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Comparison of the uptake and secretion of carotene and xanthophyll carotenoids by Caco-2 intestinal cells

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Carotenoids have been shown to have potential beneficial effects on human health which has led to an increasing interest in the study of their bioavailability. A Caco-2 cell model, as previously described, was employed to examine the percentage transfer of the carotenoids α-carotene, β-carotene, lycopene, astaxanthin, β-cryptoxanthin, lutein and zeaxanthin through an intact, highly differentiated Caco-2 cell monolayer at a range of different amounts. Our results show that astaxanthin, a carotenoid with powerful antioxidant capacity, had the highest percentage transfer overall. We examined the cellular uptake and secretion of lutein and zeaxanthin to compare two structurally similar carotenoids. Both were efficiently transported through the monolayer with a range between 5·1 (SEM 1·2) % to 20·2 (SEM 3·3) % and 5·5 (SEM 2·5) % to 13·4 (SEM 4) % for lutein and zeaxanthin, respectively. These carotenoids were compared to each other at each added amount and no significant difference was observed between the two xanthophylls. The carotene carotenoids α-carotene, β-carotene and lycopene and the xanthophyll β-cryptoxanthin were also examined and had lower uptake and secretion values when compared to lutein, zeaxanthin and astaxanthin. The xanthophyll β-cryptoxanthin was also not significantly different when compared to the carotene carotenoids. Data generated from this study compares well with *in vivo* bioavailability studies. Furthermore, the model provides comparative data on the relative absorption and transfer of seven different carotenoids. Our data indicate that lower amounts of carotenoids were absorbed and transferred more efficiently than higher amounts suggesting a saturation effect at higher exposure.

Carotenoids: Caco-2 cell model: Bioavailability: Intestinal absorption

Carotenoids are a class of fat-soluble plant compounds that are responsible for the colouration of fruits and vegetables. Interest in the bioavailability of carotenoids has been encouraged by research, demonstrating an association between the intake of fruits and vegetables and a reduced risk of chronic conditions such as CVD, certain cancers, age-related macular degeneration and cataract formation (Furr & Clark, 1997). Carotenoids can be divided into two main groups based on their structure; carotenes and xanthophylls. β-Carotene, α-carotene and lycopene are the predominant members of the carotene group which includes carotenoids mainly composed of carbon and hydrogen atoms. Xanthophylls contain at least one oxygen atom. Lutein, zeaxanthin, β-cryptoxanthin and astaxanthin are common xanthophylls with hydroxyl and keto groups as structural elements (Stahl & Sies, 2005). Bioavailability of carotenoids has been investigated using both animal and human studies. Human studies include plasma carotenoid response and intake–excretion balance studies. Animals that have similar metabolism of β-carotene to that of man include the ferret, the preruminant calf and the gerbil, but the specialised care of these animals does not make this method widely accessible (Garrett *et al.* 1999). More recent approaches using stable isotopes and mass spectral analysis of carotenoids and their metabolites are potential methods of studying human intestinal absorption but they are intricate and expensive (During & Harrison, 2004).

An *in vitro* intestinal cell culture model mimicking the *in vivo* intestinal absorption of carotenoids was recently reported (During *et al.* 2002) and is employed in the present study with modifications. The Caco-2 cell model consists of a 3-week-old differentiated Caco-2 cell monolayer grown on a permeable membrane. Caco-2 cells are human colonic adenocarcinoma cells that, when differentiated, exhibit both functional and morphological characteristics similar to enterocytes such as the secretion of brush border enzymes and the presence of microvilli (Sambury *et al.* 2001). The cell monolayer is stimulated to produce chylomicrons according to the method of Luchoomun & Hussain (1999). These conditions mimic the *in vivo* postprandial state. Carotenoids are delivered in a Tween 40 micelle and are incubated for a total of 16 h (During *et al.* 2005). The carotenoids are absorbed by the Caco-2 cells, incorporated into chylomircons and are secreted into the basolateral chamber of the transwell plate. The amounts of carotenoids that were chosen for this study were based on the calculation that During *et al.* (2002) reported to be physiologically realistic. These amounts are based on a daily intake of 5 mg β-carotene and the surface absorption,

Abbreviations: HBSS, Hanks balanced salt solution; TEERS, trans-epithelial electrical resistance.

