  for 18 h at 25°C as previously reported (21). The lipids and CARs recovered in the pure core or surface phases were measured as follows. In the case of the ZEA-containing concentrated emulsion, the triolein, the egg PL, and the ZEA recovered in the core and surface phases were separated by TLC as described above. The radiolabeled triolein and the radiolabeled ZEA were determined by dual liquid scintillation counting; the PL were determined spectrophotometrically by phosphorus measurement (36). In the case of the  $\beta$ -CAR-containing concentrated emulsion, the samples were diluted in ethanol (core samples) or in distilled water (surface sample). The radiolabeled triolein, the PL, and the  $\beta$ -CAR were measured by scintillation counting, by phosphorus measurement, and by HPLC, respectively. The surface-to-core distribution coefficients were defined as before (21) as the weight fraction of a com-

ponent in the surface phase to the weight fraction of the component in the core phase.

## Measurements of the transfer of the CARs from emulsion's lipid droplets to aqueous phase

*Preparation of emulsions.* The proportions of the emulsions' lipids were chosen in the range of a human's usual food intake, i.e., 100 g TG and 2.5 g PL. The CAR to TG mass ratio (0.1 g/100 g TG) was reasonably high to allow an accurate measurement of the CARs solubilized in the aqueous phase. Thus, aliquots of the stock solutions containing 400 mg triolein, 3000 dpm/ $\mu\text{mol}$  (carboxy  $^{14}\text{C}$ )-triolein, 10 mg egg PL, and 0.4 mg CAR were mixed. After the lipid mixture had been dried under a stream of nitrogen, 66 mL of a 10 mmol/L sodium phosphate buffer, pH 7.0, was added and concentrated emulsions were prepared as described above. The concentrated emulsions contained 0.071 g  $\beta$ -CAR ( $\beta$ -CAR-containing emulsion) or 0.017 g ZEA (ZEA-containing emulsion) per 100 g triolein.

*Medium composition.* The composition of the medium (bile lipid buffer) was chosen to mimic that found in the human duodenal content during digestion (39). Therefore, the mixed bile salt and the TG concentrations in the incubation medium showed a physiological TG-to-mixed bile salts molar ratio of 2.4 and the PL concentration in the bile lipid buffer increased 2.1 times the PL-to-triolein molar ratio in the incubation medium as compared to the emulsion's ratio. Finally, the cholesterol-to-triolein molar ratio (14.4) recovered in the incubation medium was comparable to that found in the human duodenum. Consequently, the incubation medium was a 600  $\mu\text{L}$  mixture containing: 150 mmol/L NaCl, 6 mmol/L  $\text{CaCl}_2$ , 25 mmol/L Tris-HCl buffer, pH 7.50, 8 mmol/L mixed bile salts (2.32 mmol/L glycocholate, 3.2 mmol/L glycodeoxycholate, 0.64 mmol/L taurocholate, 1.84 mmol/L taurodeoxycholate), 0.6 mmol/L egg PL, 1.33 mmol/L free cholesterol, 0.05% sodium azide. An aliquot of the concentrated emulsions provided 11.5  $\mu\text{mol}$  triolein, 0.33  $\mu\text{mol}$  egg PL, and 13.4 nmol  $\beta$ -CAR (the  $\beta$ -CAR-containing emulsion) or 3.0 nmol ZEA (the ZEA-containing emulsion).

*Incubation protocol.* The concentrated emulsions were incubated in micro test tubes (Eppendorf GmbH, Hamburg, Germany) under gentle stirring at 37°C. In a first control, they were incubated for 30 min in distilled water, without lipase and colipase. In another control, they were incubated for 30 min in the bile lipid buffer and without lipase and colipase. In the other cases, 34.7 nmol/L porcine pancreatic lipase and 333 nmol/L porcine pancreatic colipase were added to catalyze TG lipolysis. Because the pH of the incubation could be lowered by the free fatty acid produced during the lipolysis, it had been controlled to remain stable

throughout the 30-min lipolysis. The lipolysis was stopped after 15 and 30 min and 300  $\mu\text{L}$  of the incubation medium was taken to measure the overall radiolabeled lipid composition (TG, diglycerides, monoglycerides, and free fatty acids). Of the remaining incubation medium 280  $\mu\text{L}$  was immediately put in a pre-frozen polyallomer tube containing 5.72 mL distilled water, at 5°C (preliminary experiments had allowed us to establish that these conditions completely stop the lipolysis). The polyallomer tube was ultracentrifuged for 18 h at 50,000  $g$  at 5°C in a SW 40 Ti swinging bucket rotor (the ultracentrifuge was operated without braking to minimize perturbation of the separated phases). Such separation consistently produced an isotropic mixed micellar subphase, without creating an appreciable bile salt concentration gradient throughout the polyallomer tube (40). The floating remaining lipid droplets were discarded after the polyallomer tube had been sliced. Two mL of the aqueous infranatant was taken with a syringe. The pelleted fraction was obtained first by removing all the infranatant, then by washing the bottom of the tube with 1 mL stabilized tetrahydrofuran. The radiolabeled lipids recovered in the infranatant and in the pellet were extracted (41), separated, and measured as described above. The PL and bile salts recovered in the infranatant were measured by enzymatic procedures. The PL and bile salts recovered in the pellet were extracted (41), dried, dissolved in distilled water, and measured by enzymatic procedures. The infranatant and pellet CARs were extracted by stabilized hexane and measured as described above. The lipid composition of the floating remaining lipid droplets was calculated by subtracting the infranatant and the pellet lipids from the whole lipids measured on an aliquot of the incubation medium that had been taken before the ultracentrifugation.

