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ORIGINAL CONTRIBUTION

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Kinetics of gastro-intestinal transit and carotenoid absorption and disposal in ileostomy volunteers fed spinach meals

Summary *Background* Reports of low carotenoid absorption from food sources has undermined their postulated 'protective' role as one of the active agents in diets rich in vegetable matter. *Aims of the study* This study quantified β-carotene and lutein absorption from a representative green vegetable with different degrees of processing, using both mass balance and metabolic modelling of triglyceride-rich lipoprotein plasma fraction (TRL) response. *Method* Whole or chopped-leaf cooked spinach was fed to volunteers (n = 7, paired) with vegetable oil (40 g) in yoghurt. Blood and ileal effluent samples were collected for up to 24 h. Effluent and TRL samples were analysed for lutein and β-carotene by HPLC. A digesta transit model was used to describe meal transit and a single compartment model used to predict percentage absorption from the plasma TRL response. *Results* Mass balance showed 25 % of lutein and β-carotene were absorbed from chopped spinach, compared with 25 % β-carotene and 40 % lutein from whole-leaf spinach. Increased lutein absorption correlated to slower gastrointestinal (GI) transit for the whole-leaf meal. An area under the curve (AUC) response for the TRL fraction, found in 50 % of cases, was not confined to those with the greatest percentage absorption. Absorption by mass balance and TRL AUC indicate a half-life of newly absorbed carotenoid around 11 min *Conclusion* GI residence time appears to have an effect on the absorption of lutein but not β-carotene. Rapid clearance is probably the main reason for absence of measurable plasma concentration excursions. Lack of plasma response cannot be interpreted as lack of carotenoid absorption without knowledge of the absorption and disposal kinetics.

Key words Lutein – β-carotene – absorption – ileostomy – model

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Abbreviations

AUC	Area under the curve
BMI	Body mass index
GI	Gastro-intestinal
HDL	High-density lipoproteins
HPLC	High performance liquid chromatography
LDL	Low-density lipoproteins
SD	Standard deviation
SI	Small intestine
TGWM	Total gastrointestinal washout method
TRL	Triglyceride rich lipoproteins
VLDL	Very low density lipoproteins
IV	Intravenous

Introduction

The absorption of carotenoids from test meals is reported to range between 2–90 % depending upon the test material, the model used to assess absorption and interpretation of data obtained [1]. Reports of low absorption values [2, 3], obtained using changes in plasma concentration have undermined the hypothesis that the carotenoids are one of the active protective constituents of diets high in vegetables and fruits, and that supplemental green vegetable feeding may be ineffective in relieving retinol deficiency [4]. Plasma concentrations of the carotenoids may be elevated significantly in chronic dosing studies [5, 6] but the absolute amount absorbed cannot be quantified by this approach.

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The absorption of carotenoids has been attempted in human volunteers by a number of methods. Oral-faecal mass balance [7], whole plasma response after chronic [3, 8–10,] or acute [11, 12] dosing, triglyceride-rich lipoprotein (TRL) response after an acute dose [2, 13], radioactive tracers [14, 15], stable isotope tracers [16–18], total gastrointestinal washout, mass balance method (TGWM) [19] and the ileostomy mass balance model [20].

Of the mass balance methods, the oral-faecal approach has been the most commonly used. However, the method is prone to losses of carotenoid through their exposure to the microflora of the large intestine and precise faecal collection may be a problem. Estimates of absorption by this method are therefore predicted to be high and variable.

A second, frequently used approach is measuring carotenoid concentration in whole plasma following an acute (single meal), or chronic, dose (multiple meals over several days/weeks). Plasma response after a single meal has identified many individuals who do not appear to 'respond', i. e. no measurable change in plasma concentration; sometimes interpreted as no, or low, carotenoid absorption. This interpretation has been challenged following consideration of plasma clearance kinetics and the likelihood, or not, of observing a perturbation in the plasma pool after a single meal [20]. In chronic feeding studies, plasma concentrations of carotenoids are more likely to be significantly elevated, but the amount absorbed still cannot be quantified without a knowledge of the clearance kinetics from all appropriate plasma pools and re-exportation kinetics from liver and other tissues. Furthermore, changes in plasma concentration after feeding a 'standard' isolated compound vs. a food are usually assumed to follow a linear dose response. At best, absorption from the food can only be expressed as a percentage of the absorption from the 'standard' dose.

