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A study of the biological utilization of carotenoids of carrot and swamp cabbage in rats

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The biological utilization of carotenoids is related to their provitamin A activity and the efficiency of their absorption and conversion to retinol and also their function as antioxidants. For practical purposes, various authorities have taken the efficiency of conversion of β-carotene to retinol to be in the ratio of 6:1, and that of other provitamin A carotenoids as 12:1. This paper presents results of a study carried out to determine the bioavailability of carotenoids present in two carotenoid-rich vegetables (namely carrot and swamp cabbage, *kangkong*) and aims to provide further information on the nutritional value of these important sources of provitamin A compounds. The growth and the levels of retinol and carotenoids in the liver and serum of rats provided with these carotenoids in the daily diet were monitored. These results were compared with the biological utilization of crystalline retinol and β-carotene concentrates. Two different experimental procedures were adopted for the study, namely supplementation and depletion–repletion procedures using the rat as the test animal. Both procedures showed that the bioavailability of the major carotenoids in carrot (α- and β-carotene) and *kangkong* (β-carotene) was high, as evidenced by the accumulation of retinol in the liver of the experimental rats, in relation to crystalline retinol concentrate. The provitamin A activity of α- and β-carotenes in the vegetables approximates the commonly used factors of 1/12 and 1/6 that of retinol, respectively. Either one of the experimental procedures may be used for the study of bioavailability of carotenoids in plant sources. The supplementation procedure, however, takes a shorter time to carry out and could be advantageous. The 4-week supplementation period was quite sufficient to obtain clearly observable differences. To further reduce time and cost of analysis, a 2-week supplementation would suffice, and liver retinol and carotenoid concentration should be determined before and after supplementation. Such studies on the bioavailability of carotenoids should be extended to human subjects. Copyright © 1996 Elsevier Science Ltd.

INTRODUCTION

It has long been recognized that the content of nutrients in foods is not the sole determinant of their nutritional value. Several factors within and outside of the food can influence the availability of nutrients to the body. Even if a nutrient is present in high concentrations, its utilization may not be entirely satisfactory if there are factors that interfere with its availability. Thus, studies on bioavailability of nutrients are important in order to provide true information on the nutritive value of foods.

The biological utilization of carotenoids is a complicated subject, involving at least two broad issues. First, the carotenoids as a group vary greatly in provitamin A activity, depending on the chemical structure of the carotenoid. All-*trans*-β-carotene, with two unsubstituted β-ionone rings, with an attached polyene side-chain, is expected to have the full potential for conversion to retinol. Many carotenoids not meeting these structural requirements possess less or no vitamin A activity. The second issue relates to the biological utilization of the carotenoids present in the diet, which has to take into consideration the efficiency of their absorption into mucosal cells as well as their conversion to retinol. The bioavailability of carotenoids can be influenced by a host of factors, including the character of the mixed diet. For practical purposes, various authorities have taken the efficiency of conversion of β-carotene to retinol to be in the ratio of 6:1, and that of other

provitamin A carotenoids as 12:1 (WHO, 1967). Studies have been carried out by various investigators to verify this utilization rate in order to arrive at more accurate estimates of the nutritional value of carotenoids (e.g. Sweeney & Marsh, 1974; Mokady & Cogan, 1988; Dimitrov *et al.*, 1988; Brown *et al.*, 1989).

This paper presents results of a study carried out to determine the bioavailability of carotenoids present in two carotenoid-rich vegetables (namely carrot and swamp cabbage) by monitoring the growth and levels of retinol and carotenoids in the liver and serum of rats provided with these carotenoids in the daily diet. These results were compared with those from the biological utilization of crystalline retinol and β-carotene concentrates. Two different experimental procedures (rat supplementation and depletion–repletion) were adopted for the study of the bioavailability of carotenoids. This study is one of a series of studies carried out by the authors on the analytical and nutritional aspects of carotenoids in Malaysian foods (Tee & Lim, 1991, 1992; Ong & Tee, 1992), and aims to provide further information on the nutritional value of these important sources of provitamin A compounds.

MATERIALS AND METHODS

Materials

In selecting the vegetables for the study, it was ensured that they were: (a) commonly consumed items; (b) rich in provitamin A carotenoids, preferably containing other carotenes besides β-carotene (by referring to earlier findings of Tee & Lim, 1991); and (c) acceptable to the rats. Based on these considerations, the two vegetables selected were carrot (a root vegetable) and *kangkong* (swamp cabbage, a green-leafy vegetable). A few other β-carotene-rich green-leafy vegetables, including *cekor manis*, mustard leaf and spinach, were found to be poorly accepted by the experimental animals in preliminary screening studies carried out and were thus excluded in the study.

The two vegetables were freeze-dried by a commercial firm able to handle the large amount of samples required for the feeding trials. The freeze-dried vegetables were ground to a powder and analysed for carotenoid content using the HPLC method developed by Tee & Lim (1991) to calculate the amount of the vegetable to be mixed into the rat pellet for feeding the animals. Freeze-dried carrot and *kangkong* were mixed into the ground pellet and given to the designated rats daily. About 1 g of the dried carrot or 3 g of the dried *kangkong* had to be given to the rats daily to provide the required amount of vitamin A activity. The amount of carrot to be given was calculated and based on α- and β-carotene content of the vegetable; the combined vitamin A activity was calculated according to the formula: (concentration of β-carotene/6) + (concentration of α-carotene/12). The amount of *kangkong* given was based on β-carotene content divided by 6.

Crystalline retinyl acetate and β-carotene in vegetable oil were purchased from United States Biochemical Corp. (Ohio, USA) to serve as positive controls. The vitamin A activities of the preparations were analysed using the HPLC method mentioned above and appropriately diluted with corn oil before administering 0.2 ml to the animals. The volume of oil administered was kept to a minimum so as not to contribute a significant amount of energy intake to the rats.

Animals

Four-week-old Sprague–Dawley male rats maintained in the Division of Laboratory Animal Resources, Institute for Medical Research, Kuala Lumpur, were used for the studies.

Experimental procedures

Two approaches were taken in this study of bioavailability of carotenoids using experimental rats, namely: (a) supplementation of rats with different sources of carotenoids; and (b) repletion of experimental animals with various sources of carotenoids after a 4-week vitamin A depletion period.

Supplementation of rats with different sources of carotenoids

Four-week-old Sprague–Dawley male rats were weaned and animals weighing from 60 to 70 g were selected for the study. The selected animals were divided into seven groups, and were given commercial rat pellets as their daily diet. The retinol and carotenoid contents of the pellet were determined by the HPLC procedure described by Tee & Lim (1991) and found to provide about 5200 μg RE per kg of diet, or 52 μg RE per rat per day, if each rat consumed 10 g of the diet daily. Only 9% of this total vitamin A activity was provided by carotenoids, mainly β-carotene. The energy content of the pellet was analysed and found to be 3200 kcal per kg of feed, close to the recommended concentration of 3800 kcal per kg of feed (NAS, 1978).

