3. Metabolism, Kinetics and Genetic Variation

The metabolism of carotenoids has been studied since the early 1920s, when certain yellow plant pigments were found to stimulate the growth of vitamin A-deficient rats. Later in that decade, Thomas Moore showed that β-carotene, the most active of these plant pigments, was converted to vitamin A in rats *in vivo*. Until recently, carotenoids were considered in mammalian physiology only in terms of their provitamin A activity. The bioavailability and metabolism of carotenoids are significantly affected by dietary, physiological and matrix-

associated factors (Erdman *et al.*, 1993; Olson, 1994; Wang, 1994; de Pee & West, 1996; Parker, 1996, 1997), including consumption of dietary fat together with the carotenoids, release of the bile acids necessary to form lipid micelles, the amount of carotenoids present in the diet, the dietary matrix and crystalline structure of the carotenoid in the matrix, processing and cooking, nutritional status (vitamin A and carotenoid concentrations), individual host-related factors such as disease states and genetic factors.

Most of the early studies of carotenoid metabolism focused on β-carotene. More recently, other carotenoids, such as lutein, canthaxanthin, lycopene, 4,4′-dimethoxy-β-carotene, ethyl β-apo-8′-carotenoate, and β-apo-8′-carotenal, have been studied both in humans and in experimental animals. The metabolism of carotenoids has been reviewed extensively (Erdman *et al.*, 1993; Olson, 1994; Wang, 1994; de Pee & West, 1996; Parker, 1996; Furr & Clark, 1997; Parker, 1997). Although there is little information on carotenoids other than β-carotene, it is clear that the absorption and metabolism of different carotenoids varies markedly. They may also mutually influence their bioavailability, as has been demonstrated for β-carotene, lutein and zeaxanthin (High & Day, 1951; Micozzi *et al.*, 1992; Kostic *et al.*, 1995; Gärtner *et al.*, 1996).

3.1 Humans

3.1.1 Intestinal digestion and absorption

Dietary carotenoids in foods exist in two major forms: as true solutions in oil, as in red palm oil, or as parts of the matrices of vegetables and fruit. The matrix is usually complex, consisting of fibre, digestible polysaccharides and proteins. Because carotenoids are often not fully disrupted during food preparation or during their passage through the intestine, their bioavailability can vary from < 10%, such as in largely intact raw carrots, to > 50% in oily solutions or in synthetic, gelatin-based, commercial preparations (Olson, 1994; Parker, 1996; Furr & Clark, 1997). Methods for evaluating the extent of absorption have been reviewed (Bowen *et al.*, 1993; Parker, 1997). Hydrocarbon carotenoids, like β-carotene and lycopene, are solubilized in the lipid core of micelles in the gut lumen or alternatively form small clathrate complexes with conjugated bile acids, of which deoxycholate and cholate are the most effective (Olson, 1994). Xanthophyll esters must be hydrolysed before absorption. As in membranes, xanthophylls and carotenes associate differently with micelles (Furr & Clark, 1997): hydrocarbon carotenoids tend to associate with the lipophilic core of membranes and micelles. The absorption process does not seem to involve special epithelial transporters.

The factors that lower the bioavailability of carotenoids, apart from incomplete release from food matrices, include the presence of fibre, particularly pectins, in the diet; lack of fat in the diet; the presence of undigested lipids, including fat substitutes; inadequate bile flow; various clinical conditions involving lipid malabsorption and reduced gastric acidity (Olson, 1994; Parker, 1996; Furr & Clark, 1997). The absorption and conversion efficiency of carotenoids in humans and animals decreases as the amount ingested increases (Brubacher & Weiser, 1985; Furr & Clark, 1997).

Carotenoids appear to be absorbed by duodenal mucosal cells by a mechanism involving passive diffusion, similar to that for cholesterol and the products of triglyceride lipolysis (Parker, 1996). One of the earliest human experiments to investigate the events that occur during intestinal absorption of β-carotene was reported in 1966 (Goodman *et al.*, 1966a). Tritiated β-carotene (plus 47 or 91 μg unlabelled β-carotene) dissolved in 2 ml olive oil and emulsified in 50 ml skimmed milk was fed to two patients in whom polyethylene cannulae had been inserted in the thoracic duct in the neck. Radiolabel was absorbed into the lymph 3–10 h after feeding; about 20% of the label was recovered, of which 70–80% was in the chylomicrons, and 60–70% was present as vitamin A esters, indicating that the human intestine has an extremely limited ability to absorb unchanged dietary β-carotene into the lymph.

When carotenoids are present in substantial amounts, they can interfere with each other's absorption. The action is not mutually competitive, however, since β-carotene inhibits canthaxanthin and lutein absorption, whereas the

latter have lesser or no effects on β-carotene absorption (White *et al.*, 1994; Kostic *et al.*, 1995). The mechanism of this interaction has not been defined. Vitamin E and carotenoids also interact, since vitamin E supplements tend to lower plasma carotenoid concentrations, although small amounts of vitamin E may prevent carotenoid oxidation in the gastrointestinal tract. β-Carotene supplements have been reported to decrease, to increase or not to affect plasma tocopherol concentrations (Furr & Clark, 1997).

The results of several large trials of the effects of supplemental β-carotene on the serum concentrations of other carotenoids are now becoming available. Several investigators have reported statistically significant increases in α-carotene concentrations with β-carotene supplementation (Omenn *et al.*, 1993a; Wahlqvist *et al.*, 1994; Albanes *et al.*, 1997; Mayne *et al.*, 1997). [The Working Group noted that small amounts of α-carotene may be present in β-carotene preparations; furthermore, the increases seen could be due to di-*cis* isomers of β-carotene, which are difficult to separate analytically from α-carotene, or they could involve a sparing effect of β-carotene on α-carotene.] The results for the effects of supplemental β-carotene on other carotenoids are less consistent, but there do not appear to be reductions in the concentrations of any of the other major circulating carotenoids.

In a nutritional context, the large differences in the bioavailability of carotenoids make it difficult to define their equivalence as precursors of vitamin A. The molar equivalence between retinol and small amounts of β-carotene in oil is approximately 0.5, whereas that with carotenoids in rapidly stir-fried vegetables is very poor (< 0.05). Carotenoids in fruits seem to be used more efficiently. The results of studies of bioavailability, which are often inconclusive and conflicting, have been reviewed (de Pee & West, 1996).

The absorption of carotenoids and their presence in plasma are influenced by their geometrical isomeric form. The all-*trans* isomer of β-carotene is well absorbed and appears in plasma, whereas the 9-*cis* isomer, although fairly well absorbed, is found in plasma only at low concentrations (Gaziano *et al.*, 1995a). In contrast, the *cis* isomers of lycopene seem to be better absorbed into plasma than the all-*trans* form (Stahl & Sies, 1992). Thus, the 'isomer effect' depends on the carotenoid being studied and the species being used (Olson, 1994; Parker, 1996; Furr & Clark, 1997).

In general, polar carotenoids are absorbed better by humans than non-polar ones. Thus, lutein is absorbed about twice as well as β-carotene (Kostic *et al.*, 1995), and β-apocarotenals and β-apocarotenols seem to be absorbed better than less polar carotenoids (Zeng *et al.*, 1992). The proportions of lutein and zeaxanthin are greater in the chylomicra than in the ingested carotenoid mixture (Furr & Clark, 1997). These conclusions are based on the assumption that the metabolism of carotenoids in the intestinal mucosa represents only a minor part of their transfer into plasma and that their relative rates of clearance from plasma are similar. These assumptions may not hold for all-*trans*-β-carotene or for other provitamin A carotenoids.

When a moderate to large dose of β-carotene is administered orally to humans, most subjects respond by a marked increase in the β-carotene concentration in plasma, which peaks at about 6 h, decreases and then rises to a higher concentration with a second peak at about 24 h (Furr & Clark, 1997). Some subjects, however, show little or no increase in the plasma concentration of β-carotene after a single dose (Furr & Clark, 1997), and they have been called 'poor' or 'non-responders' (Bowen *et al.*, 1993). Of the various explanations that might be given for the lack of response, the most likely is that 'non-responders' are in fact rapid converters of β-carotene into vitamin A. This explanation does not hold for lycopene, however, as the responses of individuals differ (Stahl & Sies, 1996). Different individual responses to lutein were also observed in one small study (O'Neill & Thurnham, 1998), but not in another (Kostic *et al.*, 1995).