In the present study the ileostomy approach has been selected as the preferred mass balance method for obtaining quantitative data on the absorption of carotenoids from an important food source (green leafy vegetable). Whilst there may be some microbial activity in the SI through colonisation of the terminal ileum, appropriate collection and storage of effluent minimises the potential confounding influence of microbial action, and variable residence time in the large bowel.

As an alternative to mass balance measurements, the study described here also included carotenoid analysis of whole plasma and the triglyceride-rich lipoproteins to produce comparative data between loss of carotenoid from ileal effluent and appearance of carotenoids in blood. Newly absorbed carotenoids, from a meal given after an overnight fast, appear in the TRL. The AUC for the TRL-carotenoid response is measured, clearance rate assumed to be the same as for chylomicrons and carotenoid absorption quantified [13]. However, the actual clearance kinetics for chylomicron remnant half life [21–23], or chylomicron triacylglycerol half life are not known for the individual volunteers, and have to be assumed from limited published information [24], gives rise to large differences in carotenoid absorption values, depending on the half life value selected. By quantifying the amount of carotenoid lost in ileal effluent, together with the size of the TRL excursion, calculation of the carotenoid half-life was possible and assumptions relating to chylomicron remnant half-life tested.

In summary, the objectives of the study were to quantify β-carotene and lutein absorption from a representative green vegetable with different degrees of processing; using both mass balance and metabolic modelling of TRL response. This dual approach allowed comparison of the data sets obtained and testing of assumptions relating to clearance kinetics of carotenoids from the TRL fraction.

Experimental

A group of seven ileostomy volunteers who had minimal ileal resection (< 15 cm) for ulcerative colitis (5 women and 2 men), mean (SD) age 51 (7.6) y, weight 80.1 (16.4) kg gave informed written consent to the study which was approved by the Norwich District Ethics Committee. Volunteers had BMI values of 19–27, fasting plasma cholesterol ≤ 6.5 mmol/l, fasting plasma triglycerides ≤ 2.3 mmol/l, plasma β-carotene 0.1–1.0 μmol/l and plasma retinol ≥ 0.1 μmol/l, were non-smokers, not taking medication or dietary supplements. All but one consented to provide serial blood samples over the duration of the 2 study periods. Volunteers attended the Human Nutrition Unit on two occasions, at least 6 weeks apart, having avoided excessive carotenoid intake (a list of foods was provided) for 24 h before the study day. They arrived at 08.00 h having fasted from 19.00 h the previous evening and having performed their usual morning routines.

Volunteers were cannulated (antecubital/cephalic), provided a baseline fasting blood sample (20 ml), emptied their appliances (baseline effluent collection) and were then given approximately 150 g of either cooked whole, or cooked finely chopped, leaf spinach prepared from the same harvest. The spinach meal contained approximately 15 mg lutein and 10 mg β-carotene. The spinach, which had been blanched, drained, frozen and stored at –40 °C in heat sealed foil laminate pouches, was reheated in boiling water to a core temperature in excess of 72 °C for 3 min. A sub-sample of the spinach meal was retained at –70 °C for analysis. The spinach meal was followed by 400 g of skimmed milk yoghurt containing 40 g of low vitamin E sunflower oil, 20 g of sucrose and chocolate flavouring. The study was timed from when

the spinach was consumed (t = 0). Defined carotenoid-free midday (t = 4.5h) and evening (t = 10 h) meals were provided and carotenoid free drinks were freely available at all times. The midday meal provided 20 g of fat (25 % of energy) and the evening meal 42 g of fat (28 % of energy).

Volunteers remained seated in an armchair for the duration of the study except for toilet visits. This procedure was adopted to harmonise physical activity in all volunteers although it is recognised that it may have had an impact on gut motility. Blood samples (20 ml) were drawn every 2 h from t = 0 up to 12 h, into lithium heparin blood tubes, centrifuged, the plasma separated and frozen at –70 °C before further treatment. Total ileal effluent was collected every 2 h up to 12 h and then as discrete timed samples up to 24 h at the volunteers' own convenience. All effluent samples and retained food samples, collected into polythene bags, were spread into thin sheets within the bag, frozen on solid CO_2, weighed and stored at –70 °C. Plasma (5 ml) was density adjusted with potassium bromide, layered into saline (1.006 sg) and ultracentrifuged for 4 h at 64,000 g [25] to separate the lipoproteins into TRL, LDL, and HDL fractions. The fractions were then aspirated from the centrifuge tube and stored at –70 °C.