The rats were supplemented with retinol or different sources of carotenoids as indicated in Table 1.

Retinol and β-carotene concentrate or corn oil were administered to the animals orally using an especially

Table 1. Feeding schedule for supplementation of experimental rats

Group	Treatment (per rat per day for 4 weeks)
1	Control group—0.2 ml corn oil
2	Retinol concentrate (60 μg RE) in 0.2 ml corn oil
3	Retinol concentrate (90 μg RE) in 0.2 ml corn oil
4	β-Carotene concentrate (60 μg RE) in 0.2 ml corn oil
5	β-Carotene concentrate (90 μg RE) in 0.2 ml corn oil
6	Freeze-dried carrot (60 μg RE)
7	Freeze-dried swamp cabbage (60 μg RE)

A total of 16 rats were allocated to each group.

assembled force-feeding apparatus comprising the following: (a) a 0.25-ml glass syringe, graduated in 0.01 ml; (b) a piece of spring attached to the plunger of the syringe, adjusted such that the syringe delivered 0.2 ml; (c) a large-gauge (e.g. 17 G) needle with its sharp end blunted and attached to the end of the syringe barrel; and (d) a 1-cm, soft, non-pyrogenic feeding tube of diameter 17 mm attached to the end of the needle. During feeding, the tube was placed just in front of the oesophagus of the animal. The tube was changed daily to avoid contaminating the animals. The apparatus was assembled specifically for the purpose and was found to be suitable for force-feeding the rats rapidly and accurately, without causing too much discomfort to the rats. The accuracy of the apparatus was regularly checked by weighing the volumes dispensed which were compared with volumes dispensed by accurate pipettes.

The rats were housed individually in stainless-steel cages measuring 38 cm long, 22 cm wide and 20 cm high, with a front-opening door. Each cage had a wire-mesh floor and a removable aluminium tray at the bottom. The tray was layered with tissue paper to absorb urine and spilt water, and to collect faeces and spilt food. The cages were stacked on a trolley, placed in a room with 12-h light-and-dark cycles controlled by an automatic timer.

All rats were weighed weekly using a top-pan electronic balance. Before commencement of the feeding schedule, four weanling rats were randomly selected and sacrificed for determination of baseline serum and liver retinol and carotenoid contents. The animals were anaesthetized with diethyl ether, and their blood was collected by heart puncture using a 5-ml disposable syringe. The livers of the animals were removed, dabbed with tissue paper to remove traces of blood and weighed. The blood was allowed to clot and the serum separated by centrifugation. Serum and liver samples were stored at −20°C prior to analysis. At weekly intervals, four rats from each of the seven experimental groups were randomly selected and similarly sacrificed for analysis of retinol and carotenoids.

Repletion of animals with various sources of carotenoids after a 4-week vitamin A depletion period

Four-week-old Sprague–Dawley male rats were weaned and placed on commercial rat pellets for 2 weeks to boost their liver vitamin A stores. This step was thought to be necessary to avoid high mortality rates among the rats during the depletion period. It has been reported that after 5–6 weeks of vitamin A deficiency, the weight of a weanling rat levels out for about a week and then drops rapidly until the animal dies (NAS, 1978). The 6-week-old rats were weighed and those deviating by more than 10% of the median weight were rejected. The selected rats were divided into four groups. All the rats were depleted of vitamin A by providing a diet devoid of the vitamin (AIN semi-purified rat–mouse diet 76, vitamin A omitted, from United States Biochemical Corp., Ohio, USA) for 4 weeks. The vitamin A activity of the diet was analysed and found to provide about 15 μg/kg diet or 0.15 μg RE per rat per day. Compared with a requirement of 30 μg RE per rat per day (NAS, 1978), the contribution of vitamin A from the diet was minimal. Based on the ingredients' listing given by the manufacturer, the energy content of the diet was calculated to be 3850 kcal per kg of feed. At the end of the depletion period, the rats were repleted with retinol or carotenoids from different sources as indicated in Table 2.

Table 2. Feeding schedule for repletion of experimental rats

Group	Treatment (per rat per day for 5 weeks)
1	Control group—0.2 ml corn oil
2	Retinol concentrate (60 μg RE) in 0.2 ml corn oil
3	β-Carotene concentrate (60 μg RE) in 0.2 ml corn oil
4	Freeze-dried carrot (60 μg RE)

A total of 20 rats were allocated to each group.

Other aspects of the experiment were similar to the supplementation study described above, including methods of feeding and housing the animals, weekly weighing of the animals, and killing of the rats at baseline (commencement of repletion) and weekly for the determination of serum and liver retinol and carotenoid concentrations.

Analysis of retinol and carotenoids in rat serum and liver

The HPLC method developed for the simultaneous determination of retinol and carotenoids in foods (Tee & Lim, 1992) and serum (Tee *et al.*, 1994) was used. Whenever sufficient blood was obtainable, 1.0 ml of serum was used for analysis. For younger animals, from which not more than 3 ml of blood was obtained, 0.5 ml serum was used. Liver samples were well homogenized using a mortar and pestle, and an aliquot of 1–2 g was taken for analysis.

RESULTS AND DISCUSSION

Supplementation study

Supplementation with two levels of retinol or β-carotene concentrate

The growth performance of the rats supplemented with two different levels of retinol and β-carotene concentrate was not different from that of the unsupplemented rats (Fig. 1). Vitamin A in the rat pellet was expected to provide a sufficient quantity of the vitamin for growth. The satisfactory growth pattern of the animals also showed that the method of force-feeding employed did not adversely affect the consumption of feed by the rats.

In contrast, the growth of rats provided with diets supplemented with carrot or *kangkong* was seen to be slightly lower than that of the aforementioned five groups of animals. The provision of these vegetables appeared to have reduced the total amount of diet (pellet)

that was consumed by the animals. Especially in the case of *kangkong* (3 g provided daily), the displacement of diet could be significant.

Supplementation with retinol or β-carotene was found to increase liver retinol concentrations, compared with control rats (Table 3 and Fig. 2). Liver retinol concentrations continued to rise throughout the 4-week supplementation period. It was also observed that supplementation with 90 μg RE of retinol or β-carotene did not give rise to a higher storage level of retinol in the liver of rats supplemented with 60 μg RE of retinol or β-carotene. This finding is in agreement with that of other investigators; for example Erdman (1988) in his review pointed out that, with increasing amounts of β-carotene intake, the efficiency of utilization and conversion to retinol decreases. Krinsky *et al.* (1990) also reported an inverse relationship between retinol accumulation in the liver and β-carotene content of the diet. Rats fed an excess of vitamin A were also found to retain significantly less vitamin A than the amount administered (Leo *et al.*, 1989). These investigators suggested enhanced catabolism as a potential mechanism of some degree of homeostatic regulation of hepatic vitamin A accumulation.