3.1.2 Transport in plasma

Newly absorbed carotenoids, retinyl ester and small amounts of retinol are transported on chylomicra from the intestinal mucosa via the

lymph into the general circulation. Lipoprotein lipase hydrolyses much of the triglyceride in the chylomicron, resulting in a chylomicron remnant (Furr & Clark, 1997). The latter, which retains apolipoproteins B48 and E on its surface, interacts with receptors on hepatocytes and is taken up by those cells. Small amounts of chylomicron remnants may also be taken up by other tissues. The hepatocytes then incorporate much of the dietary carotenoids into lipoproteins, primarily very-low-density and low-density lipoproteins, whereas xanthophylls are distributed more or less equally between high-density and low-density lipoproteins (Olson, 1994; Furr & Clark, 1997). This distribution accords with the hydrophobicity of the carotenoids and of the lipoproteins. Specific mechanisms of incorporation, such as the α-tocopherol-transport protein of liver, have not been identified for carotenoids. A β-carotene-binding protein has recently been characterized in ferret liver (Rao *et al.*, 1997). If a similar protein exists in human liver, it may play some role in the incorporation of β-carotene into lipoproteins. High-density lipo-proteins may arise both from de-novo synthesis in the liver and from the pinching off of excess surface components from chylomicra in plasma during triglyceride hydrolysis; however, xanthophylls are probably incorporated primarily into high-density lipoproteins in the liver (Olson, 1998).

In plasma, very low-density lipoproteins are rapidly converted by lipoprotein lipase to low-density lipoproteins, which retain the carotenoids and apolipoprotein B100. Receptors for the latter are present on cells of many peripheral tissues and particularly those of the adrenal gland and testes as well as of the liver. High-density lipoproteins pick up cholesterol and possibly xanthophylls from peripheral tissues and apolipoprotein E from other plasma lipoproteins before being taken up by the liver. Except as noted above, carotenoids do not seem to be transferred from one lipid class to another, at least *in vitro* (Furr & Clark, 1997). Thus, carotenoids are involved in a complex and probably cyclical metabolic pathway involving the intestine, chylomicra, the liver, plasma lipoproteins and peripheral tissues.

3.1.3 Serum carotenoid concentrations

Carotenoids are commonly found in the plasma of fasting subjects. Of the 20 or more carotenoids present (Khachik *et al.*, 1995), the major six, which comprise 60–70% of the total (Barua *et al.*, 1993), are lycopene, lutein, zeaxanthin, β-cryptoxanthin, β-carotene and α-carotene. Because carotenoids are not covalently bound to lipoproteins and apparently are not homeostatically controlled, their concentrations in plasma are highly dependent on the diet. In a more physiological context, their steady-state plasma concentrations depend on the amounts in the diet, their efficiency of intestinal absorption, their uptake by tissues, their release from tissues back into the plasma and their catabolic rates. Plasma carotenoids represent < 10% of the total body pool (40–150 mg; Bendich & Olson, 1989), of which only 10–30% is β-carotene (Thurnham & Flora, 1988; Bendich & Olson, 1989). Because the distribution of carotenoids in a typical population is skewed, median concentrations are generally used in analyses.

The median values for the plasma concentrations of the six main carotenoids in a British and in an American population are summarized in Table 4. The reference ranges are broad since they include individuals who ingest various amounts of carotenoids. Lutein and zeaxanthin, which tend to run closely together on HPLC traces, are grouped; in general, the ratio of lutein to zeaxanthin in the plasma is 4 or 5:1 (Peng *et al.*, 1995). Although the distribution and amounts of carotenoids in individuals differ markedly, each person maintains a fairly constant pattern for up to one year (Cantilena *et al.*, 1992; Peng *et al.*, 1995), probably reflecting a fairly uniform diet during that period, abetted by the presumed buffering effect of tissue carotenoid concentrations. Depending on the variety of dietary sources of carotenoids, there can, however, be marked seasonal changes in serum concentrations (Rautalahti *et al.*, 1993). Carotenoid concentrations measured in the morning were 6–10% higher than those in the evening (Cantilena *et al.*, 1992).

Median carotenoid concentrations also vary with age, but not in the same way for all carotenoids (Briefel *et al.*, 1996). The plasma

concentrations of most carotenoids except lycopene tend to increase with age (Thurnham & Flora, 1988; Gregory *et al.*, 1990). In the United States, lycopene is generally found at the highest concentrations in plasma, followed by lutein and zeaxanthin, β-carotene, β-cryptoxanthin and α-carotene, in that order (Briefel *et al.*, 1996). The pattern differs with region, depending on dietary intake. People who ingested canthaxanthin as a tanning agent had canthaxanthin in their plasma (Gunson *et al.*, 1984). Women have higher plasma concentrations of β-carotene, α-carotene and β-cryptoxanthin than men (Stacewicz-Sapuntzakis *et al.*, 1986; Ito *et al.*, 1987; Stryker *et al.*, 1988; Thurnham & Flora, 1988; Thurnham *et al.*, 1988a; Heseker *et al.*, 1991; Olmedilla *et al.*, 1994), while the concentrations of lutein and lycopene tend not to differ between the sexes (Ito *et al.*, 1987; Thurnham & Flora, 1988; Olmedilla *et al.*, 1994). It has been reported that the carotenoid concentrations in women are at their lowest during the menses and that of β-carotene peaks in the late follicular phase, the other carotenoids following the same trend (Forman *et al.*, 1996); however, other workers have been unable to detect any significant changes associated with menstrual periodicity (Tangney *et al.*, 1991; Rock *et al.*, 1995).

Body mass index and serum glutamyl transferase activity were inversely correlated with both the baseline plasma concentrations of β-carotene and those after supplementation with β-carotene (Albanes *et al.*, 1991), while the serum concentration of cholesterol was positively correlated but only after supplementation. Dietary interventions, including a high intake of vegetables and a low intake of fat, increased the serum concentrations of β-carotene, α-carotene, lycopene and lutein but not that of β-cryptoxanthin (Rock *et al.*, 1997), and the correlations were statistically significant in the case of β- and α-carotene and lutein. In people fed a diet containing few, if any carotenoids, the plasma concentration decreases in approximately a first-order manner for 14–30 days and then tends to reach slowly declining plateau values (Rock *et al.*, 1992).

Table 4. Median concentrations (μmol/L) of serum carotenoids in adults in Great Britain and the United States

Carotenoid	Men	Women	Reference range (5th–95th percentile)
Great Britain[a]			
Lycopene	0.25	0.25	0.06–0.68
Lutein and zeaxanthin	0.29	0.29	NR
β-Cryptoxanthin	0.13	0.16	0.03–0.51
β-Carotene	0.24	0.32	0.07–0.84
α-Carotene	0.06	0.07	0.02–0.21
United States[b]			
Lycopene	0.47	0.41	0.13–0.82
Lutein and zeaxanthin	0.35	0.35	0.16–0.72
β-Cryptoxanthin	0.13	0.13	0.05–0.38
β-Carotene	0.22	0.28[c]	0.09–0.91
α-Carotene	0.065	0.081[c]	0.02–0.22

NR, not reported

[a] From Gregory *et al.* (1990)

[b] Based on data from Briefel *et al.* (1996)

[c] Extrapolated from values for nonsmoking persons

3.1.3.1 Effects of β-carotene supplements

When a single, fairly large supplement (15–30 mg, 28–56 μmol) of all-*trans*-β-carotene is administered orally to adults, the concentrations of β-carotene in plasma increase to a peak (1.5–3-fold higher than baseline) at about 24–30 h and then decline, with a half-life of seven to nine days (Brown *et al.*, 1989). [The Working Group noted that the serum concentrations achieved depend on the composition of the formulation used as a supplement.] The β-carotene concentration returns to a baseline level after about 18 days (Kostic *et al.*, 1995). When large daily supplements of β-carotene (30–300 mg, 56–560 μmol) are given orally, the plasma concentrations increase in a first-order fashion to reach a plateau, usually 7–20-fold higher than baseline, at about 25 days (Meyer *et al.*, 1985; Dimitrov & Ullrey, 1990; Prince *et al.*, 1991; Albanes *et al.*, 1992; Micozzi *et al.*, 1992; Wahlqvist *et al.*, 1994; Manetta *et al.*, 1996; Albanes *et al.*, 1997). The plateau was proportional to the dose given, but interindividual differences in plateau levels have been observed after repeated administration of β-carotene

(Albanes *et al.*, 1992). As already indicated, the bioavailability of β-carotene in oil or in commercial beadlets is far greater than that of β-carotene in vegetables (Micozzi *et al.*, 1992), and ingestion of fat with β-carotene optimizes its absorption. The distribution of carotenoids in the plasma lipoproteins of hypercarotenaemic persons was similar to that in untreated subjects (Mathews-Roth & Gulbrandsen, 1974). Elderly persons may either absorb β-carotene better or clear it more slowly from the plasma than young subjects (Maiani *et al.*, 1989). The concentration of β-carotene in tissues is generally proportional to that in plasma (Dimitrov & Ullrey, 1990; Peng *et al.*, 1995; Manetta *et al.*, 1996).

Supplements of β-carotene might affect the concentrations of other fat-soluble components of the plasma. β-Carotene supplements have little or no effect on plasma retinol concentrations in well-nourished individuals (Albanes *et al.*, 1997), primarily because the plasma retinol concentration is homeostatically controlled. Particular attention has been paid to serum α-tocophenol since the report of Xu *et al.* (1992) that daily doses of β-carotene (15–60 mg per day) for six to nine months lowered α-tocopherol concentrations by 40% in a group of 45 subjects. A similar effect was found in a study of 58 subjects (Mobarhan *et al.*, 1994); however, much larger studies with longer durations of supplementation have shown no effect of β-carotene supplements on serum α-tocophenol concentrations (Albanes *et al.*, 1992; Goodman *et al.*, 1994; Nierenberg *et al.*, 1994; Fontham *et al.*, 1995; Albanes *et al.*, 1997; Mayne *et al.*, 1997).