Plasma and plasma fractions were extracted using hexane and the carotenoid content assayed by HPLC [6] with a limit of quantification < 1 ng. Effluent and spinach samples (ca. 8 g) were broken from the frozen sheets, placed in 50 ml screw top glass centrifuge tubes and 20 ml of acetone added. The effluent was thoroughly dispersed using a small homogeniser (Ultra Turrax), the mixture centrifuged for 10 min at 2000 g to pellet the solids and the supernatant transferred to a 100 ml volumetric flask. The pellet was re-suspended in 20 ml acetone and the process repeated 3 more times. The acetone extract was made up to 100 ml, thoroughly mixed and a filtered (No.1 paper. Whatman) sub-sample (20 ml) stored at –20 °C. A sub-sample (1 ml) of the filtered acetone extract was dried under N_2, made up in HPLC mobile phase, diluted if needed, and assayed by HPLC as above.

Statistics

One tailed Student's paired t-test was used to determine whether carotenoid absorption (within subject) was significantly different ($p < 0.10$, because of the low value of n) between the whole leaf and finely chopped leaf spinach test meals. A one-tailed test was used because it was believed that absorption from the chopped spinach would be greater than from whole leaf spinach because of the more extensive tissue disruption. A 'within subject' correlation of the absorption of β-carotene from whole leaf and chopped leaf spinach was undertaken to test if the magnitude of absorption was volunteer consistent, i. e. whether a higher absorption from whole leaf spinach predicted a higher absorption from chopped leaf spinach, and *vice versa*. Likewise, a correlation for lutein absorption was also undertaken. Fasting plasma concentrations of β-carotene and lutein were correlated with percentage absorption to test if there was a relationship between habitual plasma concentration and absorption from the test meal. Regression analysis was used to assess if physical performance of the meal in the GI tract (lag phase, rate, $t_{1/2}$) was related to absorption.

Data treatment

To characterise the appearance of the spinach meal in the ileal effluent, lutein was used as a marker. The percentage of the original spinach meal collected at each time point was calculated from the total recovered lutein and the amount of lutein found in each collection. Normalised cumulative collection (percentage) of the spinach meals were plotted against time to provide a transit profile of the spinach and the data fitted using a logistic model to calculate the t1/2 of the gastrointestinal residence time. The portion of the curve that appeared to be steeply increasing with time was fitted with a regression model. The slope is equivalent to the effluent production rate, the intercept (x at y = 0 %) the lag time and time for complete passage of the meal (x at y = 100 %).

The total loss of lutein and β-carotene to the effluent and the amount in the spinach meal were calculated and expressed as percentage absorption of the carotenoid content of the meal.

Digesta transit model

The normalised cumulative effluent loss (y) vs. Time (t) data was fitted using the following 2-parameter equation:

$$y = 100 \cdot \left[\frac{e^{-a+b \cdot t}}{1+e^{-a+b \cdot t}} \right]$$

The time taken to reach 50 % loss is given by:

$$t_{1/2} = a/b$$

The slope and y-intercept were found from the linear regression of the data points in the central (linear) section of the normalised cumulative effluent loss vs. time plot. The regression model can be summarised as:

$$y = mt + c$$

where (m) is the slope and is equivalent to the effluent production rate and (c) is the y-intercept. The x-intercept of this line is the lag time and is calculated from:

$$\text{lag time} = -c/m$$

100 % transit time (y = 100) can be calculated from:

$$t = \frac{100 - c}{m}$$

TRL area under the curve model.

The areas under the curve (AUC_{oral}) of these plots were calculated using the Altman trapezoidal approximation method [26]. The integrated area under the curves for the TRL response in two of the volunteers who gave complete TRL curves within the 12 h blood sampling period were calculated and compared to the theoretical TRL AUC that would have been obtained if the carotenoids had been given as an intravenous bolus. The half life of the carotenoids in the TRL is not known, thus the theoretical TRL AUC from the bolus was calculated at a number of time points between 2 and 11 min to embrace the chylomicron triacylglycerol $t_{1/2}$ of 2–5 min, up to the chylomicron remnant $t_{1/2}$ of 11 min.