### Statistical analysis

The statistical evaluation of the data was done by Student's *t*-test or by the analysis of variance (ANOVA) and Fischer's test at a probability of 95% (42). The correlation coefficients were evaluated at a 95% probability level. The Statview program (Abacus, Berkeley CA) was used.

## RESULTS

### Emulsification of different TG molecular species

The sizes of the pure TG droplets were not measured because they were very unstable. The CARs solubilized in the PL-stabilized TG droplets (emulsions) did not affect the emulsion median diameters (data not shown). As shown in **Table 1**, the emulsion made with fish oil

TABLE 1. Median diameter and specific area of the phospholipid-triglyceride emulsions

| Emulsion Triglyceride | Median Diameter      | Specific Area         |
|-----------------------|----------------------|-----------------------|
|                       | $\mu\text{m}$        | $\text{m}^2/\text{g}$ |
| Tricaprylin           | $2.78 \pm 0.56^a$    | $3.33 \pm 0.26^a$     |
| Triolein              | $2.11 \pm 0.25^{ab}$ | $4.56 \pm 0.64^a$     |
| Trilinolein           | $1.68 \pm 0.31^{ab}$ | $5.02 \pm 0.76^{ab}$  |
| Fish oil              | $1.26 \pm 0.07^b$    | $6.98 \pm 0.21^b$     |

Values are mean  $\pm$  SEM of three measurements. Aqueous-lipid mixtures were sonicated for three periods of 15 sec each to prepare emulsions. The median diameters and the specific areas of the emulsions were calculated by the particle-sizer analyzer's software. Different superscript letters (a,b) indicate significant differences between median diameters or specific areas, as determined by analysis of variance (ANOVA) for unpaired values and Fischer's test ( $P < 0.05$ ).

TG has a median diameter significantly lower and a specific area significantly higher than the emulsion made with tricapryline. We found a negative correlation between the fatty acid chain length (8, 18, 18, and 20 fatty acid carbon numbers were attributed to tricaprylin (TC), triolein (TO), trilinolein (TL), and fish oil TG (F), respectively) and the emulsion median diameter ( $r = -0.59$ ,  $P < 0.05$ ,  $n = 16$ ) or the emulsion specific area ( $r = -0.58$ ,  $P < 0.05$ ,  $n = 16$ ). There was also a negative correlation between the degree of fatty acid unsaturation (0, 1, 2, and 5 fatty acid double-bond numbers were attributed to TC, TO, TL, and F, respectively) and the emulsion median diameter ( $r = -0.65$ ,  $P < 0.01$ ,  $n = 16$ ) or the emulsion specific area ( $r = -0.74$ ,  $P < 0.005$ ,  $n = 16$ ). These results agree well with those of a recent study (43) showing a relationship between emulsification susceptibility and triacylglycerol molecular species.

### Solubility of some common CARs in bulk TGs at 20°C

**Table 2** lists the solubility of some common CARs in bulk triglycerides at 20°C. Among all the CARs tested,  $\beta$ -cryptoxanthin had the highest solubility in triolein whereas lycopene had the lowest. Note that  $\beta$ -CAR solubility in long-chain TGs was about three times higher than the ZEA. Note also that the solubility of  $\beta$ -CAR, ZEA, and lycopene was significantly higher in tricaprylin than in long-chain TGs and that there was no effect of the fatty acid unsaturation degree of the long-chain TG on the CAR solubility.

### Amount of CARs associated with triglyceride droplets or with phospholipid-stabilized triglyceride droplets during emulsion preparation

It should be stressed that when the CARs were associated with lipid-in-water droplets, the normal absorption spectrums were replaced by very different absorption spectrums (data not shown). Moreover, the amounts of CARs associated with pure TG droplets (**Table 3**) were about 10 times higher than the amounts

TABLE 2. Solubility of some common carotenoids in bulk triglycerides at 20°C

| Carotenoid      | Carotenoid Solubility (wt %)  |                              |                              |                              |
|-----------------|-------------------------------|------------------------------|------------------------------|------------------------------|
|                 | Tricaprylin                   | Triolein                     | Trilinolein                  | Fish Oil                     |
| β-Carotene      | 0.141 ± 0.006 <sup>a,f</sup>  | 0.118 ± 0.006 <sup>a,g</sup> | 0.114 ± 0.009 <sup>a,g</sup> | 0.112 ± 0.003 <sup>a,g</sup> |
| Zeaxanthin      | 0.088 ± 0.019 <sup>b,f</sup>  | 0.022 ± 0.012 <sup>b,g</sup> | 0.030 ± 0.010 <sup>b,g</sup> | 0.034 ± 0.008 <sup>b,g</sup> |
| Lycopene        | 0.001 ± 0.0001 <sup>c,f</sup> | <0.0001 <sup>c,g</sup>       | <0.0001 <sup>c,g</sup>       | <0.0001 <sup>c,g</sup>       |
| β-Cryptoxanthin | ND                            | 0.337 ± 0.003 <sup>d</sup>   | ND                           | ND                           |
| Echinenone      | ND                            | 0.041 ± 0.001 <sup>e</sup>   | ND                           | ND                           |