3.1.3.2 Effects of tobacco smoking

Tobacco smoking has been associated with lowered concentrations of several serum carotenoids, increased turnover of ascorbic acid and a lesser effect on α-tocopherol concentrations. Decreased serum β-carotene concentrations have been reported to be associated with the number of cigarettes smoked per day, the years of smoking and whether the person is a current or an ex-smoker (Stryker *et al.*, 1988; Pamuk *et al.*, 1994; Goodman *et al.*, 1996; Albanes *et al.*, 1997). The concentrations of α-carotene, β-carotene and cryptoxanthin were lowered by about 30% in heavy smokers (> 10 cigarettes per day), whereas that of lutein/zeaxanthin was less affected, i.e. by 10% (Pamuk *et al.*, 1994; Albanes *et al.*, 1997); the concentrations of lycopene were not affected (Stryker *et al.*, 1988; Thurnham, 1994). Former smokers showed no differences from non-smokers in carotenoid concentrations. Serum retinol concentrations were affected little if at all.

The lowered serum carotenoid concentrations have been attributed to the presence of β-unsaturated aldehydes and of a large number of free radicals, estimated as 10^{15} per puff, in cigarette smoke (Eiserich *et al.*, 1995; Handelman *et al.*, 1996). Protein–carbonyl adduct formation exceeded lipid peroxide formation *in vitro*. The order of disappearance of carotenoids in plasma exposed to gas-phase cigarette smoke *in vitro* was lycopene > β-carotene > lutein/zeaxanthin = cryptoxanthin > retinol (Handelman *et al.*, 1996). α-Tocopherol was affected more *in vitro* than *in vivo*.

3.1.3.3 Effects of alcohol drinking

In the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) study (see section 4.1.1.1), consumption of > 12.9 g alcohol per day was associated with a 10–38% decrease in serum carotenoid concentrations, particularly those of α-carotene, β-carotene and β-cryptoxanthin, in both people receiving and not receiving supplements (Albanes *et al.*, 1997). In the Beta-Carotene and Retinol Efficacy Trial (CARET), alcohol consumption was negatively correlated with serum β-carotene concentrations ($r = -0.14$; $p < 0.05$) and positively associated with serum retinol concentrations ($r = 0.10$; $p < 0.05$; Goodman *et al.*, 1996). In a smaller study in Boston, USA, consumption of > 20 g alcohol per day lowered the serum β-carotene concentrations by 24% in men and 11% in women (Stryker *et al.*, 1988). In 98 volunteers in southern France, a negative correlation was reported between the plasma concentration of β-carotene and alcohol intake ($r = -0.35$; $p < 0.001$) (Saintot *et al.*, 1995). No consistent relationship was seen between alcohol intake and plasma β-carotene concentrations in a small group of alcoholics. Supplementation with 30–60 mg/d β-carotene was reported to increase the plasma concentrations, but the

people with liver cirrhosis had a lower response than those without (Ahmed *et al.*, 1994). Thus, alcohol ingestion has generally been associated with decreased serum carotenoid concentrations.

3.1.3.4 Effects of other modulators

Other factors affect serum carotenoid concentrations, primarily by inhibiting the intestinal absorption of carotenoids. Thus, acidic fibres, like pectins, lower carotenoid absorption in humans (Rock & Swendseid, 1992). The cholesterol-lowering drug, cholestyramine, which binds bile acids, has a similar effect, either by reducing the bile-acid concentration in the lumen or by directly binding carotenoids (Morris *et al.*, 1994). The fat substitute Olestra®, which is not absorbed, reduces serum concentrations of carotenoids, presumably by sequestering the compounds in the lipophilic phase (Peters *et al.*, 1997).

3.1.4 Tissue carotenoid content

The tissue concentrations of carotenoids reflect their intake in the diet and/or supplements.

3.1.4.1 Effects of dietary intake

Carotenoids are found in all tissues of the body (Blankenhorn, 1957; Stich *et al.*, 1986; Parker, 1988; Dimitrov & Ullrey, 1990; Kaplan *et al.*, 1990; Schmitz *et al.*, 1991; Stahl *et al.*, 1992; Mobarhan *et al.*, 1994; Johnson *et al.*, 1995; Kohlmeier & Kohlmeier, 1995; Peng *et al.*, 1995; Clinton *et al.*, 1996; Nair *et al.*, 1996; Redlich *et al.*, 1996; Virtanen *et al.*, 1996; Sanderson *et al.*, 1997; Table 5). On the basis of the relative weights of tissues in the adult human body (Long, 1961), carotenoids clearly are mainly present in fat, liver, skin and plasma. Some relatively small tissues, such as testes and adrenal glands, and parts of some tissues like the corpus luteum (112 nmol/g) have very high concentrations of carotenoids, whereas some major organs like muscle and brain have very low concentrations. The only major human tissues not cited in Table 5 are those of the skeleton and gastrointestinal tract. The former does not seem to have been studied in this regard, and the latter, which deteriorates rapidly after death, has not been examined in human autopsy specimens.

The total amount of carotenoid in any individual depends of course largely on intake. Thus, the ranges of specific carotenoids in tissues are very broad, and the mean concentrations in fat and liver vary widely in different studies. Some information is available about cells isolated from the gastrointestinal system. Intact buccal mucosal cells have been reported to contain 0.016 nmol of total carotenoids per 10^6 cells (Peng *et al.*, 1995) and exfoliated buccal cells about half as much (Stich *et al.*, 1986). A factor of 40 can be used to convert 10^6 cells to grams wet weight of tissue. Thus, the buccal mucosa contained 0.64 nmol/g, similar to the amount found in other internal organs. Stomach epithelial cells contained 0.5 nmol/g (Sanderson *et al.*, 1997), and colonic and rectal epithelial cells obtained at biopsy contained about 0.060 and 0.040 nmol β-carotene per g (Mobarhan *et al.*, 1994; Maiani *et al.*, 1995). If the ratio of total carotenoids to β-carotene is assumed to be 5, the total carotenoid concentrations in these cells would be 0.30 and 0.20 nmol/g, respectively. Exfoliated colonic cells contained much smaller concentrations of carotenoids, indicating that they are probably incorporated into crypt cells and then are slowly lost during the cell's migration up the villus (Nair *et al.*, 1996). This conclusion is supported by the finding that ingestion of carotenoid-rich vegetables for five to seven days maximizes the carotenoid concentration in exfoliated colonic cells, and this period concords with the migration time in villi. The concentration of total carotenoids in cells obtained by bronchoalveolar lavage (0.52 nmol/g) was similar to that in lung tissue (0.63 nmol/g; Redlich *et al.*, 1996). It has also been shown that β-carotene is secreted into bile at concentrations that reflect those in plasma (Leo *et al.*, 1995).

The total carotenoid concentrations in adipose tissue in various studies were 9.2 (Johnson *et al.*, 1995), 5.7 (Parker, 1988) and 4.2 nmol/g (Virtanen *et al.*, 1996). Because adipose tissue is the main site of storage for carotenoids, biopsies of this tissue are recommended for determining long-term carotenoid status (Kohlmeier & Kohlmeier, 1995). The carotenoid concentration in adipose tissue is higher, however, in women than in men and is inversely associated

Table 5. Estimated carotenoid contents of selected human tissues

Organ	Mean concentration[a] (nmol/g)	Approximate percent of body weight[b]	Mean total amount (μmol)[c]	Percent of total amount[d]
Fat	3.3 (15.6)[e] (0.8)[f]	18.8	42.2	65.39
Liver	5.0 (14.1)[e] (5.1)[f] (21.0)[g]	2.3	7.8	12.09
Muscle	ND[h] (0.07)[i]	42.8	2.04	3.16
Adrenal	33.7 (9.4)[f]	0.02	0.46	0.71
Plasma	ND[h] (1.1)[f] (1.6)[j]	4.9	4.5	6.97
Pancreas	3.7	0.16	0.40	0.62
Spleen	0.96 (5.9)[e]	0.25	0.16	0.25
Kidney	0.98 (1.2)[e] (0.9)f (3.1)[g]	0.41	0.27	0.42
Heart	0.81 (0.84)[e]	0.42	0.23	0.36
Testes	26.3 (7.6)[e]	0.04	0.72	1.12
Lung	ND[h] (1.9)[g]	0.73	0.94	1.46
Thyroid	0.79	0.04	0.021	0.03
Ovary	2.6 (0.9)[f]	0.01	0.018	0.03
Prostate	ND[h] (1.3)[k]	0.024	0.021	0.03
Skin	ND[h] (0.98)[j]	7.0	4.7	7.28
Brain	ND[h] (< 0.04)[f]	2.0	0.054	< 0.08
Total	–	79.9	64.54	100

[a] Mean concentrations, unless otherwise noted, from Kaplan *et al.* (1990). The average molecular mass of mixed carotenoids is assumed to be 543; thus, 1 μg = 1.84 nmol.