After correcting for the background TRL concentration of carotenoid (lutein and β-carotene), a plot of TRL concentration against time was constructed which represents the TRL response to the oral dose. A single compartment model (Fig. 1) can be assumed with only disposal (k_1) from the TRL fraction, because TRL was the only pool to have an input exclusively (theoretical or real) of newly absorbed carotenoid.

By assuming that a single compartment model (Fig. 1) will approximate chylomicron clearance from the plasma, various plasma half-lives ($t_{1/2}$) were simulated using the SAAM II modelling package [27] to investigate the absorption of an oral dose of carotenoid.

For each $t_{1/2}$, the plasma response to an intravenous (IV) dose was simulated using the above model. The plasma volume of each volunteer was estimated [28] and given as an input parameter in the model. The IV dose was adjusted until the simulated area under the curve (AUC_{iv}) was the same as that found experimentally from the oral dose (AUC_{oral}). Under these conditions, the simulated IV dose was the same as the amount of carotenoid actually absorbed from the oral test meal.

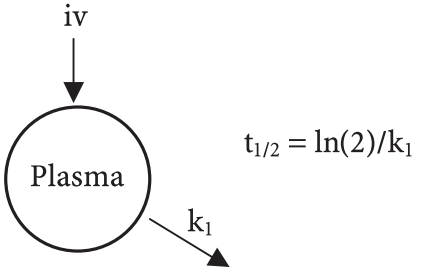


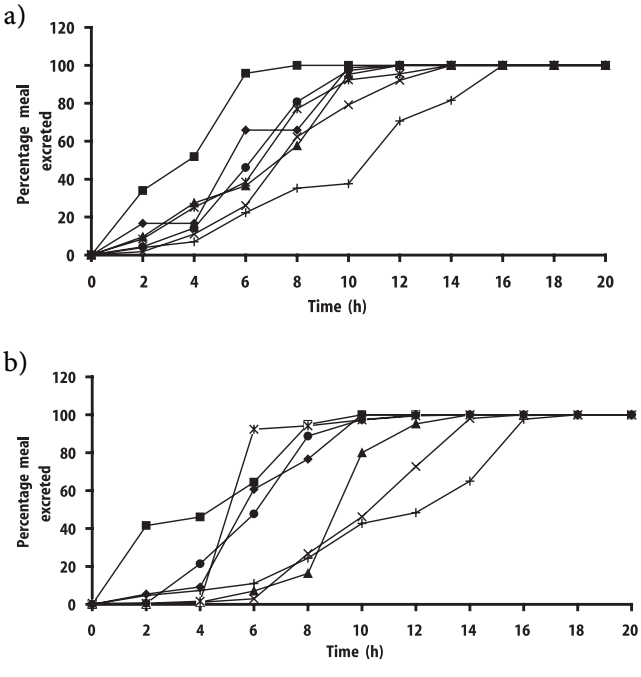
Fig. 1 Single compartment model. k_1 represents the clearance rate of the carotenoids entering the plasma pool as a theoretical intravenous (IV) dose. The half-life ($t_{1/2}$) of the carotenoid in the plasma pool is given by the equation

Results

Meal behaviour in the GI tract

Figs. 2a and 2b show the normalised appearance of spinach meal in the ileal effluent.

The calculated mean lag phase of the initial appearance of the effluent from the chopped leaf spinach meal (2.6 h, range 0.1–4.5 h) was shorter (p = 0.078, n = 7) than that from the whole leaf spinach meal (3.6 h, range 0–6 h). The mean half-life of the chopped spinach meal in the stomach and small intestinal (SI) tract (6.5 h, range 3.4–10 h) was shorter (p = 0.08, n = 7) than that for the whole leaf meal (7.4 h, range 4–11.3 h). The mean rate of mass transit through the SI tract as estimated from the slope of the cumulative collection of spinach in the ileal effluent was the same with both meals. The chopped spinach meal passed the whole SI (12.6 h, range 8–16 h) more rapidly (p = 0.099, n = 7) than the whole leaf spinach meal (13.4 h, range 10–18 h). The transit



Volunteers, A ◆, B ■, C ▲, D ×, E ✶, F ●, G +.