As expected, the liver β-carotene concentration of rats supplemented with both levels of retinol concentrate was low and was not different from that of the rats in the control group (Table 4 and Fig. 3). Supplementation of the rat feed with β-carotene resulted in an accumulation of the carotenoid in the liver, while the unsupplemented rats showed very low baseline levels of the carotenoid. Supplementation of 90 μg RE of β-carotene resulted in higher concentrations of liver β-carotene, compared with supplementation with 60 μg RE of β-carotene. This accumulation of β-carotene declined after 2 weeks of supplementation.

The literature appears to be divided regarding the accumulation of β-carotene in the liver of rats. Current understanding ofthe metabolism of β-carotene is that, following absorption, conversion to retinol takes place in the intestinal wall and is thence transported away. Ribaya-Mercado *et al.* (1989) reported no storage of β-carotene in the liver of rats supplemented daily with β-carotene at 4 or even 20 mg/kg body weight. These amounts of supplementation correspond to about 667 and 3333 μg RE/kg body weight respectively, or 80 and 400 μg RE per rat of about 120 g body weight, respectively. It should, however, be pointed out that the rats

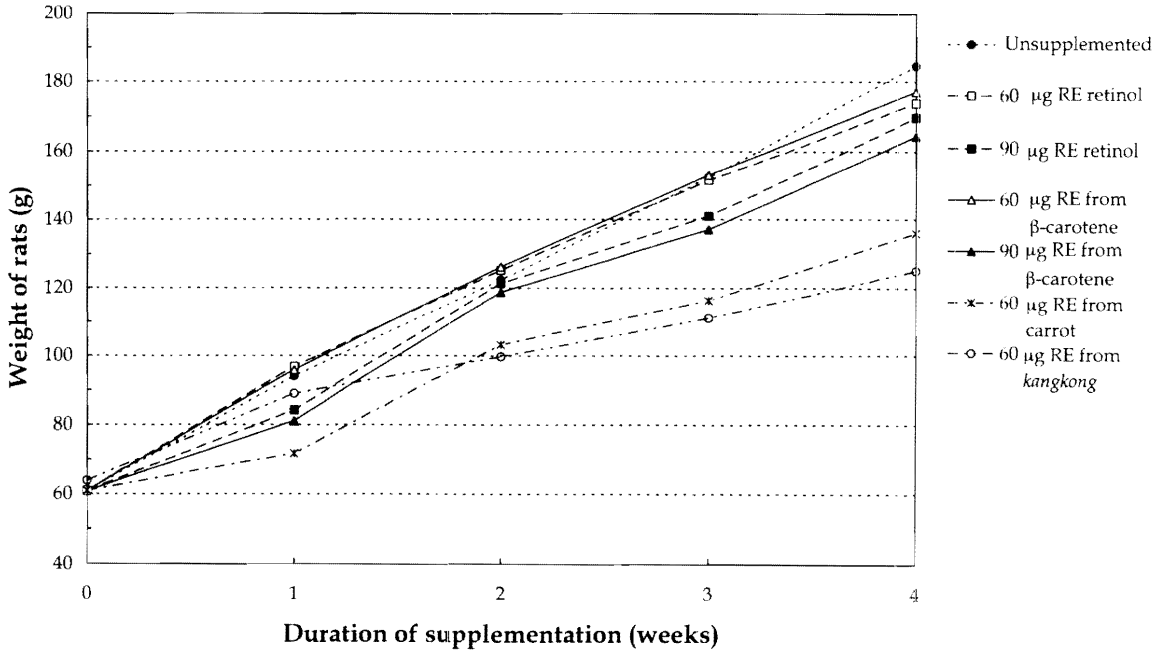

Fig. 1. Growth of rats supplemented with different sources of retinol or carotenoids.

Table 3. Liver retinol concentration (mean ± SD) of rats supplemented with different sources of retinol or carotenoids[a,b]

Weeks of supplementation	Sources of vitamin A						
	Nil (unsupplemented)	60 μg RE retinol	90 μg RE retinol	60 μg RE from β-carotene	90 μg RE from β-carotene	60 μg RE from carrot	60 μg RE from *kangkong*
1	90 ± 2.2	136 ± 12.8	122 ± 5.1	134 ± 11.4	93 ± 8.3	129 ± 22.7	127 ± 6.6
2	86 ± 15.0	161 ± 4.8	122 ± 9.5	132 ± 10.4	104 ± 11.0	161 ± 3.6	134 ± 14.8
3	113 ± 6.4	213 ± 24.2	231 ± 33.5	172 ± 27.7	165 ± 15.0	193 ± 11.9	147 ± 44.2
4	144 ± 14.5	271 ± 23.0	251 ± 7.4	232 ± 11.4	180 ± 17.5	269 ± 10.9	155 ± 8.6

[a]Each value is the mean from four rats, expressed as μg retinol/g liver.
[b]Mean liver retinol concentration before supplementation was 60.8 ± 5.5 μg/g liver.

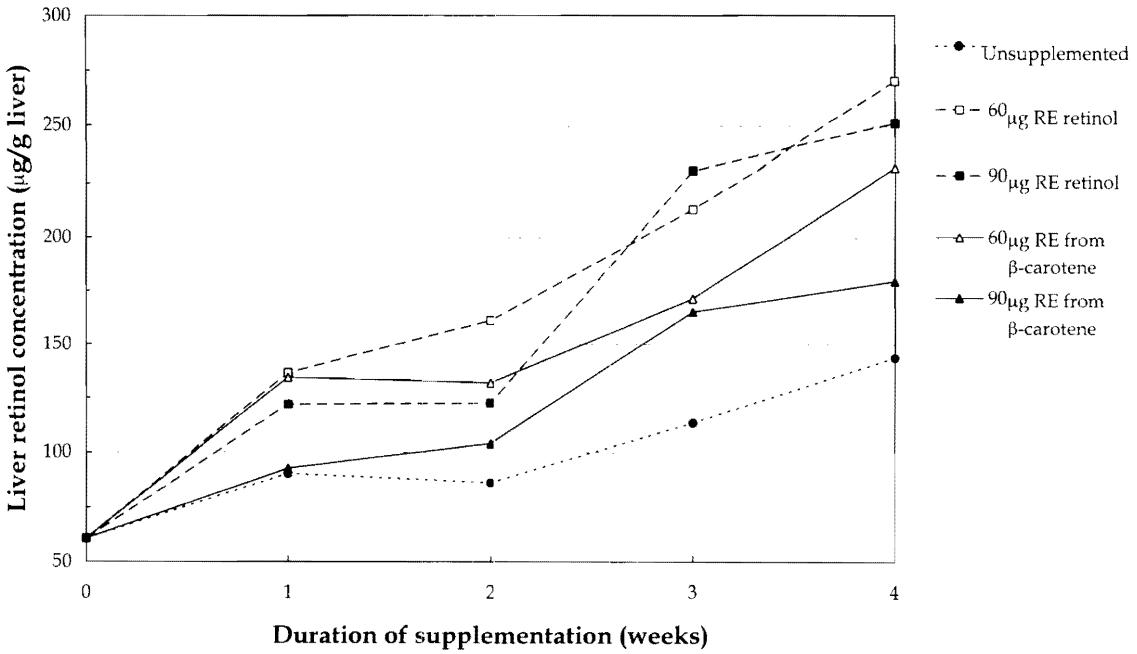

Fig. 2. Changes in liver retinol concentration of rats supplemented with retinol or β-carotene.