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length and diameter of the upper region of the small intestine and are considered representative of the normal range of carotenoids from diet and supplements.

The main objective of this study was to examine the percentage transport of various carotenes and xanthophylls through a differentiated Caco-2 cell monolayer cultured on a 0·4 μm pore size membrane and to compare the differences in absorption of individual carotenoids at varying amounts. The carotenoids studied, β-carotene, α-carotene, lycopene, lutein, zeaxanthin, β-cryptoxanthin and astaxanthin, were delivered to the cells in an emulsion. This model, when used in conjunction with an *in vitro* digestion procedure, provides an approach to assess the relative availability of carotenoids from food sources and synthetic formulations.

Materials and methods

Materials

β-Cryptoxanthin was obtained from LGC Prochem (Middlesex, UK). α-Carotene was obtained from Carbon 14 Centralen (Hoersholm, Denmark). Lutein and zeaxanthin were purchased from Fluka (Buchs SG, Switzerland). Foetal bovine serum was purchased from GIBCO BRL Technologies Ltd. (Paisley, Scotland). All other chemical reagents were purchased from Sigma Aldrich Ireland Ltd (Dublin, Ireland) unless otherwise stated. Tissue culture plastics were supplied by Greiner Bio-One (Kremsmunster, Austria). Transwell plates were purchased from Costar (New York, USA). All solvents employed were of HPLC grade.

Preparation of carotenoids

All carotenoid analysis was performed under amber light to avoid photo-oxidation of carotenoids. The carotenoids α-carotene, β-carotene, β-cryptoxanthin, lutein, lycopene, zeaxanthin and astaxanthin were prepared using the 'Tween 40' method as described by O'Sullivan *et al.* (2004). Briefly, the amounts (0·5 μg, 1·0 μg, 1·5 μg, 2·0 μg, 2·5 μg) of each carotenoid were determined spectrophotometrically using the published extinction coefficients, in either hexane, ethanol or methylethylketone. Each preparation was dried down under N_2. The carotenoids were reconstituted in 50 μl Tween 40 (200 ml acetone/l). These solutions were again dried down under N_2. Residues were reconstituted in 2 ml serum free media containing 0·5 mM taurocholate, 1·6 mM oleic acid and 45 mM glycerol for the stimulation and secretion of chylomicrons. Carotenoid enriched media was passed through a surfactant-free cellulose acetate filter (0·2 μm; Millipore, Bedford, MA, USA). before addition to cells (at final concentration of carotenoids ranging from approximately 0·5 to 2·3 μM).

Cell Culture

Caco-2 cells (without 15, 15′ dioxygenase activity) were purchased from the European Collection of Animal Cell Cultures (Salisbury, Wilts., UK). Cells were maintained in Dulbecco's Modified Eagle's Medium supplemented with 10 % foetal bovine serum and 1 % non-essential amino acids. Cells were grown at 37°C and 5 % CO_2 in a humidified incubator, in the absence of antibiotics. For experiments, cells were seeded at a density of $1{\cdot}25 \times 10^5$ cells/ml on transwell plates (6-well plate, 24 mm diameter, 0·4 μm pore size membrane). Cells were grown for 21–25 d to obtain a differentiated cell monolayer. Trans-epithelial electrical resistance (TEERS) measurements were taken twice weekly by a TEERS voltohometer to ensure the monolayer was intact. Diffusion of phenol red was also performed to ensure that cell monolayers were undamaged (results not shown). Differentiation was confirmed by measuring the activity of the brush border enzymes alkaline phosphatase and amino peptidase, which have higher activity in enterocytes compared with undifferentiated Caco-2 cells (results not shown). At the beginning of each experiment, the apical side of the transwell plate received 2 ml carotenoid enriched media (containing chylomicron stimulating compounds). The basolateral chamber received 2 ml serum free media. The incubation time for all experiments was 16 h which is optimised for chylomicron production. After the incubation time, media from each side of the membrane were removed, the monolayer was washed twice with Hanks Balanced Salt Solution (HBSS) and cells were scraped into 1 ml HBSS. Cell samples were sonicated for 30 s on ice. Samples were stored at − 80°C for further analysis.

