Predicting the bioavailability of antioxidants in food: the case of carotenoids

Professor Susan Southon and Dr Richard Faulks, Institute of Food Research, Norwich

1 Introduction

With the advent of present-day analytical techniques and instrumentation, it is possible to describe the complex chemical nature of our foods with an ever-increasing degree of accuracy and sensitivity. However, the types and quantities of either the nutrient, or non-nutrient, components of foods may have very little bearing on their potential contribution to our nutrient or ‘health’ status. This is particularly true of many of the proven, or purported, antioxidant compounds. The reason for this is that only a proportion (sometimes a highly variable proportion depending upon the food matrix, processing and storage) of these food components can be absorbed and utilised. Understanding this concept of bioavailability is essential to all involved in food production, nutritional assessment and determining diet:health relationships. The term bioavailability means many things to many people. For the purposes of this chapter, bioavailability is defined as the proportion of a nutrient (food component) that is digested, absorbed and utilised in normal metabolism; however, measurement of bioavailability relies heavily upon estimates of amounts absorbed.

The absorption and transport processes of many of the potentially bioactive components of foods (including those with antioxidant capacity) are complex and not fully understood; thus, prediction of their bioavailability is problematic. This is particularly true of the lipid-soluble antioxidants. In this chapter carotenoids have been chosen for special focus, because they serve as an excellent example of food components where too little understanding of complex behaviour within the food and within human tissues can lead to misinterpretation of study results.
Carotenoids and related compounds are the colours of nature. They consist of a group of over 600 naturally occurring coloured pigments that are widespread in plants, but only about 24 commonly occur in human foodstuffs. In the plant, they serve two essential functions: as accessory pigments in photosynthesis and in photoprotection. These functions are achieved through the polyene structure of carotenoids (Fig. 7.1), which allows the molecules to absorb light and to quench, or inactivate, singlet oxygen and free radicals. In most cases, the carotenoid (normally present as the all trans form) is associated intimately with the light-harvesting complex in the thylakoid membranes of the chloroplast where it is found as an ordered structure in association with binding protein. In the case of the carrot root and tomato fruit the β-carotene and lycopene, respectively, occur as membrane bounded semi-crystalline structures derived from chromoplast or chloroplast structures. It might be expected that in plants the lipophilic carotenoids would be found in association with sub-cellular lipid structures but it is also known that there are associated binding proteins.1 Such a complex environment has implications for their extraction, analysis and behaviour during digestion.

The hypothesis, based on epidemiological evidence, that health benefits arising from the consumption of coloured fresh fruit and vegetables are at least in part due to their carotenoid content has led to interest in the non-provitamin, as well as the provitamin, A carotenoids. At present, there are no quantifiable biochemical or physiological markers of carotenoid ‘status’ (other than in relation to the vitamin A activity). Neither carotenoid ‘deficiency’ nor ‘toxicity’ is recognised. However, low plasma

The half-lives of the various lipoprotein carriers are approximately as follows: ¹τ1/2 Chylomicrons = 2–5 min, ²τ1/2 Remnants = 11.5 min, ³τ1/2 VLDL = 120 min, ⁴τ1/2 LDL = 24 h.  
CH = Chylomicrons, BC = β-Carotene

7.1 Lipoprotein carriers.
Carotenoid concentration is used as an indicator of those ‘at risk’ of chronic disease based on the direct association between the intake of carotenoid containing vegetables and fruit, plasma and tissue concentrations of carotenoids\textsuperscript{2–4} and the development of chronic disease states, particularly cardiovascular disease\textsuperscript{5} and cancer of various organs.\textsuperscript{6} A possible negative impact on health, at least for β-carotene, is indicated by the ATBC (α-tocopherol: β-carotene) trial where increased risk of lung cancer was reported in smokers receiving relatively high dose β-carotene supplements for several years.\textsuperscript{7} Epidemiological and experimental evidence from cell culture and human studies points to a ‘window of wellness’ with respect to β-carotene intake, with beneficial responses being associated with amounts available from diets rich in coloured fruits and vegetables.\textsuperscript{8} Most recently, it has been shown in a prospective cohort study that there is a strong negative correlation between the intake of tomato and tomato products, but not tomato juice, and the incidence of prostate cancer and it is concluded that the effective agent is lycopene.\textsuperscript{9} The difference in response to different tomato products has been ascribed to differences in lycopene bioavailability.