[b] From Long (1961); main unlisted tissues are stomach and intestine (7–10%) and skeleton (12–15%)

[c] Based on a reference body weight of 68 kg

[d] Based primarily on Kaplan *et al.* (1990)

[e] From Blankenhorn (1957)

[f] From Stahl *et al.* (1992); plasma concentration in nmol/ml

[g] From Schmitz *et al.* (1991)

[h] Not determined by Kaplan *et al.* (1990)

[i] From Dimitrov & Ullrey (1990)

[j] From Peng *et al.* (1995)

[k] From Clinton *et al.* (1996); lycopene and β-carotene only

with body size and composition, including the body mass index, and with smoking and alcohol consumption. The associations were stronger with some carotenoids than with others, and carotenoid concentrations were little affected by age (mean age, 53 ± 9 years for men and 62 ± 6 years for women); however, a negative correlation with lycopene was seen in men (Virtanen *et al.*, 1996). Negative correlations between plasma lycopene and age were also seen in adults in Great Britain and the USA (Gregory *et al.*, 1990; Brady *et al.*, 1996), but in a study on 46 women with breast cancer and 63 controls, the concentrations of β-carotene, lycopene and lutein/zeaxanthin in breast adipose tissue were not correlated with the respective estimated intakes (Zhang *et al.*, 1997). More than 20 structurally different carotenoids have been detected in the breast milk of lactating women (Khachik *et al.*, 1997), showing that human milk is an important source of carotenoids and vitamin A for infants (Thurnham *et al.*, 1997).

Since ≥ 90% of the carotenoids in the body are found in tissues and < 10% in plasma, the contents of various tissues are of interest.

Lycopene and β-carotene occurred at the highest concentrations in nearly all of the tissues listed in Table 5, lutein/zeaxanthin at intermediate concentrations and cryptoxanthin and α-carotene at lower levels. The pattern of carotenoid concentrations in tissues usually reflects their distribution in plasma. Some exceptions are the preferential accumulation of β-carotene in the pineal gland (Shi *et al.*, 1991) and in the corpus luteum (Moore, 1957) and of lutein/zeaxanthin in the macula of the eye (Handelman *et al.*, 1988).

The relationship between the concentrations of carotenoids in tissues and in plasma is crucial. A significant relationship has been found for both total and specific carotenoids in most studies (e.g. Peng *et al.*, 1995), but a poorer correlation is found between tissue concentrations and dietary intake of carotenoids, possibly because of uncertainties in estimating dietary intakes accurately (Peng *et al.*, 1995). β-Carotene concentrations in the cord blood of fetuses increase from about 0.0019 μmol/L at 20 weeks of gestation to 0.056 μmol/L at 40 weeks. The concentrations in the blood of neonates were lower in mothers who smoked (Moji *et al.*, 1995). The relationship between the concentrations of retinol and β-carotene in maternal serum, cord serum and placentae was assessed at parturition in women whose vitamin A status was adequate (n = 15; serum retinol, > 0.7 μmol/L) and inadequate (n = 16; serum retinol, < 0.7 μmol/L). The concentrations of β-carotene in maternal serum (0.22 mmol/L), cord blood (0.019 μmol/L) and placenta (0.0075 mmol/g) were unaffected by maternal vitamin A status (Dimenstein *et al.*, 1996). A cross-sectional study of 30 women showed that the serum cholesterol concentration was increased during the early follicular phase of the menstrual cycle and that of α-carotene was increased in the mid-luteal phase, but only if uncorrected for total cholesterol. The concentrations of lutein, β-cryptoxanthin, lycopene and β-carotene did not change during the cycle (Rock *et al.*, 1995).

As the concentrations of total and specific carotenoids in plasma and tissues are highly skewed towards lower concentrations (e.g. Stich *et al.*, 1986; Virtanen *et al.*, 1996), either non-parametric methods or log transformation of the data before parametric analysis should be used in statistical analyses.

3.1.4.2 Effect of supplemental intake

Thirteen healthy volunteers were given a daily dose of 30 mg synthetic β-carotene [formulation unspecified] and 7.4 mg of lycopene as vegetable juice for 11 days. The buccal mucosal concentration of β-carotene was increased, but not those of lycopene, lutein, cryptoxanthin or α-carotene (Peng *et al.*, 1994).

The carotenoid concentrations in human lymphocytes were 4.5-fold lower in lung cancer patients (n = 19) than in healthy controls (n = 23), while the serum concentrations were nonsignificantly lower (Bakker Schut *et al.*, 1997). Supplementation with 100 mg/d of crystalline β-carotene in capsules (25 mg, Merck) for 17 days levelled the difference in serum concentrations between one control and two lung cancer patients, but the concentrations of carotenoids in the lymphocytes of cancer patients remained lower. Supplementation with β-carotene (30 mg/d; formulation unspecified) for six weeks markedly increased the concentration in cervicovaginal cells of 24 healthy women (Palan *et al.*, 1992). Patients with cervical intraepithelial neoplasia (n = 30) who received a similar dose for six months also had a significant increase in the β-carotene concentrations of vaginal mucosa over the baseline level (Manetta *et al.*, 1996).

3.1.5 Kinetics

The kinetics of an orally administered dose (73 μmol) of octadeuterated β-carotene *in vivo* has been carefully analysed, although in only one male adult (Novotny *et al.*, 1995, 1996). A model consisting of 11 compartments and a gastrointestinal delay parameter of 4.5 h was devised on the basis of measurements of octadeuterated β-carotene and tetradeuterated retinol in plasma at various times from 0 to 57 days, although measurements were made up to 113 days. A set of fractional transfer coefficients was defined on the basis of several feasible assumptions, with SAAM 31 software (Novotny *et al.*, 1995, 1996). The main features of the model were: slowly and rapidly turning over

pools of β-carotene and of retinol in the liver; enterocytic and extrahepatic tissue pools of carotene; pools of β-carotene and retinyl ester in plasma chylomicra; a pool of β-carotene in plasma lipoproteins and a pool of retinol in holo-retinol-binding protein in plasma. The model predicts that 22% of the β-carotene dose will be absorbed, that the hepatic reserves of β-carotene and vitamin A are 7.5 μmol and 324 μmol, respectively, and that 57% of the conversion of β-carotene into vitamin A takes place in the liver and 43% in the intestinal mucosa. With average dietary intakes of 3–7 μmol/d β-carotene, however, the intestine may well play a larger role in the conversion process.

The mean sojourn, or residence, time is defined as the mean time that tracer molecules spend in the system from the moment of entry until the time of irreversible exit. The values were 51 days for β-carotene and 474 days for retinol (vitamin A), which agree well with estimates based on other data. The empirical mean sojourn times in the plasma were only 9–13 days for β-carotene and 26 days for retinol (Novotny *et al.*, 1995, 1996). These differences in mean sojourn time values may well reflect efficient recycling of retinol, and probably of carotenoids as well, in and out of tissue depots. The mean empirical sojourn times for other carotenoids in plasma are similar to that of β-carotene: dimethoxy-β-carotene, six days; ethyl-β-apo-8′-carotenoate, nine days and canthaxanthin, eight days (Zeng *et al.*, 1992; Furr & Clark, 1997). Experimentally, the values for carotenoids are little affected by the dose given (Furr & Clark, 1997).

3.1.6 Metabolism

Carotenoids are oxidized in plants and microorganisms to a variety of compounds with fewer carbon atoms, including β-apocarotenals, abscissic acid, trisporic acid, bixin and crocetin, some of which have important physiological functions in plants (Olson, 1993). Thus, nature can modify carotenoids in a variety of ways at almost every carbon atom in the molecule.

3.1.6.1 β-Carotene

(a) Physiological pathways

β-Carotene was first shown to be converted biologically into vitamin A in mammals in 1930 (Moore, 1957). On the basis of the symmetry of the β-carotene molecules, Karrer and Eugster (1950) suggested that central cleavage was the most logical means for conversion of β-carotene into vitamin A. For many years, however, the pathways for its conversion were unclear, largely because the rate of conversion is relatively slow, cell-free preparations of tissues show little or no activity, β-carotene is rapidly oxidized chemically to various derivatives and the resolving power and sensitivity of the available methods were limited.

In 1960, Glover suggested two pathways for the cleavage of carotenoids into vitamin A, namely, central cleavage to yield two molecules of retinal or asymmetric cleavage to yield a shorter and a longer β-apocarotenal, the latter of which is sequentially shortened by the removal of C_2 and C_3 fragments to yield retinal. The evidence now suggests that central cleavage is the predominant reaction in humans. The possible role of excentric cleavage in human physiology is not known.

(b) Conversion of β-carotene to vitamin A

Because the mechanism for the conversion of carotenoids to vitamin A appears to be similar in humans and animals, data from both sources are referred to in this section. β-Carotene and other provitamin A carotenoids are converted to vitamin A in many tissues of the body. The intestinal mucosa is the main site of conversion after usual dietary intake of carotenoids, but other organs, particularly the liver, convert significant amounts when greater quantities of carotenoids are ingested. Most human tissues contain carotenoids, and retinoic acid, the major retinoid that is biologically active in cellular differentiation, can be formed both from the carotenoids present in a given tissue and from retinol taken up from the plasma.