Table 4. Liver β-carotene concentration (mean ± SD) of rats supplemented with different sources of retinol or carotenoids[a,b]

Weeks of supplementation	Sources of vitamin A						
	Nil (unsupplemented)	60 μg RE retinol	90 μg RE retinol	60 μg RE from β-carotene	90 μg RE from β-carotene	60 μg RE from carrot	60 μg RE from *kangkong*
1	4.9 ± 0.9	3.8 ± 1.0	8.4 ± 3.4	26.9 ± 4.8	37.5 ± 7.0	29.1 ± 16.9	41.2 ± 12.3
2	1.7 ± 1.3	3.7 ± 0.8	3.1 ± 2.1	27.7 ± 8.7	44.1 ± 16.6	37.6 ± 4.4	54.5 ± 24.1
3	3.1 ± 0.6	2.6 ± 1.3	2.0 ± 2.1	21.9 ± 10.2	33.7 ± 22.6	40.9 ± 7.5	92.9 ± 20.8
4	4.3 ± 0.8	2.9 ± 1.9	2.0 ± 1.3	13.6 ± 2.4	12.4 ± 5.8	56.5 ± 7.8	78.6 ± 18.1

[a]Each value is the mean from four rats, expressed as μg β-carotene/100 g liver.
[b]Mean liver β-carotene concentration before supplementation was 3.6 ± 0.7 μg/100 g liver.

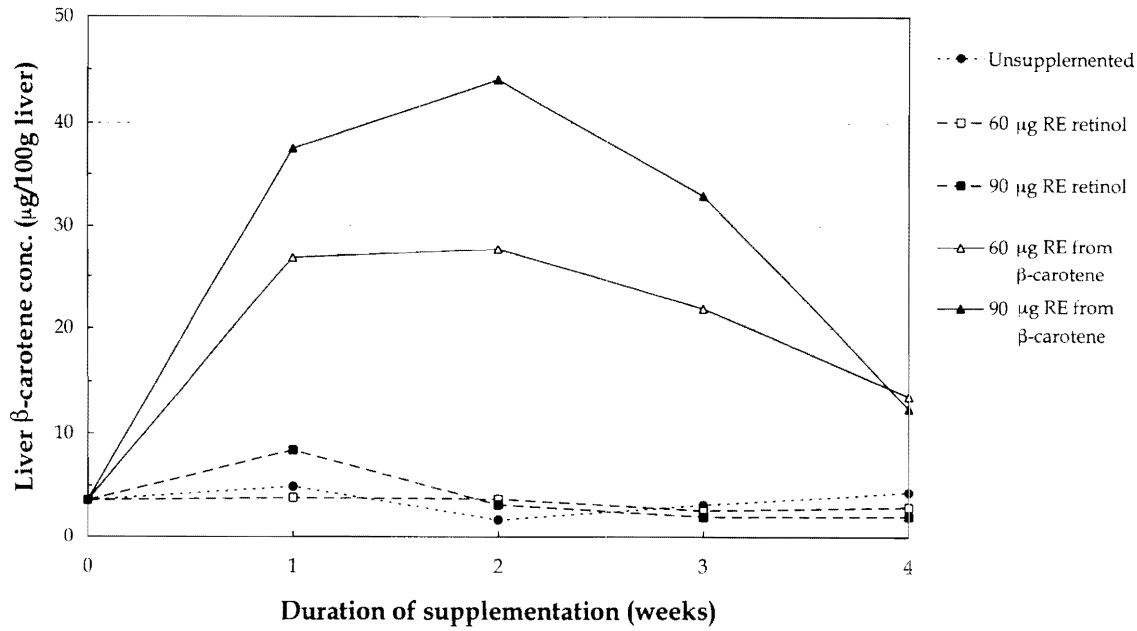

Fig. 3. Changes in liver β-carotene concentration of rats supplemented with retinol or β-carotene.

Table 5. Serum retinol concentration (mean ± SD) of rats supplemented with different sources of retinol or carotenoids[a,b]

Weeks of supplementation	Sources of vitamin A						
	Nil (unsupplemented)	60 μg RE retinol	90 μg RE retinol	60 μg RE from β-carotene	90 μg RE from β-carotene	60 μg RE from carrot	60 μg RE from *kangkong*
1	41.6 ± 0.6	44.0 ± 6.3	42.4 ± 5.6	39.5 ± 5.6	48.0 ± 13.8	45.1 ± 4.1	45.4 ± 5.0
2	46.9 ± 0.9	45.8 ± 2.5	45.7 ± 9.7	44.2 ± 5.2	51.4 ± 6.1	40.5 ± 6.1	36.7 ± 5.5
3	46.7 ± 1.8	49.6 ± 4.4	41.2 ± 4.7	50.2 ± 5.1	55.0 ± 7.5	28.6 ± 7.1	32.3 ± 5.0
4	51.6 ± 1.8	42.7 ± 2.0	50.3 ± 2.2	45.3 ± 3.0	52.3 ± 7.0	32.2 ± 1.1	43.7 ± 14.8

[a]Each value is the mean from four rats, expressed as μg retinol/dl serum.
[b]Mean serum retinol concentration before supplementation was 35.7 ± 6.7 μg/dl serum.

used by these investigators were 10-month-old rats, much older than the animals used in the present study. Findings from the study of Krinsky *et al.* (1990), however, showed that while there was no accumulation of carotenoids in the unsupplemented rats, the [C^{14}] label was detected in the liver and other organs of animals supplemented with [C^{14}] β-carotene. These investigators are of the opinion that rodents can accumulate carotenoids in blood and organs when they are given sufficiently high doses. The present findings are therefore in agreement with those reported by Krinsky *et al.* (1990).

In contrast to the accumulation of retinol and β-carotene in the livers of animals previously administered with retinol or β-carotene, there was no difference in serum retinol concentrations of rats supplemented with retinol or β-carotene (Table 5 and Fig. 4). The *XY* scatter of serum vs liver retinol concentration (Fig. 5), plotted using all the 29 pairs of data in Table 3 and Table 5 shows that there was no correlation between these two parameters. This finding confirms previous understanding that serum retinol concentrations are not good indicators of vitamin A status, unlike liver retinol levels.