Since it is recognised that the carotenoids are not actively absorbed by the gut but are passively absorbed along with lipids, the efficiency of absorption of the carotenoids is dependent on getting the lipophilic molecules and crystals to dissolve into dietary lipids both during processing or domestic preparation and during the digestive processes. It is now recognised that this is a key process in absorption and may well be the single most important factor governing the rate and limit of absorption. It is not surprising therefore to find greater bioavailability from heat-treated foods that have also been comminuted, or co-processed, with oils.\textsuperscript{10,11}

Whilst emphasis in this chapter is placed on carotenoids, many of the approaches presented and discussed have relevance in determining the bioavailability of a much wider range of food components.

The chapter will cover aspects of:

- Carotenoid metabolism – the knowledge required for prediction of absorption.
- Experimental systems for measuring absorption – their strengths and weaknesses.
- Maximising bioavailability – food processing and dietary interactions.
- Sources of further information – a current European initiative.

### 2 Metabolism

#### 2.1 Digestion

The carotenoids must be disassociated from their native environment in the plant tissue and, since the carotenoids are lipophilic, they must also be dissolved in dietary lipids before they can be absorbed. The physical processes
involve disruption of the physical structure of the food and the dissolution of the carotenoid into the bulk lipid phase, lipid emulsion or mixed micelles. The mass transfer, therefore, involves both the dietary lipid and its hydrolysis products (free fatty acids, mono- and diacyl glycerols), phospholipids and the bile salts needed to emulsify the lipid to form mixed micelles.

2.2 Gastric and lumenal events
The lumenal environment and the form of the carotenoid play a crucial role in determining how much of the carotenoid is presented for absorption. Both the hydrocarbon (e.g. β-carotene) and hydroxy carotenoids (e.g. zeaxanthin) have very limited solubility in bulk lipid (0.112–0.141 % and 0.022–0.088 %, respectively). Additionally, the hydrocarbon carotenoids are found mainly in the core of the lipid droplets whereas the hydroxy carotenoids are preferentially located at the surface because of their different polarities. This has implications for the absolute amount and type of carotenoid that can be carried by emulsified lipid droplets and in the much more highly structured micelles. These differences in physical properties and their preferred lipid domains will also control the possible transfer of carotenoid between lipid structures both in the gut lumen and post absorption.

2.3 Absorption
Carotenoids are passively absorbed from the micellar phase. However, it is not known if all the carotenoid present in a mixed micelle is absorbed, or whether some is left behind in association with unabsorbed bile salts and cholesterol, to be absorbed perhaps more distally or lost to the large intestine. Factors that increase the thickness of the unstirred layer on the surface of the gut, for example soluble dietary fibre, act to attenuate the absorption of dietary fats and may, therefore, also inhibit the absorption of carotenoids.

Disease states which impair lipid absorption, for example cystic fibrosis and coeliac disease also lead to low plasma carotenoid levels, although in some cases persistent inflammation may be a significant factor in reducing plasma levels of carotenoids. The mass transfer of the carotenoids from digesta to absorbable species is clearly a limiting step in their bioavailability on the basis that free carotenoids given orally (as supplements, solution or suspension in oil) are much better absorbed than those from foods and there is evidence that homogenisation of the food and heat treatment enhance absorption.

2.4 Transport
As already stated, the carotenoids are passively absorbed from mixed micelles at the brush border along with dietary lipids, lipid hydrolysis prod-
ucts, sterols and bile salts. The absorbed carotenoids are transported through the enterocyte from the luminal side to the serosal side, where they are re-excreted in chylomicrons into the thoracic duct and hence find their way into the circulating blood. The cleavage of some of the retinol precursor (provitamin A) carotenoids by 15,15’dioxygenase occurs in the enterocyte. The resulting retinal is reduced to retinol and subsequently esterified to the retinyl ester (mainly palmitate) which also enters the mesenteric lymph with the chylomicrons. It is probable that some of the retinol produced in the enterocyte is excreted into the portal blood in association with retinol binding protein. It is not known if any of the non-provitamin A carotenoids are competitive inhibitors of 15,15’dioxygenase but the cis-isomers of β-carotene can give rise to the corresponding cis-retinol.

The chylomicrons have a biologically-controlled composition with regard to protein (2%, consisting of apoproteins A-1, A-2, A-4 and B-48) and lipid (dietary derived), and appear to carry the carotenoids (and other lipid soluble components) passively in the mesenteric lymph. The chylomicrons are acted upon by endothelial lipoprotein lipase in the extrahepatic capillary bed. Some of the lipid is absorbed as free fatty acids and the glycerol is metabolised. It is not known if any of the carotenoid is also absorbed at this point, although clearance kinetics would imply that some is absorbed, perhaps by adipocytes. The chylomicron remnants, including residual carotenoids, are then cleared from the circulation by passage through the liver. However, lycopene in the rat and monkey model does appear to concentrate in the liver and gut and in a range of tissues in humans indicating that it disperses to tissues differently from the way that β-carotene does. This may help to explain why it is so difficult to increase plasma concentration of lycopene with chronic supplementation of 15 mg lycopene per day.