Lipoprotein fractionation

Lipoprotein fractions which include large chylomicrons, small chylomicrons and VLDL were isolated from the basolateral medium by sequential density gradient ultracentrifugation according to the methods of Luchoomun & Hussain (1999). Briefly, 2 ml basolateral chamber medium was mixed with 0·287 g KBr and then overlaid with 900 μl 1·063 g/ml density solution, 900 μl 1·019 g/ml density solution and of 900 μl 1·006 g/ml density solution. The samples were then subjected to three successive centrifugations (NVT90 rotor, Beckman Instruments Inc., CA, USA): first at 40 000 rpm for 12 min, then 40 000 rpm for 77 min followed by 60 000 rpm for 169 min. After each centrifugation, 1 ml supernatant was collected and each sample was replenished with 1·006 g/ml density solution prior to the next centrifugation. The supernatants were pooled and stored at − 80°C.

Carotenoid extraction and analysis

Apical, basolateral and cell samples were allowed to thaw and were briefly vortexed. A recovery standard consisting of 25 mM α-tocopherol acetate was added to all samples. These samples were extracted twice with hexane–ethanol–acetone (50:25:25, by volume) (Olives Barba *et al.* 2006). Samples were centrifuged (Sorvall TC6, H400 rotor, DuPont Instruments, Herts, UK) at 3000 rpm for 5 min and the supernatant layers were removed, pooled and dried down under N_2. The residues were reconstituted in 200 μl mobile phase and the carotenoid content of the samples was analysed by reverse phase HPLC. The HPLC system (Shimadzu, Kyoto, Japan) consisted of a LC10-AD pump connected to a SIL-10A auto-injector, a SPD-6AV system controller, SPD-6AV UV-visible detector and SPD-10AV UV-visible detector. The column system consisted of a Spherisorb ODS-2 C18 5 μm PEEK guard column

(Alltech Associate Applied Science Ltd; supplied by Ocon Chemicals Ltd, Cork, Ireland) connected to a Vydac 201TP54 (250 × 4·6 mm) reverse phase C18 column (Separations Group; supplied by AGB Scientific Ltd, Dublin, Ireland). Column temperature was maintained at 25°C using a column water jacket (Alltech Associates Applied Science Ltd.) with a thermostatically controlled water bath (Lauda RM6 T; Lauda-Königshofen, Germany). The injection volume was 50 μl; samples were eluted using isocratic mobile phase composed of acetonitrile–methanol–dichloromethane (75:20:5, by volume) containing 10 mM-ammonium acetate, 4·5 mM-butylated hydroxytoluene and 3·6 mM triethylamine at a flow rate of 1·5 ml/min. Carotenoids were detected at 450 nm on a visible detector and tocopherols at 292 nm on a UV detector. The mobile phase was filtered through a 0·5 μm organic filter and degassed using ultrasonic agitation. Results were collected and analysed using Millenium software (Waters Corporation, Milford, MA, USA). β-Carotene, α-carotene, lycopene, lutein, zeaxanthin, β-cryptoxanthin and astaxanthin recovered from media and cell samples were extrapolated from pure carotenoid standard curves, after correction for extraction efficiency based on recovery of α-tocopherol acetate.

Statistical analyses

Results are representative of three or more independent experiments. Errors are expressed as standard errors of the means. Carotenoids were compared at various concentrations by one-way ANOVA followed by Tukey's test. Carotenoids were compared to each other at differing concentrations by an unpaired *t* test.