Fig. 2 The normalised appearance of the spinach meals in the ileal effluent of 7 volunteers fed chopped leaf spinach (**a**) or whole leaf spinach (**b**)

time of the whole leaf spinach meal at all time points was about 1 h longer than that of the chopped leaf meal.

Absorption of lutein and β-carotene by mass balance

All the meals contained approximately 15 mg lutein and 10 mg β-carotene, the exact amount being measured by weighed intake and assay of a retained portion of the meal. The mean ratio of lutein: β-carotene was 3:2 in the whole leaf and chopped spinach. Chopping and drip loss from the prepared spinach meals did not affect this ratio.

The percentage absorption of lutein and β-carotene from leaf and chopped spinach is shown in Table 1.

Lutein absorption from whole leaf spinach (mean 44 %, range 28–58 %) was greater ($p = 0.01$, $n = 7$) than from chopped leaf spinach (mean 26 %, range 8–58 %) whereas the mean absorption of β-carotene from both meals was similar (chopped 23 %, range 4–41 %, whole leaf 26 %, range 9–58 %). Percentage lutein absorption from all meals was weakly correlated ($R^2 = 0.27$, $p = 0.05$, $n = 13$) with lag phase of initial appearance of spinach in ileal effluent by the equation: percentage absorption = 4.6 x lag phase (h) + 20.4. This indicated about 20 % lutein absorption with the shortest (> 2 h) lag phase and up to 55 % absorption with the longest (6 h) lag phase. Residence time in the SI tract altered the ratio of β-carotene: lutein absorbed. There was equal mass absorption of both carotenoids for short lag phase rising to 3:2 lutein:β-carotene at longer residence times. Absorption of β-carotene and lutein were also weakly correlated ($R^2 = 0.43$, $p = 0.01$, $n = 14$); percentage β-carotene absorbed = 0.49 x percentage lutein absorbed + 7.7.

There was no relationship between fasting plasma concentration of lutein or β-carotene and the amount absorbed measured by mass balance.

Table 1 Percentage absorption lutein and β-carotene from spinach (whole leaf and chopped leaf) meals by mass balance in ileostomy volunteers

	Lutein % absorbed		β-Carotene % absorbed	
Volunteer	Whole	Chopped	Whole	Chopped
A	33.6	28.0	22.4	30.0
B	28.0	16.0	23.0	20.6
C	51.0	26.7	32.0	21.0
D	52.7	7.3	8.7	4.3
E	29.6	14.9	13.0	14.7
F	57.0	57.9	52.1	40.9
G	58.5	33.2	33.7	32.5
Mean	44.3*	26.3	26.4	23.4
(SD)	(13.4)	(16.6)	(14.5)	(12.2)

* Whole leaf lutein is significantly greater ($p < 0.05$) than chopped leaf lutein

Plasma and lipoprotein β-carotene and lutein response

Fasting plasma β-carotene (mean ± SD) was 216 ±171 ng/ml and lutein 135 ±72 ng/ml.

None of the volunteers ($n = 6$) showed a plasma response for β-carotene or lutein with either chopped or whole leaf spinach, probably because the amounts absorbed over the time taken for the spinach to pass through the SI tract were too low (β-carotene, 2.5 mg; lutein 3.75–7.5 mg). There was also no measurable carotenoid response in either LDL or HDL fractions. However, there was a measurable TRL fraction response in 50 % of the volunteers ($n = 3$) for both β-carotene and lutein from chopped and whole leaf spinach, but complete curves were obtained for only 2 volunteers within the 12 h period of blood sampling. The absorption, measured by mass balance in these 2 volunteers, was compared to that predicted at various half life ($t_{1/2}$) values (Table 2).

Table 2 Predicted and measured absorption of carotenoids from spinach meals by TRL response. Lutein and β-carotene TRL absorption kinetics from spinach meals: Predicted percentage absorption from a single compartment model with various values of $t_{1/2}$ and absorption measured by mass balance in two volunteers

	Lutein				β-Carotene			
Volunteer Meal Type	B WL	B CL	G WL	G CL	B WL	B CL	G WL	G CL
$t_{1/2}$ (min)	Predicted Absorption %				Predicted Absorption %			
2	17	68	36	44	18	87	141	58
3	11	45	24	29	12	58	94	38
4	8	34	18	22	9	43	71	29
5	7	27	14	18	7	35	57	23
8	4	17	9	11	5	22	35	14
11	3	12	7	8	3	16	26	10
Measured Absorption%	28	16	58	33	23	21	34	33

WL Whole leaf spinach. *CL* Chopped leaf spinach

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In most cases, the measured absorption falls within the range predicted from $t_{1/2}$ values in the range 2–11 min. The exception was the measured absorption of lutein from whole leaf spinach, which was greater than could be accounted for by even the most rapid plasma clearance.