The liver re-exports lipid in the form of very low density lipoproteins (VLDL) and these contain carotenoids dissolved in the lipid portion. No specific carrier proteins have been identified. The VLDL is acted upon by endothelial lipoprotein lipases in the extrahepatic capillary bed and this removes lipid and glycerol to produce intermediate density lipoproteins (IDL) and low density lipoproteins (LDL). The IDL is converted to LDL by receptor-mediated endocytosis in the liver and the LDL is cleared from the plasma by receptor-mediated endocytosis in both liver and other tissues. The hydrocarbon carotenoids appear to remain associated with these lipoproteins, such that around 80% of β-carotene and lycopene found in fasting plasma are carried by LDL. How much of the carotenoid is distributed to the tissues by these processes is unknown, nor is it known how quickly the process occurs, but endocytosis of LDL particles by adipose tissues is likely to be a major carotenoid ‘sink’.

The hydroxy carotenoids (e.g. lutein, zeaxanthin) are found almost equally distributed between the LDL and the high density lipoproteins (HDL) in fasting subjects. HDL particles are produced in the liver and
intestine and are secreted into the blood and mesenteric lymph, respectively. Other HDL particles may arise from chylomicrons as the triacylglycerol is removed by lipoprotein lipase. The carotenoid associated with HDL may, therefore, be derived from the liver, or directly from the gut, or by exchange between lipoproteins. Unlike LDL, where the bulk of the particle is cholesterol esters, HDL contains much more protein and phospholipid. However, both particles have a hydrophobic core in which the hydrocarbon carotenoids may be carried. It is unclear, therefore, why the polar hydroxyl carotenoids partition almost equally between LDL and HDL unless both particles have similar interfacial characteristics.

If the currently accepted model of carotenoid absorption and transport is correct, then dietary carotenoids should first appear in the chylomicrons and then VLDL, LDL and HDL, depending upon the amounts of each lipoprotein and their residence time (half life) in the plasma. It would be expected that the chylomicrons would show a peak at around 4–6 h post ingestion, as the meal passes through the ileum, and would be clear at about 12 h. Studies of β-carotene absorption, using thoracic duct cannulation, 26,27 found this to be the case. Studies of the absorption of α-carotene, lycopene and lutein in the triglyceride rich lipoprotein (TRL) fraction of plasma also show a peak plasma concentration around 4–6 h which returns to baseline by 12 h post dose. 28 Appearance in the TRL would be followed by peaks in VLDL then LDL as the hepatically sequestered carotenoid is re-excreted into the circulation. Figure 7.1 (see Section 7.1) illustrates the main features of carotenoid distribution in the plasma carriers.

2.5 Isomerisation and cis-isomers

Human blood plasma contains mainly the all trans forms of the common dietary carotenoids but 5Z-lycopene (up to 50% of the total plasma lycopene) and 9Z- and 9’Z-lutein and 9Z-β-carotene 29 are also commonly found in human blood plasma. In some cases 5Z-lycopene appears in plasma in a much greater proportion than in the food. 30 This could suggest that the 5Z-lycopene is preferentially absorbed, or less rapidly cleared from the plasma, or that all trans lycopene undergoes isomerisation as a result of some biochemical interaction since simulated digestion in vitro does not cause significant acid-catalysed isomerisation.

It has been reported that 9Z-β-carotene preferentially accumulates in the lipoprotein carriers 31 but Gaziano et al. 32 found that there was a marked preferential absorption of the all trans-β-carotene in man. Supplementation of female volunteers with 15 mg per day of palm oil carotenoids (a mixture of trans- and cis-β-carotene) elicited a plasma response where the ratio of cis:trans forms was much lower than in the supplement. 18 This would indicate that the trans form is better absorbed than the cis form, or that the cis form is cleared from the plasma more rapidly. However, in ileostomists given an acute oral dose of all-trans-β-carotene and 9Z-β-carotene, both
isomers appeared to be equally well absorbed from the gut and cis–trans isomerisation did not occur during passage of the β-carotene through the GI tract. Currently it is unclear at what stage of absorption (mass transfer from food, dissolution in the lumenal lipid structures, absorption from the micelle, transport within the enterocyte and incorporation into chylomicrons) these effects occur, although simulated gastric digestion of lycopene does not produce cis isomers.

3 Systems for predicting carotenoid absorption

Various approaches have been used to assess carotenoid absorption and in each case assumptions are made in order to estimate values, either absolute or comparative. The limitations imposed by such assumptions should be borne in mind when considering data evaluation.