Results

The uptake and secretion of carotene carotenoids

The carotene carotenoids examined in this study were α-carotene, β-carotene and lycopene. These were added to Caco-2 cells at amounts of 0·5 μg, 1·0 μg, 1·5 μg, 2·0 μg, 2·5 μg. The basolateral chamber value is the amount of carotenoid that was taken up by the Caco-2 cell, packaged into chylomicrons, transported through the monolayer and secreted into the basolateral chamber of the transwell plate. This value is expressed as a percentage of the original starting amount that was added to the well. Large chylomicrons, small chylomicrons and VLDL lipoprotein fractions were isolated from the basolateral medium and the carotenoid contents of these fractions were combined and compared to the total amount of carotenoid in the basolateral chamber (data not shown). There was no difference in the carotenoid content when the lipoprotein fractions were combined and compared to the total content in the basolateral chamber. Therefore, lipoprotein fractions were no longer prepared and the entire basolateral chamber medium was analysed for carotenoid content. The percentage α-carotene that was recovered from the basolateral chamber ranged from 0·6 (SEM 0·1) to 3·8 (SEM 2·1) % (Fig. 1). This is lower than that of β-carotene where values ranged between 1·3 (SEM 0·1) and 4·2 (SEM 0·8) % (Fig. 1) and when the two carotenoids were compared, the percentage transfer values differed significantly at the 1·0 μg, 1·5 μg and 2·0 μg concentrations ($P<0·05$). Lycopene transfer was similar to that of α-carotene as there was no significant difference between the two carotenoids. The percentage carotene that remained in cells (Table 1) was combined with basolateral values (Fig. 1) and was expressed as total uptake and secretion (Fig. 2). When the percentage total uptake and secretion of α-carotene, β-carotene and lycopene were compared to each other there was no significant difference at any amount except at 2 μg where lycopene was significantly different from α-carotene and β-carotene ($P<0·05$). There were also significant differences between the 1·0 μg and the 2·0 μg lycopene amounts ($P<0·05$).

Uptake and secretion of the xanthophyll carotenoids lutein and zeaxanthin

Lutein and zeaxanthin are isomers of each other differing only in the location of a double bond on the hydroxyl end of the molecule. The percentage transfer of lutein to the basolateral chamber ranged from 2·3 (SEM 1·4) to 10·5 (SEM 2·4) % (Fig. 1). When the percentage transfer to the basolateral chamber of lutein was compared at all amounts added the 0·5 μg amount differed significantly from all others ($P<0·01$; Fig. 1). The percentage transfer of zeaxanthin to the basolateral chamber ranged from 4·5 (SEM 2·3) to 11·8 (SEM 3·9) % (Fig. 1). The 2·5 μg amount differed significantly from the 0·5 μg zeaxanthin ($P<0·05$). When the percentage transfer of lutein was compared to that of zeaxanthin at each amount, there was no significant difference between the isomers (Fig. 1). For lutein the total uptake and secretion ranged from 5·1 (SEM 1·2) to 20·2 (SEM 3·3) % and this was very similar to zeaxanthin, which ranged from 5·5 (SEM 2·5) to 13·4 (SEM 4) % (Fig. 2). These carotenoids were compared to each other at each amount and no significant difference was observed between the two xanthophylls.

Uptake and secretion of the xanthophylls β-cryptoxanthin and astaxanthin

Although astaxanthin is considered a xanthophyll carotenoid, it differs from the other xanthophyll carotenoids in this study, as it has a ketone group on the molecule. We compared the uptake and secretion of a xanthophyll carotenoid β-cryptoxanthin with the keto-xanthophyll astaxanthin in Caco-2 cells (Fig. 2). After a 16 h incubation, the percentage transfer to the basolateral chamber of β-cryptoxanthin and astaxanthin varied from 1·1 (SEM 0·1) to 3·4 (SEM 1·2) % and 2·2 (SEM 0·6) to 6·7 (SEM 0·51) % respectively (Fig. 1). The transfer of β-cryptoxanthin to the basolateral chamber was lower than that of astaxanthin (Fig. 1) and differed statistically at 0·5 μg and 1·0 μg ($P<0·05$). There was a significant difference ($P<0·05$) at all amounts when the percentage β-cryptoxanthin in cells was compared to that of astaxanthin (Table 1). The same trend was observed in the percentage total uptake and secretion of β-cryptoxanthin at every amount (Fig. 2). The 0·5 μg quantity of astaxanthin was significantly different ($P<0·05$) to all other astaxanthin amounts (Fig. 2). When both xanthophylls were compared against each other at each quantity the percentage uptake and secretion of astaxanthin was significantly higher ($P<0·05$) than that of β-cryptoxanthin (Fig. 2).