β-Carotene was not detected in serum of rats in all the groups studied. Total carotenoid concentrations in sera of rats supplemented with retinol or β-carotene were less than 1 μg/dl.

Supplementation with retinol, β-carotene or carotenoids from vegetable sources

Figures 6–8 compare the bioavailability of carotenoids from carrot and *kangkong* with that given by crystalline retinol and β-carotene, each source providing a vitamin A activity of 60 mg RE/rat/day. The liver retinol concentration of the rats supplemented with carrot was found to be similar to that given by retinol or β-carotene concentrates (Table 3 and Fig. 6). Results obtained therefore show that carotenoids in carrot possess high bioavailability. Sweeney & Marsh (1974) reported that carotene in carrot, as measured by liver and kidney storage of vitamin A in rats, was equal in availability to β-carotene dissolved in cotton seed oil. The investigators also reported that disruption of carrot cells by freezing, blending, freeze-drying or ultrasonic treatment did not increase carotene availability. Various

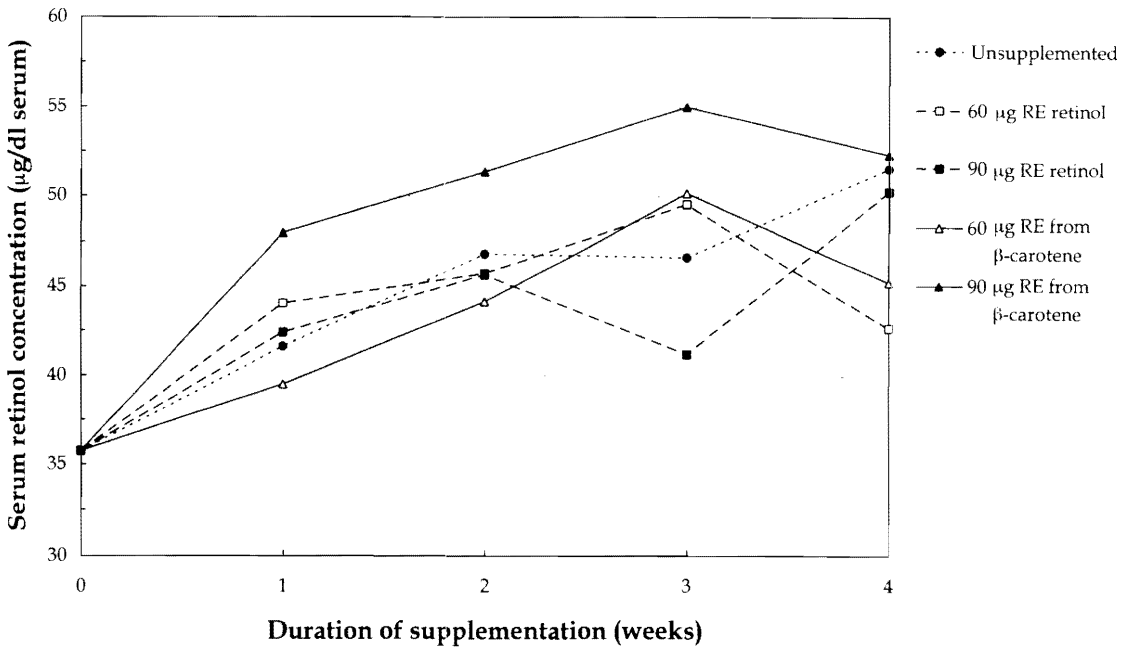

Fig. 4. Changes in serum retinol concentration of rats supplemented with retinol or β-carotene.

studies in humans have, however, shown that β-carotene is much more bioavailable in a crystalline form than from foods such as carrot (Erdman, 1988; Brown *et al.*, 1989). As indicators of bioavailability, these studies had relied on measurements of serum carotenoids after ingestion of test doses of β-carotene or carrot. In contrast, the present study (to be discussed below) has shown that serum carotenoid concentrations of rats supplemented with β-carotene, carrot or *kangkong* were low and not useful in providing information on bioavailability.

A similar response in liver retinol accumulation by rats supplemented with carrot to the group of rats supplemented with retinol concentrate suggests that calculations of vitamin A activity, based on the carotenoid concentration of the vegetable determined by the HPLC method used, are probably correct. If, on the other hand, the results obtained by the AOAC method were applied to the calculation of vitamin A activity of the carrot given, the results would have been higher, since α- and β-carotene would have been determined together as the latter. Consequently, since α-carotene in the carrot was found to contribute about 19% of the total vitamin A activity for the vegetable, the expected response in liver retinol concentration would have been higher by about 20%.

Biological conversion of β-carotene in *kangkong* to liver retinol was found to be good in the first 2 weeks of feeding (Table 3 and Fig. 6), indicating good bioavailability of the carotenoid in the vegetable. However, after 2 weeks of supplementation, accumulation of retinol in liver was seen to be lower than that for rats in the other experimental groups. As observed from the higher amount of food spillage for this group of rats, the

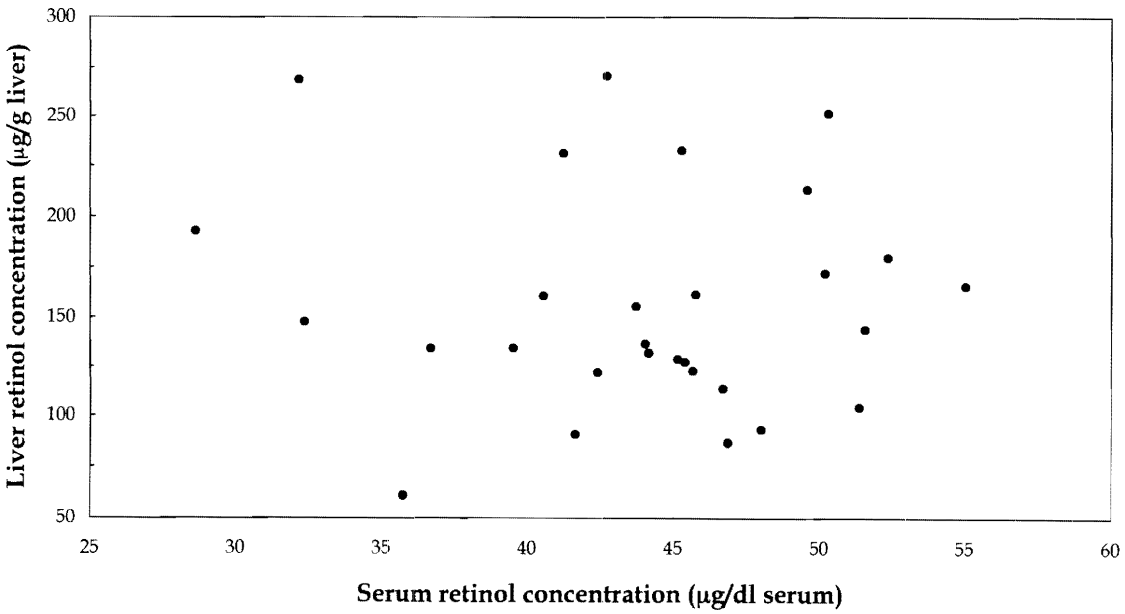

Fig. 5. Scatter diagram of serum retinol vs liver retinol concentrations of experimental rats in the supplementation study.