3.1 Mass balance methods

3.1.1 Faecal mass balance

Such studies are normally carried out over a period (5–8 d) known as the balance period. The chronic method is dependent on establishing an equilibrium between intake and excretion. This approach requires faecal markers at the beginning and end of the balance period to determine the ‘measurement’ period and, because absorption is the difference between intake and excretion, both values need to be determined with great accuracy. The use of food tables is insufficient and foods and supplements need to be assayed accurately.

If this method is applied to measuring absorption of a carotenoid from a single acute dose, the diet needs to be carotenoid free for 5 days before and during the test period and faecal collections need to be continued until no further carotenoid from the test dose is lost in the faeces. The collection period is usually 3–5 days giving a total study period of 6–10 days during which there will almost certainly be perturbation of the ‘normal’ plasma concentration of carotenoids due to the modification of the diet.

A major assumption when using the faecal balance technique to assess carotenoid absorption is that faeces are the only significant excretory mechanism of unabsorbed carotenoids, that there is no enterohepatic recycling, that the carotenoids recovered from the faeces are of dietary origin and that none of the unabsorbed carotenoid has undergone biotransformation, or otherwise been lost, due to the presence of the colonic microflora. The latter assumption gives cause for concern. Along with many other organic food components, the carotenoids are likely to be susceptible both to microbial degradation in the large bowel and to oxidative degradation. Thus, it is unlikely that unabsorbed carotenoids are quantitatively recov-
ered from the faeces. Unfortunately, much of the carotenoid absorption data from foods and isolates are based on either acute or chronic faecal mass balance methods and show great variability.

3.1.2 Gastrointestinal lavage technique
With this technique the entire gastrointestinal track is washed out by consuming a large volume (1 gallon/4.5 l) of ‘Colyte’ containing polyethylene glycol and electrolyte salts. Washout is complete with the production of clear rectal effluent (2.5–3.5h) and the volunteers then consume the test meal and are permitted only water or ‘diet’ soft drinks (non-caloric) for the next 24h. All the effluent is collected and pooled with the effluent collected on the following day when another dose of ‘Colyte’ is given to wash out the remainder of the test meal. The carotenoid recovered in the stool is subtracted from that fed to obtain an absorption figure. Difficulties associated with the method is that it is relatively time consuming, can only be applied to healthy individuals, and may give an underestimation of absorption if absorption is compromised or normal transit time is reduced due to the use of Colyte. In addition, as with the faecal mass balance, the method depends upon there being no degradation or loss of unabsorbed carotenoids. On the other hand, it has the advantage of at least standardising the residence time of carotenoids in the GI tract.

3.1.3 Ileostomy studies
In individuals who have undergone ileostomy, the colon has been surgically removed and the terminal ileum brought to a stoma on the abdominal wall. Ingested food passes through the stomach and ileum in around 6h as it would in the intact individual. The digesta (ileal effluent) can be recovered at regular intervals (2h) and all the residue from a test breakfast can be recovered in 12h if the volunteers are given carotenoid-free midday and evening meals. Test meals of either an isolated carotenoid or food can, therefore, be given to an overnight fasted volunteer at breakfast (without dietary modification) and the unabsorbed carotenoid recovered from the ileal effluent in real time without the delay of the colon and rectum, or the confounding influence of the colonic microflora. The model has the added advantage that an excretion profile can be obtained, the timing of which gives a time span for the absorption, which can in turn be compared to changes in plasma concentration over the 12h test period. Using this approach, absorption of all-trans- and 9-cis-β-carotene dispersed in a milk shake has been estimated at 75–90%.

3.2 Whole plasma methods
Measurements of absorption are usually carried out by the administration of an acute or chronic oral dose of isolated carotenoid, or carotenoid-containing food, and following the changes in plasma concentration of the
carotenoid of interest. Changes in plasma concentration are then interpreted as measures of absorption. Comparisons between plasma concentration excursions for different carotenoids should not be made without a knowledge of absorption and clearance kinetics or the form of the dose response curve. If assumptions are made these should be clearly stated and supported.

Absorption, although it comprises a major element of bioavailability, is only part of the story in that it take no account of the metabolic fate of the carotenoids. In assessing absolute absorption from plasma responses it is necessary to use a ‘disposal’ term. This disposal term takes account of the distribution of carotenoids to body ‘compartments’ other than plasma. The term does not identify these normally inaccessible body ‘compartments’ or ‘pools’ into which the carotenoids enter after leaving the plasma, but it does acknowledge their existence and influence. Lack of information results frequently in data obtained from whole plasma based methods being misinterpreted.