Solubility is expressed in g per 100 g triglycerides (wt %). Values are mean ± SEM of four measurements. ND, not determined; < 0.0001, not accurately measured because the solubility was lower than 0.0001%. One hundred mg triglyceride and 5 mg carotenoid crystals were incubated for 2 weeks in the dark and under gentle shaking. In each column, different superscript letters (a–e) indicate significant differences, as determined by analysis of variance (ANOVA) for unpaired values and Fischer’s test ( $P < 0.05$ ). In each row, different superscript letters (f–g) indicate a significant difference (ANOVA for unpaired values and Fischer’s test,  $P < 0.05$ ).

of CARs solubilized in bulk TG (Table 2). Thus it is likely that CAR microcrystals were associated with the lipid droplets during emulsion preparation. Consequently, in this experiment, we did not measure the true solubility of CAR in lipid droplets but rather the ease with which CAR monomers and CAR microcrystals were associated with lipid droplets during their preparation. The amount (expressed as g per 100 g TG) of β-CAR and ZEA associated with pure TG droplets or with PL-stabilized TG droplets increased when the fatty acid chain-length and the fatty acid unsaturation degree decreased. Furthermore, there was a negative correlation between the fatty acid chain-length of the pure TG droplets and the amount of β-CAR ( $r = -0.99$ ,  $P < 0.01$ ,  $n = 4$ ) or of ZEA ( $r = -0.97$ ,  $P < 0.05$ ,  $n = 4$ ) associated with the pure TG droplets. The amount of β-CAR associated with pure TG droplets was slightly, but nonsignificantly, higher than that with ZEA. But note that, for all the TG except for the fish oil TG, the amount of ZEA associated with the PL-stabilized TG droplets was significantly higher than the β-CAR amount associated with the PL-stabilized TG droplets. Finally, the ZEA amount in the PL-stabilized TG droplets was considerably higher than the ZEA amount associated with the corresponding pure TG droplets: 7.86% versus 1.79% for TC, 7.25% versus

0.77% for TO, 7.66% versus 0.73% for TL, 1.51% versus 0.13% for F. The increase of the amount of β-CAR associated with the PL-stabilized TG droplets as compared to the corresponding pure TG droplets was also observed but to a much lesser extent than with ZEA.

### Lipid and CAR surface-to-core distributions in PL-triolein-CAR emulsions

As shown in Table 4, and as previously found by others (20, 21), no PL was detected in the core phase of the lipid droplets. The weight fractions of triolein recovered in the surface phase:  $4.05 \pm 0.33\%$  and  $4.59 \pm 0.16\%$  for the ZEA-containing emulsion (Z-EM) and for the β-CAR-containing emulsion (β-EM), respectively, were not significantly different. Consequently, the phase distribution coefficients (the weight fraction of a component in the surface phase to the weight fraction of the component in the core phase) of triolein were  $0.041 \pm 0.003$  and  $0.046 \pm 0.001$  for the Z-EM and for the β-EM, respectively. The weight fraction of ZEA found in the surface and the core phases,  $0.0848 \pm 0.001\%$  and  $0.0019 \pm 0.0004\%$ , respectively, gave a distribution coefficient of  $52.6 \pm 10.7$ . The weight fraction of β-CAR in the core phase ( $0.0082 \pm 0.0001\%$ ) was 4.6 times as high as the corresponding ZEA weight fraction. We were unable to

TABLE 3. β-Carotene and zeaxanthin associated with triglyceride droplets and with phospholipid-stabilized triglyceride droplets at 25°C

| Droplets     | β-Carotene (wt %)        |                            | Zeaxanthin (wt %)        |                              |
|--------------|--------------------------|----------------------------|--------------------------|------------------------------|
|              | Triglycerides            | Phospholipid-Stabilized    | Triglycerides            | Phospholipid-Stabilized      |
|              |                          | Triglycerides              |                          | Triglycerides                |
| Triglyceride |                          |                            |                          |                              |
| Tricaprylin  | 2.59 ± 0.26 <sup>a</sup> | 4.67 ± 0.76 <sup>a,e</sup> | 1.79 ± 0.27 <sup>a</sup> | 7.86 ± 1.04 <sup>a,e,f</sup> |
| Triolein     | 1.10 ± 0.12 <sup>b</sup> | 3.02 ± 0.29 <sup>b,e</sup> | 0.77 ± 0.26 <sup>b</sup> | 7.25 ± 0.39 <sup>a,e,f</sup> |
| Trilinolein  | 1.17 ± 0.28 <sup>b</sup> | 2.01 ± 0.29 <sup>c,e</sup> | 0.73 ± 0.12 <sup>b</sup> | 7.66 ± 0.41 <sup>a,e,f</sup> |
| Fish oil     | 0.63 ± 0.27 <sup>b</sup> | 0.87 ± 0.11 <sup>d</sup>   | 0.13 ± 0.10 <sup>b</sup> | 1.51 ± 0.36 <sup>b,e</sup>   |

The amount of carotenoid associated with lipid droplets is expressed in g per 100 g triglycerides (wt %). Values are mean ± SEM of three measurements. Lipid droplet suspensions in water were prepared as described in the legend of Table 1. The lipid mixtures were composed of pure triglycerides or of a mixture of triglycerides and phospholipids (40/1; w/w). Note that in the conditions used for this experiment we have found that carotenoid microcrystals were associated with the lipid droplets. In each column, different superscript letters (a–d) indicate significant differences, as determined by analysis of variance (ANOVA) for unpaired values and Fischer’s test ( $P < 0.05$ ). For a given triglyceride and carotenoid, a superscript ‘ indicates a significant difference between the triglyceride droplets and the phospholipid-stabilized triglyceride droplets, as determined by Student’s *t*-test for unpaired values ( $P < 0.05$ ). For a given triglyceride and type of droplet, a superscript / indicates a significant difference between the amount of β-carotene and zeaxanthin associated with the lipid droplet, as determined by Student’s *t*-test for unpaired values ( $P < 0.05$ ).