The only fairly well characterized carotenoid cleavage enzyme in mammals is carotenoid 15,15′-dioxygenase (EC 1.13.11.21). This enzyme, found in many tissues, shows similar properties in various tissues and species (Olson, 1983). It is located in the cytosol, requires molecular oxygen, shows a K_m value for β-carotene of 1–10 μmol/L, has a slightly alkaline optimal

pH (7.5–8.5), is inhibited by metal ion chelators and by sulfhydryl-binding reagents and can be activated by glutathione. The activity of the intestinal mucosal enzyme is enhanced by vitamin A deficiency and by treatment with polyunsaturated fatty acids but is depressed by treatment with β-carotene. The activity of the liver enzyme, although seemingly less sensitive to vitamin A status, is increased by treatment with β-carotene or with polyunsaturated fatty acids (van Vliet *et al.*, 1992).

It has been postulated that, in addition to central cleavage, excentric cleavage of β-carotene might occur, yielding apocarotenals of different chain lengths, which might subsequently be shortened by the removal of C_2 and C_3 fragments to yield retinal (Glover, 1960). These fragments are presumed to be oxidized to carbon dioxide. While favouring asymmetric cleavage, Glover found, however, that the amounts of radiolabelled carbon dioxide produced by the metabolism of ^{14}C-β-carotene and ^{14}C-retinol in rats were the same, indicating that asymmetric cleavage is not a major pathway. A few years later, the sole detectable product of ^{14}C-β-carotene was found to be retinal (Goodman & Huang, 1965; Olson & Hayaishi, 1965). Indeed, the stoichiometry of the reaction—the ratio of the moles of retinal formed to those of β-carotene consumed—was found to be 1.1–1.5. Any value greater than 1.0 would, of course, favour central cleavage. The enzyme carotenoid 15,15′-dioxygenase (EC 1.13.11.21) was found to require molecular oxygen.

An interesting challenge to the idea of central cleavage was posed by Hansen and Maret (1988), who, although unable to repeat earlier findings, showed that β-carotene could be converted to β-apocarotenals chemically in the presence of oxygen under normal incubation conditions. In a subsequent re-examination of the cleavage reaction, however, Lakshman *et al.* (1989) showed that retinal is the primary, if not the sole, product of β-carotene cleavage catalysed by a partially purified enzyme preparation of rabbit and rat intestinal cytosol.

Whether asymmetric cleavage really occurs in mammals then became a key query. Wang and colleagues (Wang, 1994; Wang, X.D. *et al.*, 1991, 1996) subsequently showed that whole intestinal homogenates convert β-carotene to a group of β-apocarotenals in the presence of oxygen. Of particular interest in this regard was the formation of a pair of β-apocarotenals that are counterparts in a 13′:14′oxidative cleavage reaction. Since retinal was a relatively minor product of the reaction in their studies, they concluded that sequential asymmetric cleavage is a major pathway for the conversion of β-carotene to vitamin A.

The most appropriate way of determining the relative importance of the two pathways is to examine the stoichiometry of the reaction. Central cleavage yields 2 mol of retinal per mole of β-carotene consumed, whereas asymmetric cleavage yields, via β-apocarotenals, a maximum of 1 mol of retinal. Using whole intestinal homogenates similar to those used by Wang, X.-D. *et al.* (1991), Devery and Milborrow (1994) reported a mean molar ratio of 1.72, and Nagao *et al.* (1996) reported a molar ratio of 1.88 ± 0.08 (standard deviation [SD]). After correction for the efficiency of solvent extraction, the molar ratio in the latter study was 2.07 ± 0.09 (SD). β-Apocarotenals were detected in only trace amounts, if at all. No stoichiometric studies have been conducted that favour asymmetric cleavage as the major pathway, and the available information indicates that central cleavage is the predominant reaction in mammals. It should be emphasized that the enzyme has not been purified or crystallized.

(c) Retinoic acid formation

The formation of retinoic acid from β-carotene can proceed by central cleavage, followed by conversion of the resulting retinal to retinoic acid by one of several aldehyde dehydrogenases (Blaner & Olson, 1994). As the diterpene aldehyde, citral, inhibits the formation of retinoic acid from retinal (Wang, 1994; Wang, X.-D. *et al.*, 1996), it should inhibit the formation of retinoic acid from β-carotene, if indeed retinal is a free intermediate in the reaction. On the basis of earlier observations that retinoic acid might be produced directly from β-carotene (Napoli & Race, 1988), it was of considerable interest that β-carotene, but not retinal, is converted into retinoic acid in the

presence of citral in whole intestinal homogenates and is also found in portal blood derived from ferret intestine perfused with these substrates (Wang, X.-D. *et al.*, 1996). Thus, retinoic acid might be formed by oxidation of retinol or by oxidative cleavage of β-apocarotenoic acids.

The two forms of retinoic acid of physiological interest are all-*trans*- and 9-*cis*-retinoic acid, which are ligands for nuclear retinoid receptors. The 9-*cis* isomer can be formed either by isomerization of all-*trans*-retinoic acid or by oxidative cleavage of dietary 9-*cis*-β-carotene (Nagao & Olson, 1994; Wang, X.-D. *et al.*, 1994) followed by oxidation of 9-*cis*-retinal to 9-*cis*-retinoic acid.

Administration of large amounts of retinol is known to promote the formation of retinoids that are active in gene regulation (Tang & Russell, 1991; Norum, 1993); however, β-carotene does not appear to have an immediate effect on the concentrations of retinoic acid in plasma or adipose tissue. The concentrations in plasma were similar in vegetarians and non-vegetarians and were not changed after administration of 90 mg β-carotene for three weeks, nor were the concentrations in adipose tissue changed over 15 days after administration of 120 mg β-carotene (Johnson *et al.*, 1995).

3.1.6.2 β-Apocarotenoids

β-Apocarotenals can be converted directly to retinal and to an uncharacterized, short-chain aldehyde by the carotenoid 15,15′ dioxygenase (Olson, 1983), although slowly in some cases (Nagao *et al.*, 1996). β-Apo-8′-carotenal, and presumably other analogues, can be reduced to alcohols and then esterified in the human intestine or be oxidized to their corresponding acids (Zeng *et al.*, 1992). Several β-apo-carotenoic acids can also be converted to retinoic acid in ferret liver mitochondria, presumably by β-oxidation (Wang, X.-D. *et al.*, 1996). Ethyl β-apo-8′-carotenoate, however, did not appear to be metabolized in humans (Zeng *et al.*, 1992). This compound bears some resemblance metabolically to etretinate, which is stored in the body for long periods.

3.1.6.3 Other carotenoids

Carotenoid 15,15′-dioxygenase also cleaves the all-*trans* isomers of 3,4,3′,4′-tetradehydro-β-carotene, 5,6-epoxy-β-carotene, 5,8-epoxy-β-carotene, α-carotene, 5,6-epoxy-α-carotene, 5,8-epoxy-α-carotene and 3′,4′-dehydro-β-ψ-caroten-16′-al (Olson, 1983), but usually at rates considerably lower than for all-*trans*-β-carotene. The dioxygenase also cleaves 9-*cis*-β-carotene and possibly 13-*cis*-β-carotene, but again at lower rates than the all-*trans* isomer (Nagao & Olson, 1994; Wang, X.-D. *et al.*, 1994). Carotenoids that are cleaved either at low rates (< 5% that for all-*trans*-β-carotene) or not detectably include 5,6,5′,6′- and 5,8,5′,8′-diepoxy-β-carotene, 3′,4′-didehydro-β-cryptoxanthin, zeaxanthin, lutein, the 5,6 epoxides of several β-apocarotenals and β-apocarotenoic acids (Olson, 1983). Carotenoid 15,15′-dioxygenase has also been reported to cleave β-cryptoxanthin (Sivakumar & Parvin, 1997). The lack of activity of the dioxygenase towards β-apocarotenoic acids accords with the demonstrated β-oxidation of the latter to retinoic acid in mitochondria (Wang, X.-D. *et al.*, 1996).

In humans, 4,4′-dimethoxy-β-carotene is converted to canthaxanthin (4,4′-diketo-β-carotene) and to more polar, unidentified metabolites (Zeng *et al.*, 1992). α-Carotene is cleaved to retinal and α-retinal, presumably by carotenoid 15,15′-dioxygenase (Sivakumar & Parvin, 1997). Lycopene, although absorbed well from oily solutions and taken up by the liver and other organs, is metabolized by poorly understood pathways. Many isomers of lycopene are present in human plasma and tissues, the main ones being all-*trans*- and 5-*cis*-lycopene (Stahl & Sies, 1996). A similar pattern of isomers is found in tomato products; however, the percentage of *cis* isomers of lycopene is 9–21% in tomato products, 58–73% in plasma and 79–88% in prostate tissue (Clinton *et al.*, 1996). Thus, a clear shift from all-*trans* to *cis* isomers takes place *in vivo*, due perhaps to isomerization, preferential uptake of specific isomers or selective extraction of isomers into other liphophilic dietary compounds, such as fat and oil (see section 2.1.3.3).