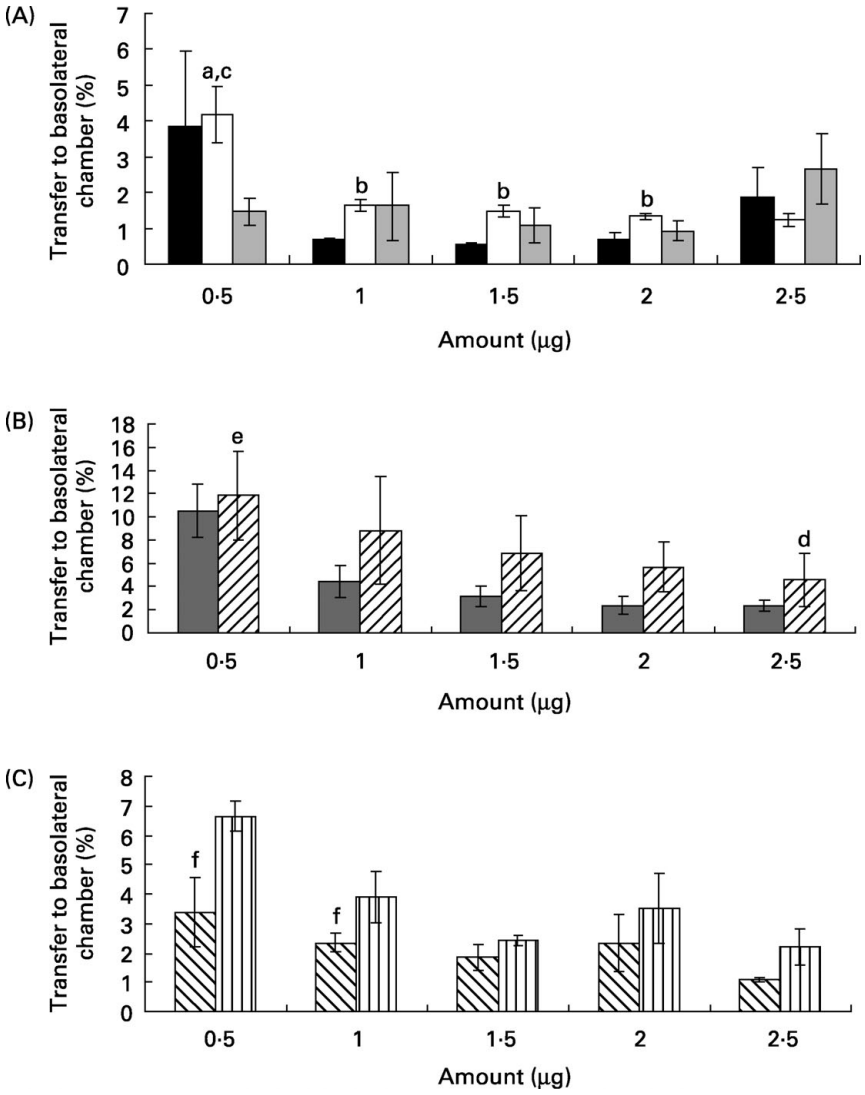

Fig. 1. The percentage transfer of carotenoids to the basolateral chamber. Data are means representative of three of more independent experiments with their standard errors represented by vertical bars. (A) ■, α-carotene; □, β-carotene; ▭, lycopene. (B) ■, lutein; ▨, zeaxanthin. (C) ▧, β-cryptoxanthin; ▥, astaxanthin. [a,b,c,d,e,f] Mean values were significantly different for the following comparisons: [a]*v.* all other β-carotene amounts ($P<0·01$); [b]*v.* α-carotene at same amount ($P<0·05$); [c]*v.* lycopene at same amount ($P<0·05$); [d]*v.* 0·5 μg zeaxanthin ($P<0·05$); [e]*v.* all other lutein amounts ($P<0·01$); [f]*v.* astaxanthin at same amount ($P<0·05$).