3.2.1 Acute doses
Such tests are usually carried out in fasted individuals who have restricted their dietary intake of the carotenoid of interest (and other carotenoids) for several days before the test day and for a period of days following. Blood samples are drawn at various time intervals after the test meal and the plasma/serum analysed for the carotenoid(s) of interest. Plasma concentration is then plotted as a function of time and the area under the curve (AUC) calculated as concentration \( \times \) time (\( Ct \)) This method cannot determine absolute absorption but it is possible to compare different doses and foods and derive some information as to the relative absorption by comparison to a standard dose, normally the isolated carotenoid. Such studies cannot normally be carried out blind because of the problem of disguising the treatment. A crossover design, with an adequate period of washout between treatments, is the most suitable approach so that each individual can act as their own control and data can be compared using a paired t-test. Each volunteer acting as their own control is essential since the AUC for the same dose in different individuals will be very variable, and such variability does not only depend upon the amount absorbed but on the absorption and clearance kinetics which may vary widely between individuals.

The measurement of absolute absorption of a carotenoid, calculated from the changes in plasma concentration following a single acute dose, is difficult and frequently misunderstood. The first point to deal with is the form and duration of the plasma response curve. Peak plasma concentration occurs at between 6h and 48h, depending upon the dose and the frequency of making the measurements. Since it is evident that the dose passes through the ileum in about 6h, the advent of plasma peaks found beyond this time can only result from delayed passage of carotenoid to the serosal
side after absorption into the enterocyte, or rapid absorption of carotenoid into the body, sequestration from the circulation, and then re-exportation to the plasma.

Evidence cited for the first case is a frequently found second plasma peak occurring following the meal. However, this is countered by lack of evidence for temporary storage in the enterocyte. There is no known storage mechanism, no ‘tailing’ of ileal loss in ileostomy patients\textsuperscript{16} and radiolabelled β-carotene absorption appears complete in less than 12\textsuperscript{h}.\textsuperscript{26,27} The second peak could simply result from an increase in the plasma lipids following a meal, providing the lipoprotein and triglyceride needed to transport carotenoid into the plasma. Alternatively, and most probably, the first peak in plasma concentration is due to the carotenoid present in the newly absorbed chylomicrons and the second peak, or prolonged duration of the first peak, results from hepatically re-exported carotenoid in VLDL and LDL.

The transfer of carotenoids from the short lived chylomicrons to the longer lived LDL and HDL (which appear to carry most of the carotenoid in fasting subjects) would also explain why the plasma concentration may remain elevated for up to and beyond 10 days post dose.\textsuperscript{35,36} Under such circumstances, the plasma AUC approach is not appropriate for the calculation of absolute absorption because the kinetics of absorption, disposal and re-exportation are not known. Even comparative studies of two sources within a single individual may not be valid unless the equation of the dose response curve is known and attenuated delivery or absorption caused by different physical characteristics of the meal is known not to occur.

3.2.2 Chronic doses

Chronic dosing with supplements or foods needs to be carried out until the plasma concentration reaches a plateau. This normally takes a period of weeks when supplementing with amounts of carotenoids in the region of 10–15 mg per day, and may increase the plasma concentration of β-carotene up to 10-fold; with other common carotenoids, particularly lycopene, showing smaller increases. Again, absolute absorption cannot be measured but the data may allow comparisons between isolated compounds and foods, and between different foods provided dose response is linear. As with the acute dose studies, it is essential that each study volunteer acts as their own control since the plasma response for the same dose in different individuals can be highly variable. Decay curves of falling plasma concentration of carotenoids, when supplementation is discontinued, may also provide some data on the half-life of the body carotenoid pool.\textsuperscript{37,38}

3.3 Plasma triglyceride rich lipoprotein (TRL) fraction approach

Newly absorbed carotenoids are initially present in plasma chylomicrons before they are sequestered by body tissues and re-exported in, or transferred to, other lipoprotein fractions (Fig. 7.1); see Section 7.1. Thus, mea-
measurement of carotenoids in this fraction, and a knowledge of the rate of clearance of the chylomicrons, should permit the calculation of rates of absorption, disposal and overall absorption based on AUC measurement.

This method has the advantage that chylomicrons present in fasting plasma are few and they are almost devoid of carotenoids. Thus, response following a carotenoid-meal is more clearly defined (Fig. 7.2). The disadvantage is that the plasma has to be ultracentrifuged to separate the lipoprotein classes. Ultracentrifugation, however, does not normally permit the separation of the chylomicron fraction free of other low density lipoproteins, particularly the VLDL, which may be the primary vehicle for the hepatic re-export of absorbed carotenoids. Recent work has shown that chylomicrons and VLDL can be separated by less vigorous centrifugal treatment.