TABLE 4. Surface-to-core distribution of the lipids and of the carotenoids in the phospholipid-triolein-carotenoid emulsions

| Emulsion Carotenoid | Triolein (wt %) |                                   | Phospholipid (wt %); |      | Carotenoid (wt %)   |                                  |
|---------------------|-----------------|-----------------------------------|----------------------|------|---------------------|----------------------------------|
|                     | Surface         | Core                              | Surface              | Core | Surface             | Core                             |
| $\beta$ -Carotene   | 4.59 $\pm$ 0.16 | 99.9918 $\pm$ 0.0001              | 95.41 $\pm$ 0.20     | ND   | ND                  | 0.0082 $\pm$ 0.0001              |
| Zeaxanthin          | 4.05 $\pm$ 0.33 | 99.9982 $\pm$ 0.0007 <sup>a</sup> | 95.87 $\pm$ 0.33     | ND   | 0.0848 $\pm$ 0.0011 | 0.0019 $\pm$ 0.0004 <sup>a</sup> |

Concentrated emulsions were prepared as described in the legend of Table 1 with a mixture of triolein-phospholipids-carotenoid 1:0.23:0.001 (w/w/w). The composition of the concentrated emulsions obtained was TG-PL-CAR 1:0.0561:0.000496 and TG-PL-CAR 1:0.0715:0.000176 for the  $\beta$ -carotene and the zeaxanthin concentrated emulsion, respectively. The core and surface phases of lipid droplets were separated using ultracentrifugation in glass capillary tubes. Values are mean  $\pm$  SEM of three measurements; ND, not detected. Lipids and carotenoids are expressed as the weight percentage (wt %) of the sum lipids plus carotenoid recovered in a given phase. A superscript *a* indicates that the lipid or the carotenoid wt % is significantly different ( $P < 0.05$ ) among the emulsions, as determined by Student's *t*-test for unpaired values.

detect  $\beta$ -CAR in all the surface phase samples. We therefore considered a detection lower limit of 1 ng  $\beta$ -CAR by HPLC and, on the basis of the lipid amount

recovered in the surface samples analyzed for  $\beta$ -CAR, we then calculated that the  $\beta$ -CAR weight fraction in the surface phase was lower than 0.45 ng per 100 g lipids. Thus, the  $\beta$ -CAR phase distribution coefficient was near 0.

#### Distribution of the lipids between emulsion droplet and aqueous phases during the TG lipolysis catalyzed by pancreatic lipase

The emulsions had a droplet median-size of  $1.7 \pm 0.3$   $\mu$ m and a specific area of  $5.4 \pm 0.5$  m<sup>2</sup>/g. When these emulsions were incubated in distilled water or in the bile lipid buffer for 30 min, their droplet median-size did not change (data not shown). Figure 1 shows the radiolabeled lipid composition of the three phases recovered after ultracentrifugation of the ZEA-containing emulsions incubated under various conditions. Similar figures, concerning the floating lipid and infranatant lipid contents, were observed after the incubations of the CAR-free emulsion and the  $\beta$ -CAR-containing emulsion. Nevertheless, note that no pellets were recovered after the incubation of these emulsions. As shown in Fig. 1, TG accounted for 94% of all the radiolabeled lipid classes recovered after incubating the emulsions in dis-