Discussion

Understanding the concept of bioavailability is essential to all involved in food production, nutritional assessment and determination of diet:health relationships. However, the absorption and post-absorptive metabolism of many of the bioactive organic components of foods is complex and not fully understood. The carotenoids provide an excellent example of where too little understanding of the complexity of the behaviour of a food component within the food matrix, during digestion, absorption and clearance and within human tissues, can lead to naïve interpretation of study results.

Faecal mass balance studies are constrained by (a) dietary modification, (b) prolonged sample collection, and (c) the assumption that loss is the same as absorption. Some of these criticisms can be overcome by using modified mass balance methods such as the ileostomy model [20] and TGWM [19].

Interpretation of plasma or plasma fraction carotenoid excursions can only be undertaken with a clear knowledge of the absorption and clearance mechanisms and by sampling the most appropriate 'pool'. Even so, there are inevitably assumptions that need to be invoked and justified when using plasma response models.

Particular care must be taken with chronic dosing when comparing 'relative bioavailability' for four main reasons, (a) the absence of a knowledge of dose response, (b) change in plasma concentration induced by a test food relative to the free compound does not provide an absolute absorption, (c) change or rate of change of plasma carotenoid concentration can be constrained if the doses exceed the capacity of the gut to absorb or the plasma to carry, thus all excessive doses will provoke the same plasma response, (d) at what point during the supplementation period are the relative plasma responses to be measured?

Acute studies also present specific problems. These relate to (a) observing small changes in plasma concentration against a high endogenous background, (b) avoiding the confounding influence of sequestration from and re-export to the plasma pool, (c) lack of knowledge of absorption and clearance kinetics, (d) lack of knowledge of dose response relationship curves.

Newly absorbed carotenoids appear in the plasma chylomicron fraction (TRL), which is virtually free of carotenoids in the fasting state; thus measurement of the TRL response avoids both the problems of quantifying small changes against a high endogenous background and the problems arising from carotenoid trading between other body pools.

The present study sought to quantify β-carotene and lutein absorption from a representative green vegetable, using two experimental systems (together with metabolic modelling) to allow comparison, and more rigorous examination and interpretation, of the data sets obtained.

Mass balance: effects of food structure

Most nutrients have specific absorption mechanisms but many minor lipophilic components are passively absorbed from the gut as an integral part of lipid absorption. Such components, if present in foods of plant origin, must be extracted from their native environment and dissolved in appropriate lipid carriers. Intuitively, breaking up the cellular structure of the food, the presence of lipid, bile salts, lipases and the correct pH should increase the probability of achieving maximum absorption. Absorption from cooked processed foods may be very different by comparison with that from raw; however, disruption of plant cell architecture, beyond that occurring during the relatively mild processing used here, did not influence the proportion of β-carotene absorbed from spinach *in vivo* (Table 1).

The increased absorption of lutein from the whole leaf spinach was unexpected, since it was assumed that the larger particle size would slow down mass transfer to absorbable lipid structures in the stomach and ileum. However, recent studies of carotenoid mass transfer in an *in vitro* gastric and duodenal environment (using samples taken from spinach as fed to human volunteers) demonstrate that whilst the rate and limit of mass transfer are the major controlling factors of transfer to the lipid phase, time is much more crucial for the transfer of lutein [polar] than β-carotene (apolar) (Fillery-Travis. A. Personal Communication). This would explain the enhanced absorption of lutein from the whole leaf spinach and the lack of self-consistency of lutein absorption (Table 1), which might be confounded by unquantified transit rate fluctuations or changes in luminal conditions which affect lutein, but not β-carotene, absorption.

Mass balance: inter- and intra-individual response

Inter-individual β-carotene absorption response to the same food was highly variable but intra-individual response was consistent ($R = 0.887$, $n = 7$, $p = 0.01$) between different forms of the same food (Table 1). A self-consistency of plasma response has also been seen in

chronic supplementation studies [6]. This could be due to two mechanisms: those individuals who exhibit low plasma concentrations of β-carotene (a) absorb less β-carotene, or (b) the β-carotene is absorbed to the same extent but is not retained in the plasma pool. The converse would be true for those showing high plasma β-carotene concentrations.