Canthaxanthin, like lycopene, is not metabolized to detectable products in rats, squirrel monkeys or humans (White *et al.*, 1994).

Lutein may be oxidized *in vivo* in humans to its 3′-keto derivative, isomerized from the 6′*R*

to the 6'*S* form or converted to 3'-epilutein and zeaxanthin (Khachik *et al.*, 1995). Although the enzymatic reactions have not been clarified, such derivatives appear in human plasma after lutein supplementation. Lutein and zeaxanthin may have a physiological role in the body, since they are specifically found in the retina. Zeaxanthin is concentrated in the macula, while lutein is dispersed throughout the retina (Bone *et al.*, 1988; Handelman *et al.*, 1988). The presence of carotenoids in the eye may improve visual acuity and may also protect against damaging photochemical reactions (Bone *et al.*, 1985, 1993). There is some dispute about the relative amounts of the two carotenoids in the retina: Bone *et al.* (1988) reported that zeaxanthin predominated in more than 90% of human eyes examined, while Handelman *et al.* (1988) reported that the concentration of lutein exceeded that of zeaxanthin in 15/16 whole retinas examined. The total amount of carotenoid recovered in the retina does not appear to be related to age, but the concentration can vary from 3 to 85 ng (Bone *et al.*, 1988) or 35 to 120 ng (Handelman *et al.*, 1988). Zeaxanthin exists as two stereo-isomers in the eye: zeaxanthin itself [(3*R*,3'*R*)-β,β-carotene-3,3'-diol] and *meso*-zeaxanthin [(3*R*,3'*S*)-β,β-carotene-3,3'diol] (Bone *et al.*, 1993). The presence of lutein and zeaxanthin in the eye does not necessarily imply that lutein undergoes metabolic or structural changes during its absorption and dispersion through the tissues, but lutein usually occurs at considerably greater concentrations than zeaxanthin in the bloodof most people in western countries, unless they consume large amounts of yellow corn. These observations led Bone *et al.* (1993) to suggest that *meso*-zeaxanthin arises from lutein by some chemical process in the retina.

Capsanthin, a major carotenoid in paprika, is a dihydroxymonoketo-carotenoid with one five-carbon cyclic ring. When given orally to men, capsanthin is well absorbed, is associated equally with high-density and low-density lipoproteins in plasma and is cleared rapidly from the circulation (Oshima *et al.*, 1997). No metabolites were identified.

9'-*cis*-Bixin, a monomethylester of an acylic C_{24} dicarboxylic acid, and its congeners are found in the seeds of the annatto plant. Extracts of these seeds are used commonly as a food colouring in Spain and Latin America. When ingested by human volunteers, 9-*cis*-bixin is well absorbed but rapidly cleared from plasma. It is both dimethylated to the dicarboxylic acid norbixin and isomerized to all-*trans*-bixin *in vivo* (Levy *et al.*, 1997).

Little is known about the metabolism in mammals of other carotenoids, such as neoxanthin, violaxanthin and astaxanthin. Although found in foods, these carotenoids have not been detected in human plasma (Khachik *et al.*, 1991).

3.1.7 Genetic variation

No clearly defined genetic defects have been observed in the metabolism of carotenoids. Differences in plasma concentrations of carotenoids after an oral dose of β-carotene may represent genetic variability, but the absence of differences in the absorption of other carotenoids complicates the interpretation of relative responsiveness.

3.2 Experimental models

Research on the bioavailability of carotenoids is hampered by the fact that most common laboratory animal species, in contrast to primates, efficiently convert β-carotene to vitamin A, and very little is absorbed intact. The situation in primates has, however, been mimicked in two animal models: the ferret and the preruminant calf. Although both models have some limitations, important information on carotenoid uptake and metabolism have been obtained. Most of the studies on carotenoid uptake, distribution and metabolism in humans and animals have focused on β-carotene, and less information is available for other carotenoids.

3.2.1 Non-human primates

Various carotenoids, including lutein, zeaxanthin, α-cryptoxanthin, β-cryptoxanthin and β-carotene, were detected in plasma, liver and other tissues of monkeys that received the usual, unsupplemented Primate Center diet (Krinsky *et al.*, 1990b; Snodderly *et al.*, 1990), indicating that rhesus monkeys absorb and

transport dietary carotenes and xanthophylls in the same way as humans.

When ^{14}C-β-carotene was given at a single dose of 1.26 mg in olive oil to rhesus monkeys, peak accumulation in the serum of labelled retinol derived from the parent was detected after 8–24 h; some unchanged labelled material was also detected. Considerable individual variation was seen in the amount of radiolabel in serum. Most of the absorbed radiolabel was detected in the liver, mainly as retinol, with only 2–8% as unchanged β-carotene. Much less radiolabel was found in other tissues. Rhesus monkeys are thus capable of absorbing β-carotene intact and of using it as a source of retinol. No other β-carotene metabolite was detected in a significant amount (Krinsky *et al.*, 1990b). In contrast, no labelled metabolic products were found when ^{14}C-canthaxanthin or β-lycopene was administered to rhesus monkeys, confirming the finding that neither compound has provitamin A activity. Peak accumulation of radiolabel in plasma was measured at 8–48 h for both compounds, but the clearance of lycopene from plasma appeared to be slower than that of canthaxanthin. The liver was the major depot organ for both carotenoids. Various amounts of the pigments were detected in other tissues, with the highest concentrations in spleen (Mathews-Roth *et al.*, 1990).

When the diet of squirrel monkeys was supplemented with zeaxanthin and β-carotene, rapid plasma responses were measured. After two weeks of increasing zeaxanthin intake, a relatively stable, higher plasma concentration of this compound was reached. In animals given a standard laboratory diet, the plasma concentrations of β-carotene were low and increased only slightly with supplementation. The plasma concentrations of lutein were not affected by zeaxanthin supplementation (Snodderly *et al.*, 1997).

In baboons, the toxicity of ethanol was reported to be enhanced when it was taken together with β-carotene. In animals fed the combination, histological changes in the liver were more pronounced, and increased blood concentrations of the mitochondrial enzyme glutamic dehydrogenase were found as compared with controls that received either ethanol or β-carotene alone. It was suggested that the effect is due to interference by ethanol with the metabolism of β-carotene to retinol and disposition of the carotenoid from the liver. Baboons that received β-carotene and ethanol had higher concentrations of β-carotene in plasma and liver than controls, and the β-carotene-induced increase in liver retinol was lower in animals that received the combination. When β-carotene treatment, was stopped, the plasma concentrations decreased more slowly in the animals fed ethanol. The half-life of β-carotene in plasma was fourfold longer when the slow elimination phase was taken into account. This experiment provides interesting information on the possible interactions between β-carotene and ethanol with respect to bioavailability and metabolism (Leo *et al.*, 1992). [The Working Group noted that the dose of ethanol was very high, supplying 50% of the total energy intake over two to five years.]

The pattern of carotenoids in the macula lutea has also been investigated in monkeys (Handelman *et al.*, 1991; Snodderly *et al.*, 1991). As in humans (Landrum *et al.*, 1997), the main carotenoids are lutein and zeaxanthin, although the ratio between the two xanthophylls differs within different regions of the macula lutea. Macaque monkeys have a consistent pattern of more zeaxanthin than lutein at the foveal centre, and a similar distribution is observed in the macula lutea of adult humans (Bone *et al.*, 1997).

Studies on the conversion of β-carotene to retinoids and apocarotenals have been performed with homogenates from various monkey tissues. β-Carotene was converted to retinoids and apocarotenals by intestine, liver, kidney, lung and adipose tissue (Wang, X.-D. *et al.*, 1991). β-Apo-13-carotenone and β-apo-14′-carotenal were identified after incubation of β-carotene with intestinal preparations from monkeys, rats, ferrets and humans (Tang *et al.*, 1991).

3.2.2 Preruminant calves and cows

The preruminant calf has been introduced as an animal model for the study of carotenoid biokinetics in humans (Hoppe & Schoner, 1987; Poor *et al.*, 1992). Preruminant calves are new-

born animals that are maintained in a monogastric state by feeding them an all-liquid diet. For most studies of carotenoids, milk substitutes with a low concentration of vitamin A are used (Poor *et al.*, 1992; Hoppe *et al.*, 1996). After a single oral dose of 20 mg β-carotene dissolved in a milk substitute, peak serum concentrations in calves of about 0.4 μmol/L β-carotene were observed after 12–30 h but no significant change in serum vitamin A concentration was found. Relatively high concentrations of β-carotene were found in the adrenal glands, with a maximum 24 h after treatment. High concentrations were found in liver, lung and spleen, also peaking 24 h after administration, whereas the highest concentrations in adipose tissue and kidney were detected 72 and 144 h after dosing, respectively. No significant change in carotenoid concentration was observed in heart or muscle. The serum β-carotene response curves of the calves were similar to those observed in humans after a single dose of β-carotene; however, about 30% of the animals did not show significantly increased serum concentrations after β-carotene administration (Poor *et al.*, 1992). A similar phenomenon has been observed in humans who are designated 'low' or 'poor responders' (Johnson & Russell, 1992; Stahl *et al.*, 1995). There is at least one significant difference between preruminant calves and humans with regard to the biokinetics of carotenoids, which may affect transport and clearance. In human blood, β-carotene is associated mainly with the low-density lipoprotein fraction (Krinsky *et al.*, 1958; Johnson & Russell, 1992), whereas in calves and cows high-density lipoproteins appear to be the major transport form (Schweigert & Eisele, 1990; Bierer *et al.*, 1993, 1995).