Table 1. The percentage carotene and xanthophyll carotenoids recovered from Caco-2 cells after 16 h incubation. Values are expressed as a percentage of the original quantities added to the cells and data are means representative of three or more independent experiments with their standard errors

	Carotene carotenoids						Xanthophyll carotenoids							
	β-Carotene		α-Carotene		Lycopene		β-Cryptoxanthin		Astaxanthin		Lutein		Zeaxanthin	
Amount(μg)	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0·5	4·5	1·9	6·4	1·7	7·6[a]	1·6	5·4	1·8	18·3[g]	2·3	9·7[f]	1·2	1·7	0·4
1·0	3·7	1·4	4·6	0·2	3·3	0·7	3·6	0·7	12·1[g]	0·2	5·7[d,f]	1·6	1·2	0·3
1·5	5·2	3·6	4·3	0·6	2·4[c]	0·3	2·9	0·5	9·0[g,h]	0·3	3·4[d,f]	0·8	0·9	0·2
2·0	3·8	0·3	4·2	0·5	2·5	0·6	2·5	0·3	8·6[g,h]	1·4	3·7[d,f]	1·3	0·9	0·4
2·5	4·0[b]	0·3	3·9	0·5	1·6	0·4	2·9	0·6	7·8[g]	1·2	2·8[d,e]	0·7	1·1	0·6

[a,b,c,d,e,f,g,h] Mean values were significantly different for the following comparisons: [a]*v.* all other lycopene amounts ($P<0·01$), [b]*v.* lycopene at same amount ($P<0·05$), [c]*v.* α-carotene at same amount ($P<0·05$), [d]*v.* 0·5 μg lutein ($P<0·01$), [e]*v.* 1·0 μg lutein ($P<0·05$), [f]*v.* zeaxanthin at same amount ($P<0·05$), [g]*v.* β-cryptoxanthin at same amount ($P<0·05$), [h]*v.* 0·5 μg astaxanthin ($P<0·05$).