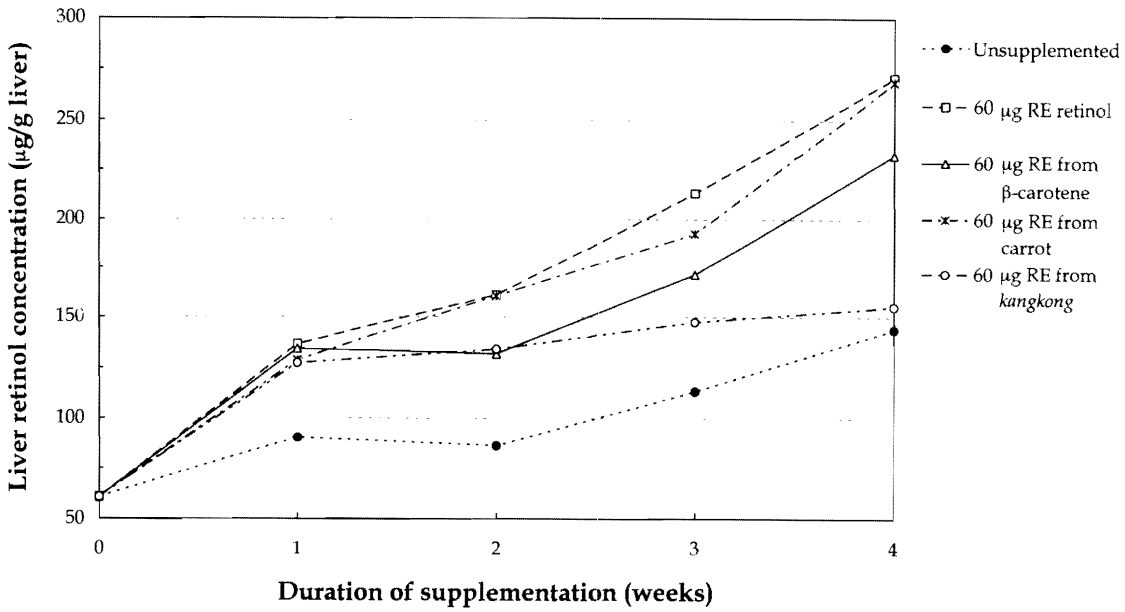

Fig. 6. Changes in liver retinol concentration of rats supplemented with retinol or different sources of carotenoids.

poorer utilization of carotenoids from *kangkong* may be attributed to the incomplete consumption of all the vegetable provided daily. As explained above, compared with carrot, *kangkong* and other vegetables (*cekor manis*, mustard leaf and spinach) were found to be poorly accepted by the experimental animals in preliminary studies carried out. Thus, the lower accumulation of retinol in the liver of rats supplemented with *kangkong* may not necessarily reflect the lower bioavailability of the β-carotene in the vegetable.

The accumulation of β-carotene in the livers of rats supplemented with carrot or *kangkong* can be seen in Table 4 and Fig. 7. These results confirm earlier comment regarding the accumulation of the carotenoid in the liver of rats supplemented with β-carotene. As expected, unsupplemented rats and animals supplemented with retinol showed very low levels of the carotene in the liver.

The accumulation of other carotenoids in the liver was also observed for the two groups of experimental animals supplemented with carrot or *kangkong*. The carotenoid accumulated reflected the type of vegetable fed. In the group supplemented with carrot, the α-carotene level in the liver was 40–80 μg per 100 g liver during the period of study, whereas in all the other groups the level of this carotenoid was less than 3 μg/

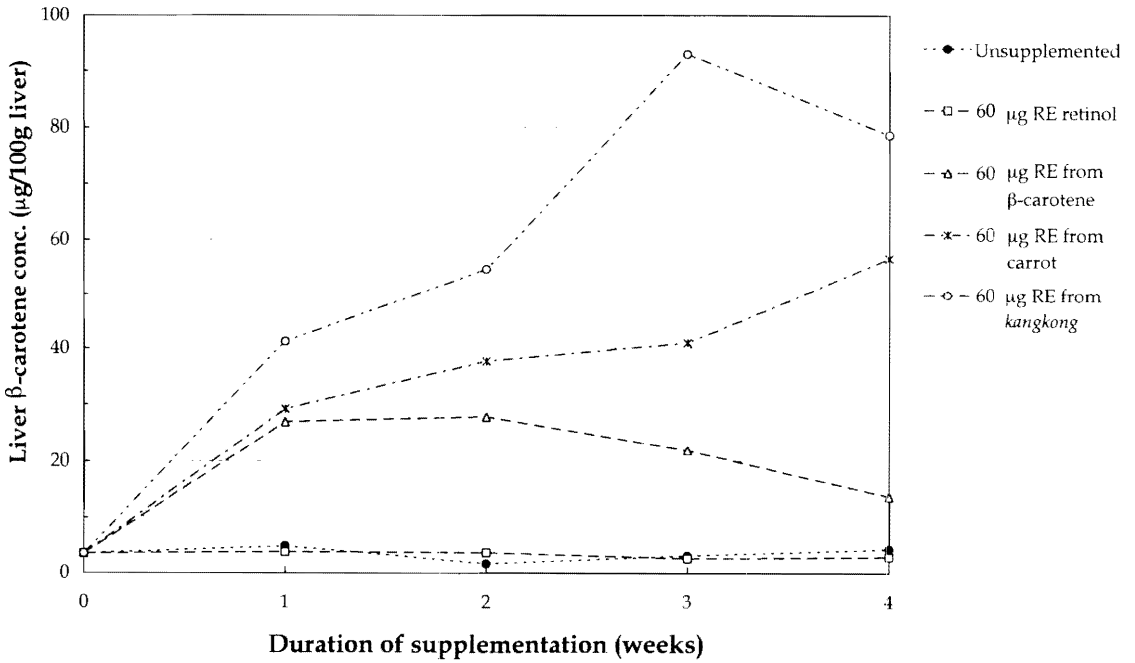

Fig. 7. Changes in liver β-carotene concentration of rats supplemented with retinol or different sources of carotenoids.