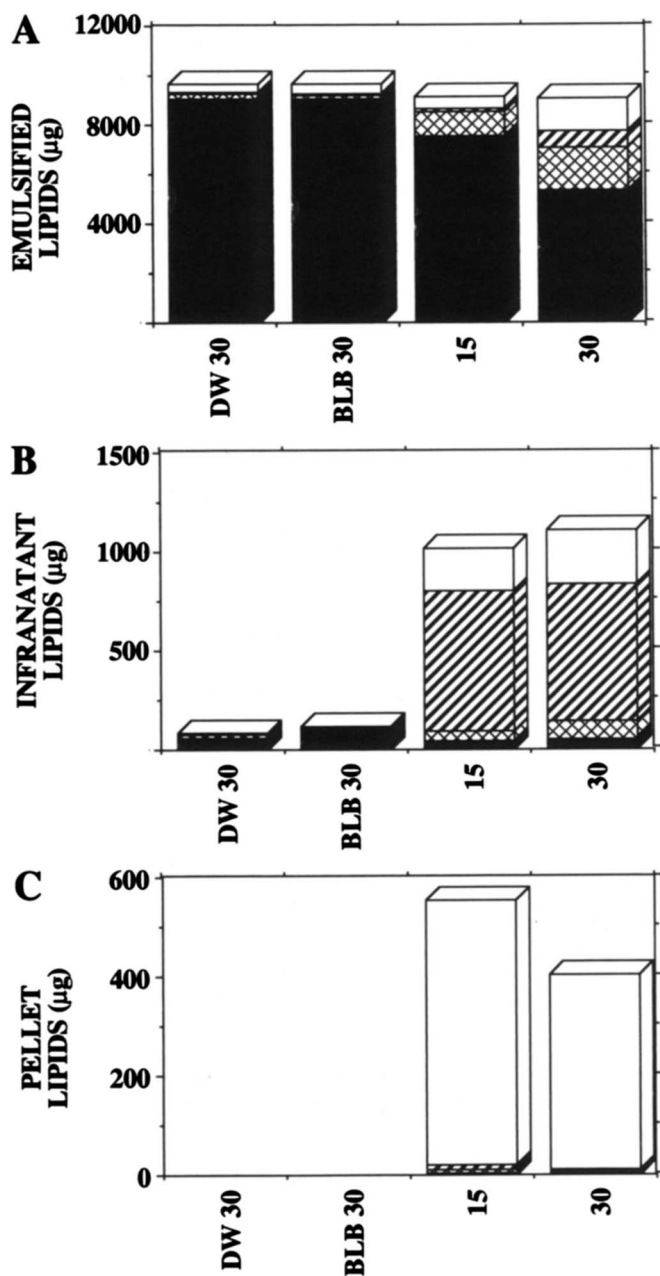


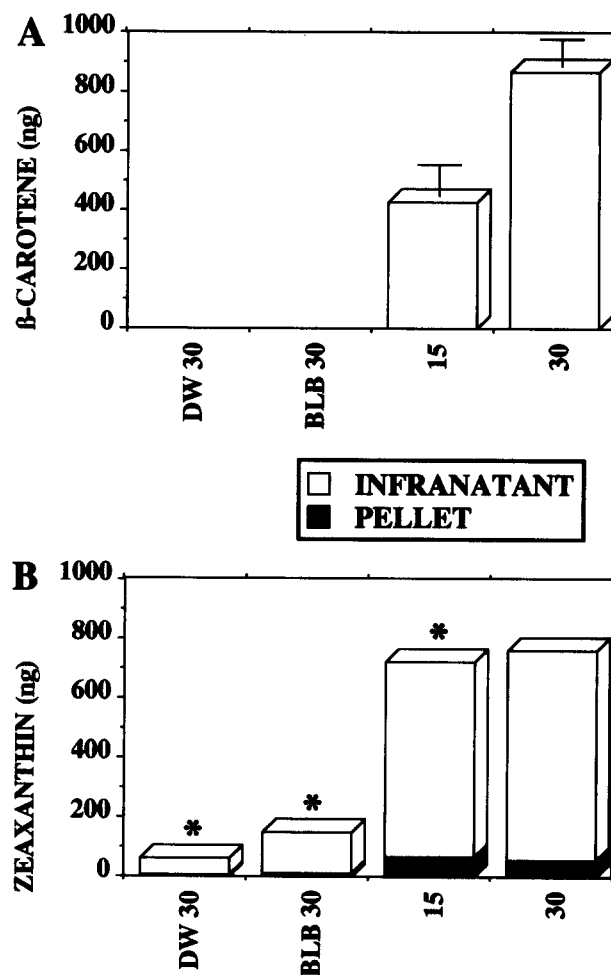
Fig. 1. Triolein and triolein lipolysis products recovered in the three phases obtained after ultracentrifugation of the zeaxanthin-containing emulsion incubated under various conditions. The results are the mean of three experiments. Emulsions composed of triolein, phospholipids, and carotenoid were prepared by sonication. The concentrated emulsions were incubated under various conditions and were ultracentrifuged for 18 h at 50,000 g at 5°C in a SW 40 Ti rotor. Three phases were recovered: a floating remaining emulsion (emulsified lipids, A), a clear subphase containing mixed micelles (infranant, B), and a pellet (C). Incubation protocol: concentrated emulsion consisting of 11.5  $\mu$ mol triolein, 0.33  $\mu$ mol egg phospholipids, and 3.0 nmol zeaxanthin was incubated in distilled water for 30 min (DW 30), in the bile lipid buffer for 30 min (BLB 30), or in the bile lipid buffer and pancreatic lipase and colipase for 30 min (15, 30). The radiolabeled lipids recovered in the infranatant (B) and in the pellet (C) were separated and quantified using TLC and scintillation counting. The floating, remaining, emulsified lipid droplet compositions were calculated by subtracting the infranatant plus the pellet lipids from the whole lipids assayed on aliquots of the reaction medium obtained before the ultracentrifugation. FFA, free fatty acids (▨); MG, monoglycerides (▩); DG, diglycerides (□); TG, triglycerides (■). Note that MG could have been slightly overestimated (see the Experimental section).

tilled water for 30 min, and only a negligible proportion (0.6%) of the radiolabeled lipids was recovered in the aqueous infranatant (containing mixed micelles) (Fig. 1B) and in the pellet (Fig. 1C). Incubating the emulsions for 30 min in the bile lipid buffer did not induce any marked change in the radiolabeled lipids distribution, as compared with the figures observed with distilled water. Conversely, incubation of the emulsions in the bile-lipid buffer and with colipase-pancreatic lipase induced a continuous decrease in the proportion of TG recovered in the floating remaining emulsion: TG accounted for 55% of the radiolabeled lipids after 30 min of lipolysis. The relative percentages of the TG lipolytic-products recovered throughout the lipolysis in our in vitro system were close to the pattern previously observed (44), even though the monoglycerides were probably slightly overestimated as a consequence of the methodology used for the separation of the lipolytic-products (see Experimental section). As TG decreased, the amounts of diglyceride, monoglyceride, and free fatty acids recovered in the three phases increased. As shown in Fig. 1B, after 15 and 30 min lipolysis there was a dramatic increase in monoglyceride amounts (accounting for 62–70% of radiolabeled lipids recovered in the infranatant) and in free fatty acid amounts (accounting for 21–35% of radiolabeled lipids recovered in the infranatant) in the aqueous phase, as compared with the monoglyceride and free fatty acid amounts recovered after the incubations without pancreatic lipase. At that time a pellet almost exclusively composed of free fatty acids (97–99% of radiolabeled lipids recovered in the pellet) appeared.