The data show wide variation between individuals with regard to lutein absorption but, unlike β-carotene, there was no self-consistency between the different forms of spinach meal despite there being a weak correlation between lag phase and amount absorbed ($R^2 = 0.27$, $p = 0.05$, $n = 13$), with the longer residence time doubling the absorption.

Modelling of TRL response

Those individuals that showed a TRL response to β-carotene and lutein, from both chopped and whole leaf spinach, were the same in both cases but were not the individuals that gave the greatest absorption. The fact that there is a measurable TRL response at all levels of absorption (depending on the individual) is a reflection of clearance from the plasma rather than absorption (confirmed by mass balance). Although all the volunteers had 'normal' plasma lipids, turnover was not measured. It would be expected that individuals with rapid clearance of chylomicrons would show the smallest change in TRL concentration of carotenoids because they would not accumulate in the plasma to any measurable extent. Those volunteers with slower lipid turnover may be those that exhibit measurable TRL carotenoid excursions. This might be the reason why the volunteers fell into consistent groups of TRL 'responders' and 'non-responders' despite the fact that carotenoid absorption occurred in all cases.

Two individuals gave complete TRL AUCs for both lutein and β-carotene, over the 12 h of blood sampling, for both whole and chopped spinach meals (Table 2). Using the kinetic model described in this paper, the measured absorption of lutein from the whole leaf meal exceeded that which is predicted from the model, whereas for the chopped leaf meal the measured absorption falls within the range of acceptable $t_{1/2}$ (2–11 min). This indicates that GI transit rate of whole leaf spinach is positively associated with loss of lutein in the GI tract, which contributes to an increased loss (elevated measured absorption) but which is not seen in the TRL response. Alternatively, TRL is not an appropriate measure for newly absorbed lutein, which, because of its more polar nature, may be partially transported in portal blood by a mechanism un-associated with the chylomicrons and thus it was not detected. For the TRL lutein response to chopped spinach the percentage absorption can be predicted if the TRL $t_{1/2}$ is in the range 2–11 min. The range of $t_{1/2}$ does not allow any decision as to whether the lutein is cleared from the TRL in the extrahepatic capillary bed along with the triacylglycerols ($t_{1/2} \approx 2$–5 min), or remains with the chylomicron remnants (mean $t_{1/2} \approx$ 11.5 min).

For β-carotene the measured absorption exceeds that which would be predicted from a TRL $t_{1/2}$ of 11 min (Table 2). This is not surprising because some of the absorbed β-carotene will be converted to retinol in the enterocytes and it will therefore not appear in the plasma as β-carotene. However, if conversion is low (≈10 %) it will not significantly reduce the TRL β-carotene concentration and could still indicate that some of the β-carotene is absorbed in the extrahepatic capillary bed and the remainder being cleared by the liver along with the chylomicron remnants indicating a shorter $t_{1/2}$. Carotenoid losses, here interpreted as absorption because it is assumed that there are no microbial or oxidative losses, may in fact have occurred. If this is the case then absorption by mass balance in the ileostomy model (and oral-faecal model) is an overestimate and $t_{1/2}$ would be longer than that indicated.

Conclusion

Whole leaf spinach has approximately a 1 h longer GI transit time than chopped leaf spinach and this doubles the loss of lutein (25 % → 40 %) but has no effect on β-carotene (25 %). The attenuated delivery of the small amounts of both lutein and β-carotene from the spinach meals did not cause a measurable plasma AUC or responses in LDL and HDL fractions. TRL carotenoid responses were seen in 50 % of the volunteers but in only 2 cases were the whole curves within the 12 h blood sampling window. Future studies with foods should be run for at least 16 h to ensure the complete curves needed for modelling. In both volunteers, the measured absorption of lutein and β-carotene exceeds that predicted from a theoretical TRL $t_{1/2}$ of 11.5 min and this could be a result of luminal losses that are taken as absorption in the mass balance model but which do not appear in the TRL fraction. In the specific case of β-carotene some of the 'loss' will be accounted for by conversion to retinol.

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