In addition, absorption data based on oral AUC in TRL, and the theoretical AUC that would be obtained if the dose had been administered intravenously (using plasma volume and chylomicron clearance half life), give results that differ. For β-carotene Van Vliet et al., calculated an absorption figure of 11% (central cleavage) or 17% (eccentric cleavage), whereas O’Neill and Thurnham calculated 3.9% and 2.5% absorption in males and females, respectively, assuming only central cleavage. Both authors assume a cited chylomicron remnant half life of 11.5 min. However, a true clearance rate of carotenoid in the TRL fraction can also be obtained from the graph of TRL carotenoid concentration vs time and this could also be used to provide a carotenoid half-life term which would be independent of assumptions based on lipid kinetics. It is worth noting that much shorter half lives (2.5–7.9 min) have been reported for the clearance of chylomicron triglyceride and use of these values, rather than those of chylomicron triglyceride, would be more appropriate.

![Graph](image-url)

### Figure 7.2
Whole plasma and TRL fraction lutein concentrations over 12 h following a spinach meal (150 g).
remnant clearance,\textsuperscript{43} has the effect of proportionally increasing the apparent absorption (percent absorption doubles every time the half life is halved). The plasma chylomicron concentration will depend both on the lipid load and the ability of the individual to clear the chylomicrons from the plasma and unless this is known it will introduce errors by the use of inappropriate half-life values. The calculation of absorption, using a theoretical plasma concentration excursion based on plasma pool size and a theoretical intravenous dose,\textsuperscript{40,41} must therefore be treated with caution unless exact clearance kinetics of the carotenoids are known.

Some difficulties in explaining carotenoid kinetics may arise from:

- The observations that the triglyceride in the TRL peaks at 2 h whereas the $\beta$-carotene peaks at 5–6 h.\textsuperscript{40}
- Individuals who are highly variable in their plasma and TRL response (or not) to oral $\beta$-carotene.\textsuperscript{35,39,44}
- Individuals with a high concentration of plasma $\beta$-carotene appear to be those that show the greatest increment in plasma carotenoid concentration on supplementation.\textsuperscript{18}

### 3.4 Isotope methods

The use of radioactive tracers in human volunteers to determine the bioavailability of the carotenoids is not now possible because of ethical constraints. There are, however, two studies\textsuperscript{26,27} in males using $^{14}$C and $^3$H. These studies provide useful information on the duration and extent of absorption of $\beta$-carotene and the degree of conversion to retinol. Absorption of radiolabelled $\beta$-carotene was found to be in the range 8.7–16.8 % but most of this was recovered as the retinyl esters. This indicated that $\beta$-carotene absorbed by this route was largely converted to retinol. Peak absorption was found to be at 3–4 h and 6–7 h for each of two volunteers, respectively, and this time coincided with maximum lactescence in the lymph as assessed visually. In both cases, despite the relatively low absorption, no further radiolabel was found in the lymph after 12 h. Transitory storage in the enterocytes, prior to transfer to the serosal side, would probably have been detected as a tailing of the absorption curve and the high level of conversion may explain why elevation of plasma $\beta$-carotene is not always seen in volunteers given small acute doses.

The use of stable isotopes is more ethically acceptable. Highly labelled $\beta$-$[^{13}]C$ carotene has been used to study the metabolism of $\beta$-carotene in man.\textsuperscript{45,46} The single acute oral dose used in these studies was 1–2 mg of purified labelled ($>$95 % $^{13}$C), dissolved in tricaprylin or safflower oil and given with a standard meal. Blood samples were drawn at intervals and the $\beta$-carotene, retinol and retinyl esters separated, quantified and purified by HPLC (high performance liquid chromatography). The $\beta$-carotene (converted to the perhydro derivative by hydrogenation over platinum oxide),
and the retinol and retinol derived from retinol esters, were subjected to
gas chromatography-combustion-isotope ratio mass spectrometry (GCC-IRMS). The method was sufficiently sensitive to track the $^{13}$C in retinol esters up to 2 days and $\beta$-carotene and retinol up to 25 days.