During the TG lipolysis, the infranatant bile salt amount was not modified while the infranatant PL amount increased (data not shown). More precisely, when the infranatant PL amount recovered after 30-min incubation in the bile lipid buffer was set at 100%, the relative PL amounts recovered in the infranatant after 15 and 30 min lipolysis were  $133 \pm 3\%$  and  $138 \pm 6\%$ , respectively. The infranatant PL amount probably increased because TG lipolytic-products were formed, allowing the lipid droplet PL to transfer from the lipid droplet surface to the aqueous phase. Whatever the incubation conditions, no PL and no bile salts were recovered in the pellets.

#### Transfer of CARs from emulsion lipid droplets to emulsion aqueous phase

As shown in Fig. 2, no  $\beta$ -CAR-containing pellet was recovered whatever the incubation conditions of the  $\beta$ -CAR-containing emulsion ( $\beta$ -EM), whereas ZEA-containing pellets were recovered after incubation of the ZEA-containing emulsion (Z-EM) with or without colipase-pancreatic lipase. No detectable amount of  $\beta$ -CAR

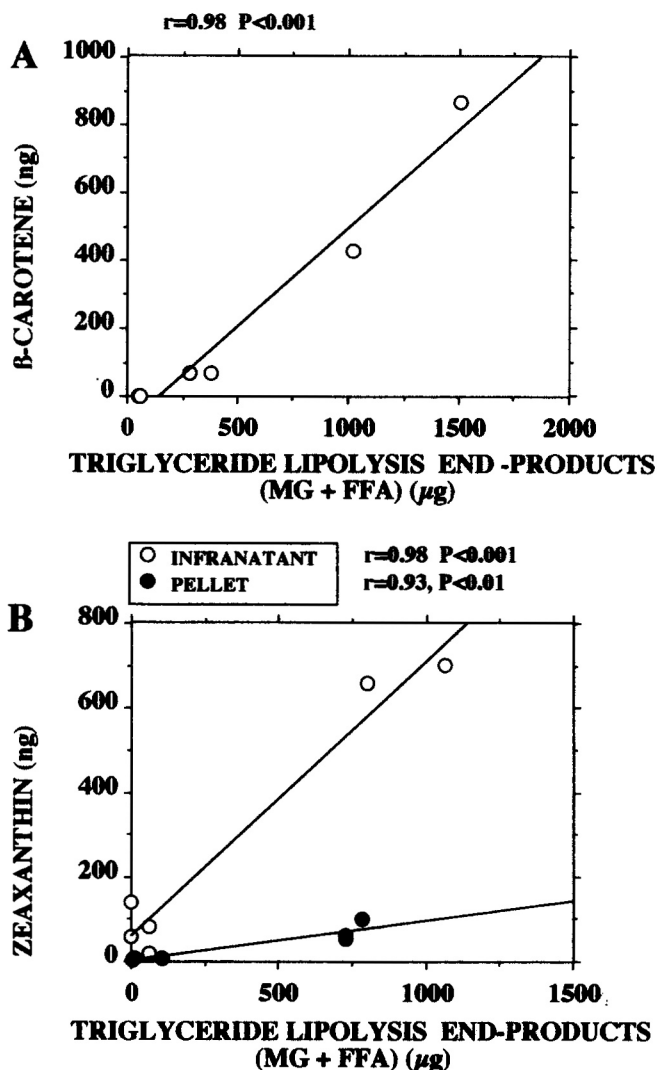


**Fig. 2.** Carotenoids recovered in the infranatants (mixed micelles) and in the pellets obtained after ultracentrifugation of the  $\beta$ -carotene-containing emulsions (A) or the zeaxanthin-containing emulsions (B) incubated under various conditions (see legend of Fig. 1). Values are mean  $\pm$  SEM of three experiments. SEM are not shown in panel B, but are in the same range as those shown in the panel A. An asterisk indicates that the amounts of  $\beta$ -carotene and zeaxanthin, recovered in the infranatants at a given incubation time, were significantly different, as determined by Student's *t* test for unpaired values ( $P < 0.05$ ).

was recovered in the aqueous phase after incubating the  $\beta$ -EM in distilled water or in the bile lipid buffer for 30 min. Conversely,  $59 \pm 19$  ng and  $141 \pm 9$  ng of the ZEA, accounting for 3.4% and 8.3% of the ZEA solubilized in the Z-EM, were transferred after 30-min incubation of the Z-EM in distilled water and in the bile lipid buffer, respectively. After TG lipolysis, there was a dramatic increase in the infranatant CAR accumulation:  $428 \pm 105$  ng and  $868 \pm 85$  ng  $\beta$ -CAR and  $658 \pm 6$  ng and  $704 \pm 23$  ng ZEA were recovered in the infranatant after 15 and 30 min lipolysis, respectively.

As shown in Fig. 3, there is a strong positive correlation between accumulation of infranatant TG lipolytic products and infranatant  $\beta$ -CAR or ZEA. Similarly, there is a strong positive correlation between occurrence of pellet lipids and pellet ZEA. Furthermore, there was a





**Fig. 3.** Correlations between lipolysis end-products (monoglycerides plus free fatty acids) and carotenoids recovered in the infranatants (vesicles + mixed micelles) or in the pellets after ultracentrifugation of (A) the incubated  $\beta$ -carotene-containing emulsions or (B) the incubated zeaxanthin-containing emulsions. The correlation coefficients ( $r$ ) and the probability levels ( $P$ ) are shown.

strong positive correlation ( $r = 0.89$ ,  $P < 0.05$ ) between PL and ZEA recovered in the incubation infranatants (data not shown) that was not observed between infranatant PL and  $\beta$ -CAR.