The dose–response relationship of β-carotene was also investigated in this model. The compound was administerd orally with a milk substitute to preruminant calves for 28 days at a dose of 0.23, 0.46, 0.92, 1.84 or 3.68 μmol/kg bw per day. Steady-state plasma concentrations were reached on day 28 and were clearly related to the logarithm of dose. At doses between 0.23 and 0.92 μmol/kg bw per day, the relationship with steady-state serum concentrations was linear, but considerable interindividual differences in these concentrations were found, with a calculated coefficient of variation of about 30%. No animals considered to be poor responders were reported. Dose-dependent accumulation of β-carotene was measured in liver, lung, heart, adrenals and adipose tissue. In the liver, the vitamin A concentrations increased with β-carotene intake. All-*trans*-β-carotene was the only isomer present in plasma and the adrenals and the major isomeric form in other tissues (Hoppe *et al.*, 1996).

The absorption and transport of β-carotene, α-carotene, lycopene, canthaxanthin and lutein in preruminant calves were compared after a single oral dose of 20 mg of each carotenoid. The serum responses indicated that all of them were absorbed but in variable amounts; the variations may be due, at least in part, to the use of different vehicles or carotenoid preparations. The peak serum concentrations of canthaxanthin and lutein occurred earlier (8 and 12 h) than those of lycopene, α-carotene and β-carotene (16, 16 and 24 h, respectively). At these times, 70–90% of the serum carotenoids were associated with the high-density lipoprotein fraction. The oxocarotenoids were cleared more quickly from serum than α-carotene, β-carotene or lycopene, and lycopene and α-carotene had slower disappearence rates than β-carotene (Bierer *et al.*, 1995).

Several studies in humans indicate that heating of a dietary source improves the bioavailability of carotenoids (Micozzi *et al.*, 1992; Stahl & Sies, 1992), but no such effect was seen in the preruminant calf model, in which mild heat treatment had only slight effects on the availability of α- and β-carotene from carrots, as shown by analyses of serum and tissue concentrations (Poor *et al.*, 1993).

β-Carotene is also distributed into the milk of cows. After parenteral administration, elevated concentrations of the parent compound were found in milk and plasma. No increase in the vitamin A concentrations of plasma was observed, whereas the vitamin A concentration of milk increased (Schweigert & Eisele, 1990).

The corpus luteum of both cows and humans has extremely high concentrations of β-carotene. In bovine corpus luteum, the highest concentrations were detected in nuclei,

lipids and mitochondria; however, much of the β-carotene was loosely bound in the nuclear and mitochondrial fractions, and in the cytosolic fraction β-carotene was associated with with high-molecular-mass proteins (O'Fallon & Chew, 1984). A study of the intracellular transport of β-carotene in bovine liver and intestine suggested that β-carotene, unlike other lipophilic compounds, is not transported by cytosolic proteins, and other mechanisms such as vesicular transport may be involved (Gugger & Erdman, 1996).

3.2.3 Ferrets

The ferret (*Mustela putorius furo*) has been used to investigate the intestinal absorption, metabolism and storage of carotenoids, and a direct comparison with rats showed that this species is a more appropriate model for humans. In the absence of supplementation, the serum concentration of β-carotene in ferrets was only about 0.01 nmol/ml, and no β-carotene was detected in liver or adipose tissue. After supplementation with 4 or 20 mg/kg bw β-carotene in corn oil for two weeks, the serum concentrations increased to 0.28 and 0.78 nmol/ml, respectively, within the range detected in human serum. Increases in β-carotene concentrations were also measured in liver and adipose tissue, with values in the liver of 1.7 and 7.7 nmol/g tissue after the repeated doses of 4 and 20 mg, respectively (Ribaya-Mercado *et al.*, 1989).

After a single dose of 10 mg/kg bw β-carotene, a peak serum concentration of 0.68 nmol/ml was observed 8 h after ingestion; within 76 h, the compound was essentially cleared from the blood. Peak concentrations of β-carotene in various tissues were detected between 8 and 16 h after administration. The highest concentration was found in the liver (1.2 nmol/g); less was found in the lung (0.04 nmol/g), kidney (0.09 nmol/g) and spleen (0.08 nmol/g). Other polar and nonpolar carotenoids were identified in ferret livers. The results show that this species can absorb carotenoids intact and transport them into various tissues (Gugger *et al.*, 1992).

All-*trans*-β-carotene accumulates preferentially in the serum of ferrets that have ingested an isomeric mixture. Similar effects were observed in humans, suggesting that the ferret is also a suitable model for intestigating the biokinetics of geometric isomers of carotenoids. In the same study, it was shown that less β-carotene is bioavailable from the natural source, carrot juice, than from a commercial beadlet preparations used as a feed additive (White *et al.*, 1993a). Canthaxanthin had specific antagonistic effects on the bioavailability of β-carotene when the two compounds were given concurrently (White *et al.*, 1993b). Furthermore, the biokinetics of carotenoids in ferrets and humans show striking differences. In ferret blood, β-carotene is associated mainly with the high-density lipoprotein fraction, whereas the major transport form in humans is low-density lipoproteins (Ribaya-Mercado *et al.*, 1993).

After long-term supplementation of ferrets with canthaxanthin at 50 mg/kg bw per day, the serum concentrations increased from 0 at baseline to about 0.1 nmol/ml after 12 months. High concentrations of canthaxanthin were also detected in liver, fat, lung and small intestine, but no detectable concentrations were found in the eyes, and the animals showed no clinical signs of toxicity (Tang *et al.*, 1995).

The ferret is also a useful model for studying the bioconversion of β-carotene (Wang *et al.*, 1992; Hebuterne *et al.*, 1995, 1996; Wang, X.-D. *et al.*, 1996). Experiments involving perfusion of the small intestine of ferrets added evidence that geometric isomers of β-carotene are isomer-selective precursors of the respective retinoic acids. After perfusion with all-*trans*-β-carotene, the resulting retinoic acid was mainly in the all-*trans* form; when 9-*cis*-β-carotene was used, about 50% of the retinoic acid formed was the 9-*cis* isomer. Interestingly, the total amount of retinoic acid was similar in the two experiments (Hebuterne *et al.*, 1995).

Interactions of β-carotene with other lipophilic antioxidants have been shown in the ferret model. α-Tocopherol at low doses enhanced the lymphatic transport of β-carotene fourfold, and this effect was even more pronounced at pharmacological doses of vitamin E. It was suggested that α-tocopherol modulates the metabolism of β-carotene and has a positive effect on its intestinal absorption (Wang *et al.*, 1995).

3.2.4 Rats

As rats convert β-carotene and other provitamin A carotenoids to retinol, this species has often been used to study the metabolism of these compounds. For moderate and higher intake, the conversion is inversely related to dose (Brubacher & Weiser, 1985). Fractions of rat tissues have been investigated for their capabilty to cleave carotenoids: after experiments with various fractions of rat liver homogenate, cleavage activity was assigned to the enzyme β-carotene 15,15′-dioxygenase (Olson, 1961; Olson & Hayaishi, 1965). A mechanism for the biosynthesis of vitamin A from β-carotene was postulated on the basis of the results of an investigation of the cleavage products in rat lymph after administration of doubly labelled β-carotene, i.e. that the formation of vitamin A occurs in a dioxygenase-like reaction after cleavage of the central double bond (Goodman *et al.*, 1966b). The primary cleavage product is retinal (Lakshman *et al.*, 1989), as shown in several studies, although the formation of retinal and other retinoids has been challenged in a single report (Hansen & Maret, 1988).

The fate of β-carotene geometric isomers remains an open question. Studies in rats and other species have added evidence that 9-*cis*-β-carotene is an isomer-selective precursor of 9-*cis*-retinal or 9-*cis*-retinoic acid (Nagao & Olson, 1994; Hebuterne *et al.*, 1995). The latter is a high-affinity ligand of retinoic X receptors, which act as ligand-dependent transcription factors (Kliewer *et al.*, 1994). Enzyme preparations partially purified from rat liver and intestine converted all-*trans*, 9-*cis*- and 13-*cis*-β-carotene to an isomeric mixture of all-*trans*, 9-*cis*- and 13-*cis*-retinal (Nagao & Olson, 1994). Relatively more *cis*-retinals were formed when β-carotene *cis* isomers were used as substrates; however, the cleavage rates determined *in vitro* were distinctively lower with 9-*cis*- and 13-*cis*-β-carotene than with the all-*trans* form.