Potentially, the use of $[^{13}$C$]$ carotenoids, either as an isolate or within a food, should permit the measurement of absolute absorption and the kinetics of disposal and conversion to other metabolites. An alternative to the use of $\beta$-$[^{13}$C$]$ carotene is octadeuterated $\beta$-carotene ($\beta$-carotene-d$_8$), an isotope that can be separated from natural abundance $\beta$-carotene by HPLC, thus, avoiding the use of mass spectrometry.$^{47}$ The retinol-d$_4$ derived from $\beta$-carotene-d$_8$ has to be separated from the plasma using a solid phase system$^{48}$ and derivatised to the tert-butyldimethylsilyl ether$^{49}$ before measurement by gas-chromatography-mass spectrometry. The method has been applied successfully to the tracking of both $\beta$-carotene-d$_8$ and retinol-d$_4$ in human volunteers for up to 24 days after an oral dose of 73 $\mu$M (40 mg).$^{47}$ Application of a compartmental model Novotny et al.$^{50}$ indicated that 22% of the carotenoid dose was absorbed; 17.8% as carotenoid and 4.2% as retinoid. This result is close to the 11% absorption of $\beta$-carotene found by Van Vliet$^{40}$ but indicates much lower percentage conversion to retinol than that found using very small oral doses of $\beta$-$[^{13}$C$]$ carotene.$^{46}$ It is worth noting that the percentage conversion to retinol is dose and retinol status dependent and that retinol palmitate measurements need to be made to allow for this. Table 7.1 summarises the range of absorptions that may be found in the literature.

### 4 Maximising the bioavailability of carotenoids

Because the carotenoids are lipophilic they need to be transferred from their aqueous environment in vegetable tissue to the lipid phase of the food or digesta before they can be effectively absorbed. Barriers to this process are the binding proteins with which the carotenoids are associated in photosynthetic structures and the physical architecture and strength of the plant tissue. Besides these physical constraints the thermodynamics of dissolution in the absorbable lipid domains from protein–carotenoid complexes, carotenoid-rich lipid droplets and membrane-bounded crystalline structures need to be considered.

Mass transfer can only occur where the lipid structure (bulk lipid, lipid emulsion, micelle) is contiguous with the carotenoid and this intimate contact can only occur once the structure has been disrupted. The interfacial characteristics of the lipid structures are also important because they will control aggregation of food particles, a prerequisite for the close physical contact needed for mass transfer. Interfacial characteristics will be dependent on pH, salt concentration, surfactant (bile salt, phospholipid) and peptides in the food or generated by digestion of dietary proteins.
Cooking and mechanical comminution are the most common method of increasing availability, but other methods, e.g., co-processing with lipid as in prepared recipes, or enzyme treatments which cause cell separation or disruption (juice production) may be used. During digestion, any disease state that compromises fat absorption will reduce the absorption of lipid-soluble micronutrients. The consumption of fat replacers, e.g., Olestra®, or cholesterol absorption inhibitors, e.g., plant phytosterols, will also have a negative impact on the absorption of carotenoids51,52 and perhaps of other lipid-soluble micronutrients.

### 4.1 Processing and storage
Carotenoids are normally present in fruits and vegetables as predominantly the all-trans (E) form although in some cases there may be a considerable proportion of cis isomers (Z) particularly in algae. During processing there are a number of physical and chemical changes that need to be considered for their possible impact on bioavailability.

Thermal processing is normally undertaken to render the product edible, to eliminate any spoilage/pathogenic organisms and to inactivate enzymes. Cooking therefore softens the cell walls so that they are easily separated or

<table>
<thead>
<tr>
<th>Carotenoid</th>
<th>% Absorption</th>
<th>Source</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Carotene&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40–98</td>
<td>Isolate in oil</td>
<td>33</td>
</tr>
<tr>
<td>β-Carotene&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1–87</td>
<td>Raw carrot</td>
<td>33</td>
</tr>
<tr>
<td>β-Carotene&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4–22</td>
<td>Raw carrot, grated</td>
<td>33</td>
</tr>
<tr>
<td>β-Carotene&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1–48</td>
<td>Cooked carrot</td>
<td>33</td>
</tr>
<tr>
<td>β-Carotene&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25–56</td>
<td>Carrot puree</td>
<td>33</td>
</tr>
<tr>
<td>β-Carotene&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9–45</td>
<td>Raw spinach</td>
<td>33</td>
</tr>
<tr>
<td>β-Carotene&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6–88</td>
<td>Cooked spinach</td>
<td>33</td>
</tr>
<tr>
<td>β-Carotene&lt;sup&gt;b&lt;/sup&gt;</td>
<td>75–98</td>
<td>Isolate in milk shake</td>
<td>16</td>
</tr>
<tr>
<td>β-Carotene&lt;sup&gt;c&lt;/sup&gt;</td>
<td>17–52</td>
<td>Isolate</td>
<td>34</td>
</tr>
<tr>
<td>β-Carotene&lt;sup&gt;d&lt;/sup&gt;</td>
<td>11–17*</td>
<td>Beadlets</td>
<td>40</td>
</tr>
<tr>
<td>β-Carotene&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.4</td>
<td>Isolate, capsule</td>
<td>41</td>
</tr>
<tr>
<td>Lutein&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.7</td>
<td>Isolate, capsule</td>
<td>41</td>
</tr>
<tr>
<td>Lycopene&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.6</td>
<td>Isolate, capsule</td>
<td>41</td>
</tr>
<tr>
<td>β-Carotene&lt;sup&gt;e&lt;/sup&gt;</td>
<td>22</td>
<td>Deuterated isolate</td>
<td>50</td>
</tr>
<tr>
<td>β-Carotene&lt;sup&gt;f&lt;/sup&gt;</td>
<td>9–17</td>
<td>Isolate, radiolabelled</td>
<td>27</td>
</tr>
</tbody>
</table>