## DISCUSSION

This study was conducted to have a better understanding of the behavior of carotenoids (CARs) in biological emulsions (BIO-EMs) such as dietary fat droplets, triglyceride-rich lipoproteins (TRLs), and intracellular storage droplets. We therefore studied: *i*) the solubility of CARs in BIO-EM models, *ii*) the CAR surface-to-core

distributions in BIO-EM models, and *iii*) the mechanisms involved in the transfer of CARs from emulsion lipid droplets to other soluble biological lipid structures such as vesicles and mixed micelles in the gastrointestinal lumen. Because we hypothesized that polar and apolar CARs have different behaviors in BIO-EM, we compared the fate of  $\beta$ -CAR (a model for the apolar CARs) and ZEA (a model for the polar CARs).

CARs are highly hydrophobic molecules, virtually insoluble in water and assumed to be very poorly soluble in fat. Yet no accurate data are available on their solubility in bulk triglycerides as well as in BIO-EM which are their natural vehicles in animals and humans. BIO-EM are composed of a TG core surrounded by a monomolecular film of PL (21). Because hydrophobic molecules can be solubilized in the TG core as well as in the monolayer PL surface of BIO-EM models (20, 21), we measured *i*) the solubility of some common CARs in bulk TGs and *ii*) we compared the amount of  $\beta$ -CAR and ZEA associated with pure TG droplets and with PL-stabilized TG droplets during the preparation of model emulsions.

### Solubility of common CARs in bulk TG is very different

CAR solubility in bulk triolein at 20°C ranged between 0.0001 and 0.337%, which is considerably lower than the free cholesterol solubility i.e., 2.8% (45), and the cholesteryl-oleate solubility i.e., 23% (45) at 21°C. The CAR solubility, i.e., 0.118% for  $\beta$ -carotene in bulk triolein, was close to the CAR solubility found in edible oils, such as palm oil, which contains about 0.2% CARs. As previously found for free-cholesterol (46), the lower the chain length of TG fatty acids, the higher CAR solubility. Note that we did not find a correlation between the CAR melting point, which has been shown to be correlated with the solubility of aromatic compounds in triglycerides (38), or the number of CAR hydroxy groups and the CAR solubilities.

### PL dramatically increased the amount of polar CAR associated with lipid droplets

The methodology used to prepare the lipid-in-water droplets led to the association of CAR microcrystals with the droplets. This explains why the amount of CAR associated with the pure TG droplets was considerably higher than the CAR solubility in bulk TG. Thus, in this experiment, we have in fact measured the ease with which polar and apolar CARs were associated with TG droplets or with PL-TG droplets during emulsion preparation. The main result is that the amount of ZEA associated with lipid droplets increased dramatically in PL-TG droplets, as compared to the corresponding pure TG droplets, while the amount of  $\beta$ -CAR associated with

lipid droplets increased only slightly in PL-TG droplets. This finding suggests that, contrary to apolar CARs, an important fraction of polar CARs is associated with the surface PL of the BIO-EMs.

### **Polar and apolar CARs have contrasting surface-to-core distributions in BIO-EMs**

We measured CAR distributions between the surface and the core of BIO-EMs in lecithin-stabilized triolein emulsions so that we could compare our results with those previously obtained for PL, triolein, and free cholesterol with the same model emulsions and validated methodology (20, 21). The surface-to-core distributions observed for PL and triolein were in the same range as those previously obtained (20, 21). The two CARs used had opposite behaviors in lipid droplets, as ZEA was preferentially solubilized in the surface PL while  $\beta$ -CAR was preferentially solubilized in the core TG. This is in agreement with the first finding of this study concerning CAR solubility in pure lipid droplets and in PL-TG droplets. Note also that the ZEA surface-to-core distribution coefficient ( $52.6 \pm 10.7$ ) was about 2-fold higher than that of free cholesterol ( $23.9 \pm 3.6$ ) (20, 21, 47) and that the  $\beta$ -CAR surface-to-core distribution was near zero. Because ZEA has two hydroxy groups, free cholesterol has one, and  $\beta$ -CAR has none, we suggest that the surface-to-core distribution of alicyclic hydrocarbons whose sole source of polarity is hydroxy groups is correlated to the number of hydroxy groups they have. In more general terms, the apolar CARs, i.e.,  $\alpha$ -carotene,  $\beta$ -CAR, and lycopene, would be solubilized almost exclusively in the BIO-EM core.  $\beta$ -Cryptoxanthin, a one-hydroxy-group polar CAR would distribute between the BIO-EM core and surface phases like free cholesterol, i.e., with a distribution coefficient of approximately 24 (20, 21, 47). Finally, polar CARs with two hydroxy groups, i.e., ZEA and lutein, would comparably distribute between the core and surface phases with the coefficient of ZEA (approximately 53).