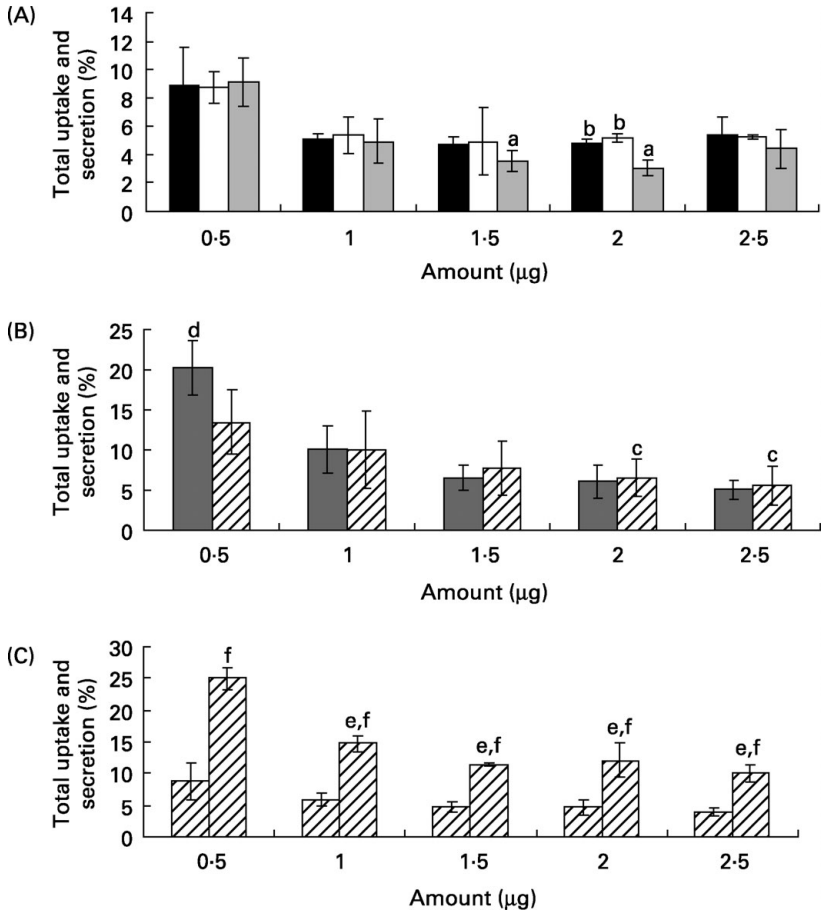

Fig. 2. The percentage total uptake and secretion of carotenoids through the Caco-2 model. Data are means representative of three of more independent experiments with their standard errors represented by vertical bars. (A) ■, α-carotene; □, β-carotene; ▤, lycopene. (B) ▩, lutein; ▨, zeaxanthin. (C) ▧, β-cryptoxanthin; ▥, astaxanthin. [a,b,c,d,e,f] Mean values were significantly different for the following comparisons: [a] *v.* 0·5 μg lycopene ($P<0·05$); [b] *v.* lycopene at same amount ($P<0·05$); [c] *v.* 0·5 μg zeaxanthin ($P<0·05$); [d] *v.* all other lutein amounts ($P<0·01$); [e] *v.* 0·5 μg astaxanthin ($P<0·05$); [f] *v.* β-cryptoxanthin at the same amount ($P<0·05$).

Discussion

Uptake and transport of the carotene carotenoids

The aim of this study was to examine individual carotenoid uptake and transport in the Caco-2 cell model. The Caco-2 cell model has previously been used to mimic the intestinal absorption of carotenoids (Garrett *et al.* 1999, 2000; Ferruzzi *et al.* 2001; During *et al.* 2002; Chitchumroonchokchai *et al.* 2004; Liu *et al.* 2004; During & Harrison, 2005; Reboul *et al.* 2005; Chitchumroonchokchai & Fallia, 2006; Ferruzzi *et al.* 2006). There are some reports on uptake and transport kinetics of β-carotene through Caco-2 cell monolayers (During *et al.* 2002, 2005); data on α-carotene, zeaxanthin, β-cryptoxanthin and astaxanthin transport, however, are limited. The amounts of carotenoids added to cells (0·5–2·5 μg) in this study are similar to the amounts that have been demonstrated to produce an anti-proliferative effect on human prostate carcinoma cells *in vitro* (Levy *et al.* 1995; Pastori *et al.* 1998) and these concentrations are also reported to be physiologically realistic (Pastori *et al.* 1998).

Data from this study indicates a 1 % difference in the percentage transport of α-carotene and β-carotene which is in line with the observations made by During *et al.* (2002) and suggests that the uptake and secretion of both carotenoids is similar. These findings are also consistent with a human study conducted by Edwards *et al.* (2002) which reported similar absorption of α- and β-carotene. However, for both α- and β-carotene the percentage transport to the basolateral chamber reported by During *et al.* (2002) was higher and differed from the results obtained in the present study by approximately 10 %. The discrepancy between the findings may be related to the differences in pore size of the membrane on the transwell plate. Differentiated Caco-2 monolayers grown on transwell plates are a well- established method for studying drug transport *in vitro* (Artursson & Karlsson, 1991). More recent studies investigating lipophilic drug transport in the Caco-2 cell model use transwell membranes having pores with a 0·4 μm diameter in order to avoid cell migration from the apical to the basolateral side (Seeballuck *et al.* 2003, 2004). In the present study, 0·4 μm pore size membranes were also employed on the transwell plates and this contrasts with the study by During *et al.* (2002)where a 3 μm pore size membrane was employed. Our data agree with the work of Edwards *et al.* (2002) who, in a human study, reported an absorption value of about 1·2–3·5 % for α-carotene after ingestion of a known concentration of this carotenoid from a carrot puree. Using a similar Caco-2 cell model, Ferruzzi *et al.* (2006) examined the uptake of β-carotene from a test meal