<sup>a</sup> faecal mass balance  
<sup>b</sup> mass balance in ileostomy volunteers  
<sup>c</sup> gastrointestinal lavage mass balance  
<sup>d</sup> calculated from plasma triacylglycerol rich fraction carotenoid excursion and hypothetical clearance kinetics  
<sup>e</sup> compartmental model based on plasma concentration excursion  
<sup>f</sup> based on recovery of radiolabel from thoracic duct  
<sup>*</sup> assuming central (11%) or eccentric cleavage (17%)
broken mechanically, all cellular membranes are destroyed and proteins denatured. The carotenoids, normally stable within the original structure, are then exposed to the external environment where they may be subject to light, atmospheric oxygen and oxidised or reactive products of other components. Lycopene (tomatoes) and lutein and β-carotene (green leaves) appear to be quite stable in the fresh tomato and green leaf even when exposed to intense sunlight. During processing the protection of the native environment is lost and the carotenoids are readily oxidised and photo-bleached. This is particularly true if the product is dried and exposed to the air and light. It has been shown that excessive thermal processing may also create cis (Z) isomers, particularly 5Z-lycopene although isomerisation can also occur at positions 9,13 and 15.β-carotene although isomerisation can also occur at positions 9,13 and 15.30,53 Cis isomers with their kinked structure tend to be more soluble in organic solvents and this change in physical properties may have an influence on the ease with which they are absorbed by the gut, their partitioning between the various lipoprotein carriers and half life in the plasma.30,53

Losses of carotenoid that occur after thermal processing and storage in anaerobic and light-free conditions (e.g. canning) are slight and may be as a result of oxidation by compounds formed enzymically or thermally during processing. Processing, however, increases the availability of lycopene for absorption, particularly if processed in the presence of lipid.10,11 It is also recognised that dietary fat itself improves carotenoid bioavailability.37,54–56

4.2 Interactions

Single acute oral doses of lutein and β-carotene have shown that the two carotenoids interact to reduce the apparent absorption of lutein as measured by the plasma area under the curve (AUC), and in some instances lutein has been shown to reduce the AUC for β-carotene.57 Similarly, a combined dose of β-carotene and lycopene does not appear to affect the absorption of β-carotene but enhances the absorption of lycopene.58 Short term supplementation of volunteers with β-carotene, either as a pure compound or as the major constituent of a natural β-carotene source, has also been found to reduce the plasma concentration of lutein.38,23,18 However, in a long term study (4 years) of β-carotene supplementation, although there was a trend, the reduction of plasma lutein was not significant.59 The other carotenoids, therefore, do not appear to affect the apparent absorption of lycopene, possibly because only β-carotene and lycopene are the main hydrocarbon carotenoids. Although they use the same carriers both in the gut lumen and in vivo (LDL) carrier capacity is probably not limiting.60

With respect to other carotenoids and vitamin E, β-carotene supplementation of volunteers with colorectal adenomas for 2 years resulted in highly significant increases in plasma lycopene and α-carotene in both men and women.61 However, short-term supplementation (15mg per day
β-carotene for 35 days) of apparently healthy volunteers had no effect on the plasma concentration of lycopene, or on plasma vitamin E.\textsuperscript{18}

In humans, concurrent feeding of acute doses of β-carotene and canthaxanthin was found to inhibit the plasma appearance of canthaxanthin but there was no converse interaction.\textsuperscript{62} The observation that the carotenoid profile in the TRL fraction is not the same as in a supplement\textsuperscript{63} clearly indicates that in order to assess bioavailability of any one carotenoid the carotenoid profile of the supplement or food needs to be defined, as does the amount and type of fat in the test meal.\textsuperscript{64} These findings suggest that β-carotene has a sparing effect on lycopene and that if this is the case other ‘interactions’ \textit{in vivo} may also occur which will need to be considered when looking at plasma carotenoid profiles and dietary habits.