### **Transfer of CARs from BIO-EM lipid droplets to aqueous-soluble lipid structures is partially spontaneous for polar CARs and totally mediated by TG lipolysis for apolar CARs**

Previous studies on the transfer of hydrophobic molecules from lipid structures to cell membranes (48–51) have shown that this process is mediated by TG hydrolysis catalyzed by a lipase. We therefore studied the role of TG lipolysis on the transfer of polar and apolar CARs between a model emulsion and mixed micelles. We think that this model can provide explanations for CAR transfer from dietary emulsions to mixed micelles as well as from TRLs to other lipoprotein classes or to cell membranes. Indeed, TRLs and dietary emulsions have

close physico-chemical characteristics, and pancreatic lipase as well as lipoprotein lipase and hepatic lipase have evolved from a common ancestral gene and therefore have a number of similar characteristics. In this study, we demonstrate that a polar CAR could be partially transferred without TG lipolysis, while an apolar CAR absolutely requires TG lipolysis to be transferred. We suggest that PL and polar CARs localized at the lipid droplet's surface can be spontaneously extracted and solubilized in the aqueous phase, while apolar CARs localized in the droplet's core absolutely require the production of aqueous-soluble lipids to be transferred from the lipid droplet to the aqueous phase. This is of high biological significance as it gives an explanation for the CAR transfer mechanism that must exist between dietary emulsions and mixed micelles during CAR digestion, or that might exist between TRLs and other lipoproteins, or between TRLs and cell membranes during CAR intravascular metabolism.

### **Biological consequences**

*Intestinal absorption of CARs solubilized in dietary lipid emulsions.* CARs and other hydrophobic molecules must be solubilized in mixed micelles to be absorbed in the intestine (29–31). In agreement with others (26, 27), we found that CARs were solubilized in the aqueous phase containing mixed micelles. Note that the ZEA to TG lipolytic products mass ratio ( $0.084 \pm 0.019\%$ ) was not significantly different from the  $\beta$ -CAR to TG lipolytic products mass ratio ( $0.063 \pm 0.012\%$ ), therefore polar and apolar CARs have close solubility in mixed micelles. Note also that the CAR to lipolytic products mass ratio in the aqueous phase was constant; therefore, the limiting step for the solubilization of CARs in aqueous phase is the amount of mixed micelle produced, which depends on the amount of TG lipolytic products produced. This can explain why, for a given amount of dietary TG, the CAR relative weight fraction absorbed decreases when the CAR amount ingested increases, and why pharmacological CAR amounts are less efficiently absorbed than physiological ones. Thus, we suggest that intestinal absorption of CARs, solubilized in BIO-EM, depends on the amount of TG lipolytic product generated by pancreatic lipase. Our findings provide an excellent explanation of why dietary fat enhances CAR bioavailability (23, 24). Finally, it must be stressed that the spontaneous transfer of the polar CARs but not of the apolar ones could have nutritional and therapeutic consequences. Indeed, subjects with chronic pancreatitis, whose pancreatic secretion is impaired, could absorb polar CARs more efficiently than apolar ones.

*CAR metabolism in lipoproteins.* The different affinity of polar and apolar CARs for TG and PL could explain their distribution between lipoprotein classes. Indeed,

it has been shown that  $\beta$ -CAR (52–54) and other CARs (19, 55) distributed differently among human serum lipoprotein classes. For example, the HDL/LDL ratio of lutein (a polar CAR) was 3.1 while the ratios of  $\beta$ -CAR and lycopene (an apolar CAR) were 0.5 and 0.4, respectively. But, these differences have not been explained. Lipoprotein classes contain various proportions of surface (PL) and core lipids (TG, esterified cholesterol): HDL has a PL to apolar lipid (PL/NL) mass ratio of 1.4, while LDL and VLDL have PL/NL mass ratios of 0.5 and 0.3, respectively. We suggest that the preferential solubility of polar CARs in PL and of apolar CARs in TG explains the CAR distribution between lipoprotein classes: polar CARs are preferentially found in lipoproteins with a high PL/apolar lipid mass ratio, i.e., HDL, while apolar CARs are preferentially found in lipoproteins with a low PL/apolar lipid ratio, i.e., VLDL and LDL.

Like other lipid molecules, CARs might be transferred from a lipoprotein class to another lipoprotein class or to cell membranes. We suggest that these transfers are affected by the CAR surface-to-core distribution. More precisely, the transfer of a polar molecule, mainly recovered at the droplet's surface, might be easier than the transfer of an apolar molecule, mainly recovered in the biological lipid droplet's core. Accordingly, it has indeed been shown that the  $\beta$ -CAR transfer rate between model and native lipoproteins is very slow (half-time > 18 h), while the transfer rates of free cholesterol and  $\alpha$ -tocopherol, which have a one-hydroxy-group, are fast: half-times of approximately 20 min and 70 min, respectively (56). No data are available concerning the transfer rate of other apolar CARs and of polar CARs, but we suggest that apolar CARs would not spontaneously transfer between biological lipid structures, that one-hydroxy-group CARs would transfer with the same rate as free cholesterol and  $\alpha$ -tocopherol, and that two-hydroxy-group CARs would transfer faster than the apolar and one-hydroxy-group CARs. These assumptions will be tested in another study.

To summarize, polar CARs are preferentially solubilized in the PL surface of the BIO-EMs while apolar CARs are preferentially solubilized in the BIO-EM TG core. Consequently, they distribute differently between the core and the surface of BIO-EM. The transfer of the polar CARs between BIO-EM and other biological lipid structures (mixed micelles and vesicles and, potentially, lipoproteins and membranes) is easier than that of apolar CARs, which requires TG lipolysis by a lipase. Such basic data further our understanding of the mechanisms governing the intestinal absorption of CARs and the distribution of CARs between various biological lipid structures, including lipoproteins and membranes.

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