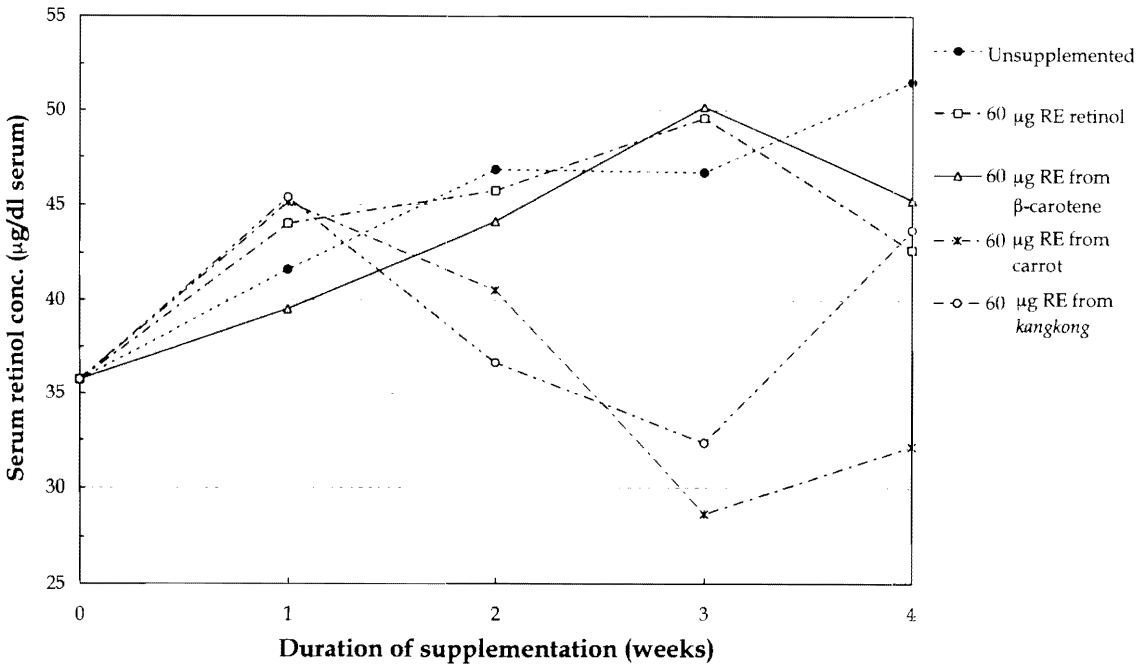

Fig. 8. Changes in serum retinol concentration of rats supplemented with retinol or different sources of carotenoids.

100 g liver. The results reflect the high α-carotene content of carrot, a characteristic feature of this root vegetable. On the other hand, in the group of rats supplemented with *kangkong*, in which more than 80% of the carotenoid is lutein, a high lutein level of about 25–60 μg/100 g liver was found. Lutein was not detected in the liver of rats in all the other experimental groups. These findings clearly confirm the ability of rats to accumulate carotenoids in the liver, irrespective whether the animals were supplemented with β-carotene, carrot or *kangkong*.

Serum retinol concentrations of rats supplemented with carrot or *kangkong* appeared to be different from animals supplemented with retinol or β-carotene. Figure 8 (and data in Table 5) shows a drop in serum retinol concentrations during the 3rd and 4th weeks of supplementation with carrot or *kangkong*.

No detectable amounts of β-carotene were observed in the sera of rats supplemented with carrot or *kangkong*. The total carotenoid concentrations in the serum were also low, at less than 4 μg/dl. These findings are similar to those observed for rats supplemented with retinol or β-carotene. The analysis of serum carotenoids is thus of no significance in these studies.

Repletion study

Vitamin-A-depleted rats when repleted with retinol, β-carotene or carrot for 5 weeks grew equally well and gained weight. In contrast, the growth of vitamin-A-depleted rats was adversely affected when there was no repletion with retinol or carotenoids (Fig. 9). Vitamin A activity in this deficient diet (providing less than 1 μg RE/rat/day) was much lower than that in the pellet (providing 52 μg RE/rat/day) given to the control group in the supplementation study which appeared to be sufficient to maintain normal growth.

Repletion with carotenoids contained in carrot was found to give a similar response to liver retinol concentration as when retinol and β-carotene were used (Table 6 and Fig. 10). These data confirm findings from the supplementation study above that the biological utilization of carotenoids in carrot, as provitamin A compounds, was high in these experimental animals. The liver retinol concentration in the unrepleted rats continued to decline to a very low level (about 2 μg/g liver) at the end of the study. However, these rats did not show clear clinical signs of vitamin A deficiency, for example in the eyes. Other signs of deficiency, for

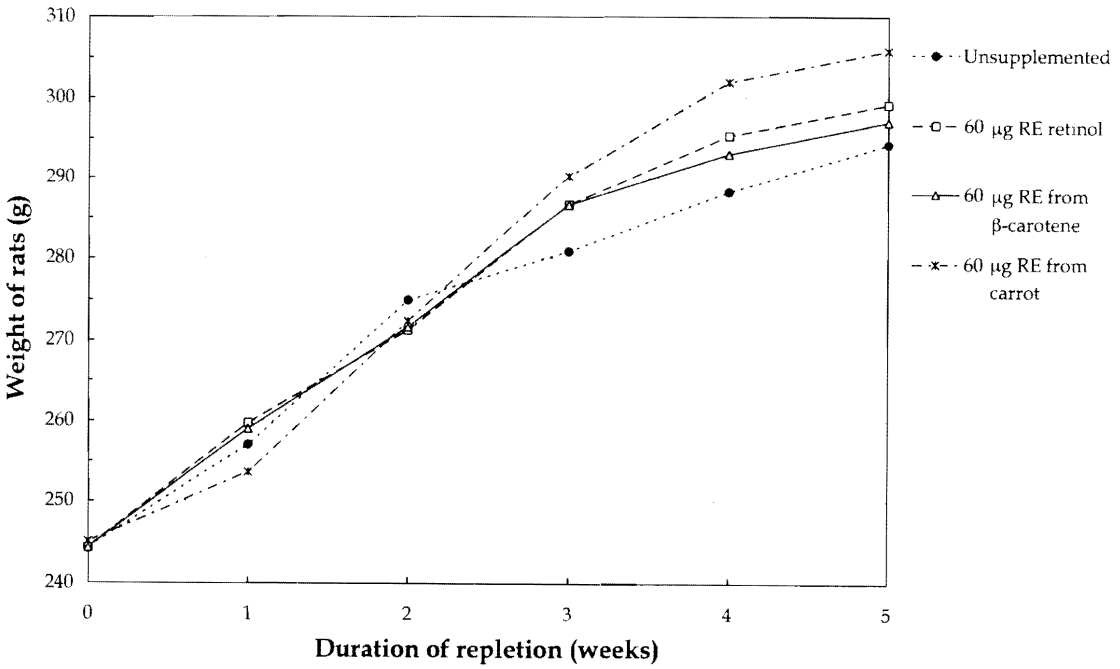

Fig. 9. Weight changes of rats repleted with retinol, β-carotene or carrot.