Fewer data are available on the cleavage of carotenoids other than β-carotene in rats. Other provitamin A carotenoids are metabolized to retinal by rat intestinal preparations, but, in comparison with β-carotene, less retinal is formed. Retinal formation from α-carotene and cryptoxanthin was 29 and 55%, respectively, that of β-carotene. *In vitro*, other carotenoids can influence the efficacy of β-carotene cleavage, as less retinal was formed from β-carotene in the presence of lutein; no effects on β-carotene metabolism were observed in the presence of lycopene (van Vliet *et al.*, 1996a). The highest activity of the enzyme β-carotene 15,15′-dioxygenase within the intestine, investigated in female Wistar rats, was found in the cytosol of mature functional enterocytes harvested from the jejunum. Retinal and retinoic acid were the only metabolites identified in this study; no apocarotenals were detected (Duszka *et al.*, 1996). The absorption and metabolism of β-carotene are affected by the vitamin A and β-carotene concentrations in the diet. The intestinal enzyme activity was higher in rats fed a diet with a low content of vitamin A than in those fed a diet with a low content. The dioxygenase activity was lower when the animals were given a diet supplemented with β-carotene than when they received an unsupplemented diet (van Vliet *et al.*, 1993, 1996b).

No β-carotene was detected in rat serum within 72 h after administration of a single oral dose of ^{14}C-labelled compound; the peak serum concentrations of labelled retinol were measured at 4 h. Small amounts of unchanged β-carotene were found in the liver, but about 90% of the radiolabel in this organ was assigned to the retinol fraction (Krinsky *et al.*, 1990b; Mathews-Roth *et al.*, 1990). Increasing plasma concentrations were reported after a single oral dose of 10 mg/kg bw of all-*trans*- or 9-*cis*-β-carotene, with peak concentrations at 4 h. When the 9-*cis* isomer was administered to rats, higher plasma concentrations of the all-*trans* form (about 0.1 nmol/ml) were measured than after application of the all-*trans* isomer (0.03 nmol/ml), but the 9-*cis* isomer was not detected after administration of the all-*trans* form, and only small amounts of this isomer (0.01 nmol/ml) were found after 9-*cis* supplementation. The results indicate that the 9-*cis* isomer is converted to all-*trans*-β-carotene before or during absorption (Suzuki *et al.*, 1996). Similar observations have been made in humans (Gaziano *et al.*, 1995a; Stahl *et al.*, 1995; You *et al.*, 1996). Rats, like humans, have considerable amounts of 9-*cis*-β-carotene in the liver (Ben-

Amotz *et al.*, 1989). Higher concentrations of *cis* isomers were found in rat liver after administration of 9-*cis*-β-carotene than its 13-*cis* analogue, and an inverse relationship was found between the corresponding liver concentrations of *cis*-β-carotene and the ability to revert symptoms of vitamin A deficiency (Weiser *et al.*, 1993).

Several studies in this animal model indicate that factors which influence the bioavailability of β-carotene in humans also affect their availability in rats. These include bile salts (El-Gorab *et al.*, 1975), fatty acids and pH (Hollander & Ruble, 1978), dietary lipids (Mokady & Ben-Amotz, 1991; Bianchi-Santamaria *et al.*, 1994) and interactions between β-carotene and other carotenoids (High & Day, 1951).

Under normal dietary conditions, very little carotenoid crosses the intestinal mucosa into the systemic circulation. Thus, adipose tissue is usually white, and other organs contain little carotenoid. After long-term supplementation with a large amount (0.2% of diet) of β-carotene, however, β-carotene was found in significant amounts in blood and tissues. Plasma was saturated within three days, whereas saturation was not reached in the liver, adrenal gland or ovary even after 21 weeks of supplementation. The largest amount of β-carotene (9.3 nmol/g) was found in liver (Shapiro *et al.*, 1984). In carcinogenicity experiments as well, long-term dietary supplementation of rodents with large amounts of carotenoids led to appreciable concentrations of the administered carotenoid in the liver and other organs (see section 4.2).

Less is known about the absorption and distribution of carotenoids other than β-carotene in rats. After administration of ^{14}C-labelled lycopene and canthaxanthin, peak accumulation of radiolabel in plasma was detected within 4–8 h. Both pigments accumulated in liver, and small amounts were found in various other tissues (Krinsky *et al.*, 1990b; Mathews-Roth *et al.*, 1990). High concentrations of canthaxanthin and β-carotene were reported in the livers of rats fed a diet containing either 0.2% canthaxanthin or 0.2% β-carotene for two weeks; smaller amounts were measured in the lung (van Vliet *et al.*, 1991).

The results indicate that the rat is not a suitable model for studying the uptake and plasma response of carotenoids, especially β-carotene. Much less of the carotenoid is absorbed unchanged, and the times at which peak concentrations are found in plasma after a single dose differ considerably, being about 4 h in rats and 24–48 h in humans. Since rats efficiently convert β-carotene to retinal, however, this species could be used to investigate the metabolism of provitamin carotenoids; rat tissue preparations are suitable sources for the cleavage enzyme β-carotene 15,15′-dioxygenase. As the fate of geometric isomers of β-carotene appears to be similar in rats and humans, this model may also be used to investigate the metabolism and tissue distribution of geometric isomers in the organism.

Some carotenoids can induce xenobiotic metabolizing enzymes. Canthaxanthin accumulates in rat liver and increases the activities of some drug-metabolizing enzymes, including cytochromes P4501A1 and P4501A2, *para*-nitrophenol-uridine diphosphoglucuronosyl transferase and quinone reductase. Similar but less pronounced effects were detected with astaxanthin. In contrast, β-carotene, lutein and lycopene did not induce the activity of these enzymes (Astorg *et al.*, 1994; Gradelet *et al.*, 1996a).

3.2.5 Other animal species

Several other species have been used to study the bioavailability and metabolism of β-carotene. Low concentrations of β-carotene but increasing amounts of retinol were found in the livers of hamsters fed various concentrations of the compound, indicating that these animals are efficient converters of β-carotene. No carotenoids were detected in the blood of rabbits fed a carotenoid-rich diet, and only small increases were seen in the concentration of vitamin A in liver, indicating that the rabbit is a poor absorber, although preparations of rabbit instestine have been used to investigate the β-carotene cleaving enzyme β-carotene 15,15′-dioxygenase. Preparations of rabbit intestinal mucosa were used to show that lycopene, lutein and astaxanthin competitively inhibit the enzyme and partially protect it from trypsin digestion (Ershov *et al.*, 1994).

The absorption, transport and tissue distribution of β-carotene have been studied in pigs given ^{14}C-labelled compound orally. The amount of radiolabel in plasma increased within 4 h, and, by 24 h, a large amount of radiolabel was found in the liver and lung. In the liver, the label was associated mainly with retinol, while in the lung large amounts of the parent compound (5.4 μg/g tissue) were detected. Thus, pigs absorb the compound intact and accumulate β-carotene in the lung (Schweigert *et al.*, 1995). Studies with preparations of pig intestinal mucosa showed that the major cleavage product of β-carotene is retinal, formed in a molar ratio close to 2, indicating mainly central cleavage. Other retinoids or apocarotenals were not detected (Nagao *et al.*, 1996).

As Mongolian gerbils absorb β-carotene intact, this species could be used to evaluate specific aspects of the biokinetics of carotenoids. After a single oral dose of about 0.15 mg β-carotene, increasing serum concentrations were detected between 0 and 4 h, with a peak concentration of 88 pmol/ml, but the concentration decreased to 0 within 72 h. In tissues, the highest β-carotene concentrations were found in liver and spleen; low concentrations were also detected in kidney, lung and adipose tissue (Pollack *et al.*, 1994).

Studies on the bioavailability of β-carotene and its geometric isomers have been performed in chickens given an isomeric mixture extracted from the alga *Dunaliella bardawil*. Chicks were fed either synthetic β-carotene (all-*trans* isomer) or β-carotene from the alga (containing about equal amounts of the all-*trans* and 9-*cis* isomers) at equivalent levels of total β-carotene. The absolute amount of β-carotene in chick liver was higher when the natural source was used, but no difference was found in the content of retinol. The ratio of 9-*cis*-β-carotene to its all-*trans* analogue exceeded that of the natural source, indicating preferential accumulation of 9-*cis*-β-carotene in chick livers. Similar data were obtained when purified isomers were used (Mokady *et al.*, 1990). In chick liver, the highest concentration of β-carotene was detected in the mitochondrial fraction, followed by lysosomes, microsomes and nuclei (Mayne & Parker, 1986).

3.2.6 Conclusion

No animal species provides a model suitable for studying all aspects of the biokinetics of carotenoids in humans. The preruminant calf and the ferret appear to be the most suitable species. The results of studies with non-human primates are promising, but more studies are needed to evaluate these models. Other species may be considered for the investigation of specific aspects of the biokinetics of carotenoids, such as their metabolism.