following *in vitro* digestion and reported that the micelles generated contained 22 139 pmol β-carotene of which 916 pmol (about 4 %) was taken up by the cells. Our results support this observation as we found the percentage of β-carotene remaining in cells ranged from about 3·7 to 5·2 % after the 16 h incubation period. These data also compare well with the results of the human study reported by Edwards *et al.* (2002) where between 0·9 and 2·4 % of a known ingested amount of β-carotene was absorbed from a test meal determined using an extrinsic reference method. A human study conducted by O'Neill & Thurnham (1998) investigated the intestinal absorption of β-carotene, lycopene and lutein and concluded that the absorption of β-carotene is a saturable process as only small amounts (about 3·5 %) of a 40 mg dose were detected in the TAG-rich lipoprotein fraction. Data from the present study are also in agreement with isotopic tracer studies conducted by Edwards *et al.* (2001) and Lin *et al.* (2000) who reported bioavailability values for β-carotene ranging from 3 to 16 % from a test meal and pure β-carotene sources, respectively. In a human study using intrinsically labelled tomatoes, Tang *et al.* (2005) examined the bioavailability of lycopene and reported that approximately 1·2 % of the administered dose was absorbed. This is similar to the results we obtained for the percentage of lycopene that was secreted into the basolateral chamber which ranged from 0·9 (SEM 0·2) to 2·6 (SEM 0·9) %. These data also agree with the work of O'Neill & Thurnham (1998) who found that about 1 mg lycopene was absorbed from a 38 mg dose administered in capsules (approximately 2·6 %) and that there was no significant difference between the absorption of β-carotene and lycopene.

Uptake and transport of the xanthophyll carotenoids lutein and zeaxanthin

We examined the cellular uptake and secretion of lutein and zeaxanthin to compare two structurally similar carotenoids. It is important to note that we looked at these carotenoids independently of each other and therefore we were not observing preferential uptake of one carotenoid over another as a number of studies have reported (Böhm & Bitsch, 1999; Garrett *et al.* 1999; Kostic *et al.* 1995; van Hof *et al.* 1999; Tyssandier *et al.* 2002). Findings from the present study are supported by the work of Chitchumroonchokchai *et al.* (2004) who found that about 6·3 % of lutein that was retained by cells was transported to the basolateral chamber after the addition of lutein containing micelles generated from *in vitro* digestion of spinach.

To date, little work has been conducted on the absorption and transport of zeaxanthin through Caco-2 cell monolayers. Chitchumroonchokchai & Failla (2006) investigated the hydrolysis of zeaxanthin esters during an *in vitro* digestion procedure and found that about 4·5 % of zeaxanthin that was taken up by cells was transported to the basolateral chamber. This was slightly lower than results obtained from this study.

Uptake and transport of the xanthophyll carotenoids β-cryptoxanthin and astaxanthin

Coral-Hinostroza *et al.* (2004) conducted a study to investigate the appearance of astaxanthin isomers in men given a mixed dose of astaxanthin diester isomers. They reported that about 6 % of the administered dose appeared in plasma after supplementation with 100 mg carotenoid. This compares well with our data, which reports a 6·7 (SEM 0·5) % transfer of astaxanthin to the basolateral chamber. When the transfer of astaxanthin was compared to β-cryptoxanthin, it was significantly higher at all amounts studied (Fig. 1). Overall, astaxanthin had the highest percentage total uptake and secretion, which ranged from 13·8 (SEM 3·9) to 26·9 (SEM 2·4) % (Fig. 2).

In conclusion, we demonstrate that the Caco-2 cell model is a useful tool to study the absorption and transport of carotenoids through a cell monolayer representative of the intestinal epithelia *in vivo*. Statistical analysis of data concludes that the xanthophyll carotenoids astaxanthin and lutein were transported more efficiently than the carotene carotenoids and that overall, astaxanthin was the most efficiently absorbed. This is the first study to compare a range of carotene and xanthophyll carotenoids using the Caco-2 *in vitro* model. Our results correlate with *in vivo* data (Kostic *et al.* 1995; Gartner *et al.* 1996; van Hof *et al.* 1999) that have also shown higher percentage absorption of xanthophyll carotenoids compared to carotenes thus providing validation of this model as an approach to assess carotenoid availability. Additionally, our findings indicate that lower amounts of carotenoids were absorbed and transferred more efficiently than higher amounts suggesting a saturation effect. Our study adds to the increasing body of literature demonstrating the usefulness of the Caco-2 *in vitro* model for obtaining information about the intestinal absorption of carotenoids. In the future we will couple the Caco-2 cell model with an *in vitro* digestion procedure to look at the availability of carotenoids from foods and supplements.

Acknowledgements

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