Table 6. Liver retinol concentration (mean ± SD) of rats repleted with retinol, β-carotene or carrot[a,b]

Weeks of repletion	Sources of vitamin A			
	Nil (not repleted)	60 μg RE retinol	60 μg RE from β-carotene	60 μg RE from carrot
1	17.6 ± 3.7	29.1 ± 5.7	31.1 ± 4.8	26.4 ± 4.0
2	14.7 ± 3.0	28.7 ± 6.3	34.5 ± 3.0	35.6 ± 4.7
3	6.1 ± 3.1	41.9 ± 2.8	34.8 ± 4.5	37.0 ± 10.1
4	5.2 ± 1.8	45.7 ± 1.7	44.1 ± 5.7	57.8 ± 5.9
5	2.3 ± 1.5	51.8 ± 9.6	51.0 ± 13.7	61.3 ± 8.8

[a]Each value is the mean from four rats, expressed as μg retinol/g liver.
[b]Mean liver retinol concentration before repletion was 21.3 ± 4.0 μg/g liver.

example bone defects, increase in cerebrospinal fluid pressure, reproductive failure, and epithelial metaplasia and keratinization (NAS, 1978) were not easily observable.

Figure 11 and data in Table 7 show a similar picture for liver β-carotene concentration in the depletion–repletion study as that found in the supplementation study (Figs 3 and 8). Table 8 and Fig. 12 again show

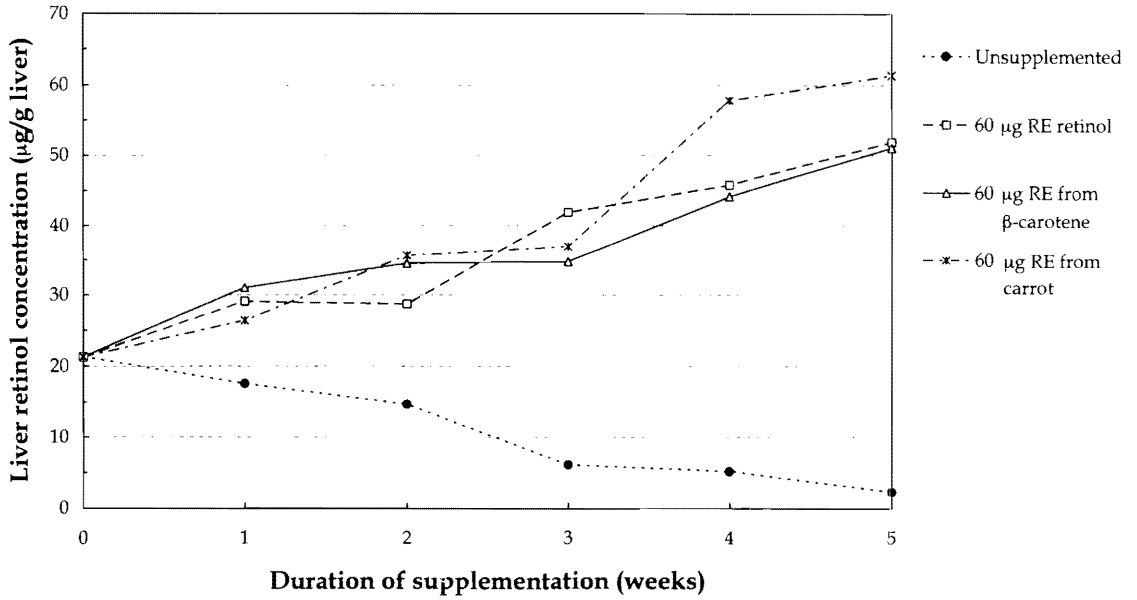

Fig. 10. Changes in liver retinol concentration of rats repleted with retinol, β-carotene or carrot.

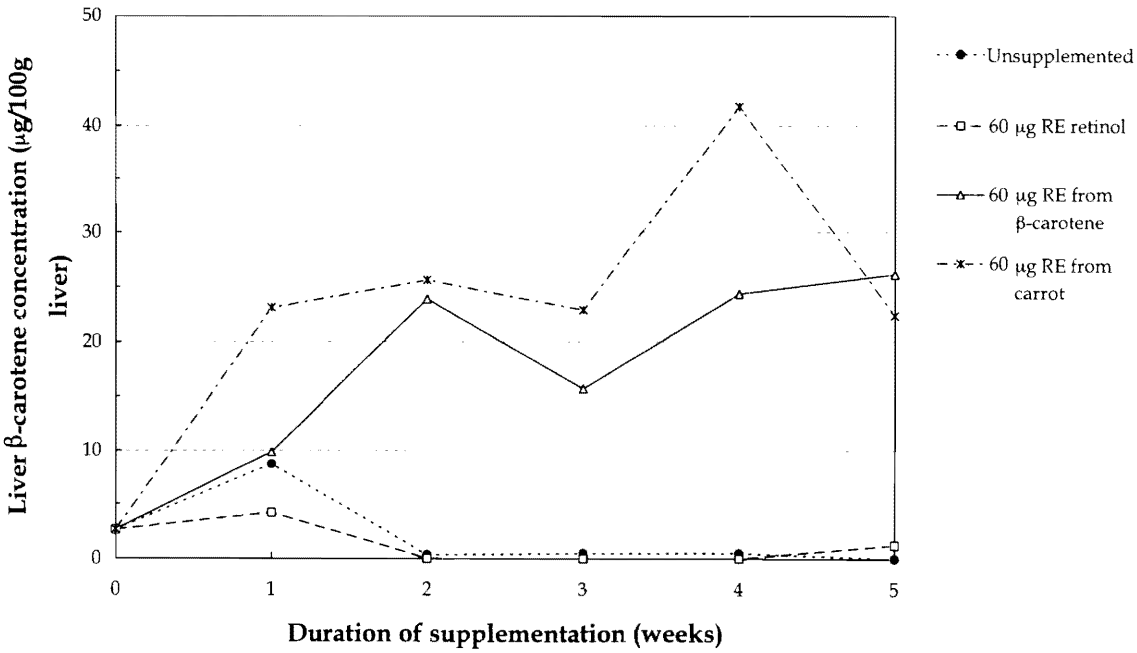

Fig. 11. Changes in liver β-carotene concentration of rats repleted with retinol, β-carotene or carrot.

Table 7. Liver β-carotene concentration (mean ± SD) of rats repleted with retinol, β-carotene or carrot[a,b]

Weeks of repletion	Sources of vitamin A			
	Nil (not repleted)	60 μg RE retinol	60 μg RE from β-carotene	60 μg RE from carrot
	8.7 ± 3.0	4.2 ± 2.8	9.9 ± 2.7	23.1 ± 13.1
2	0.3 ± 0.6	0	23.9 ± 9.0	25.6 ± 2.9
3	0.4 ± 0.6	0	15.7 ± 5.6	22.9 ± 5.7
4	0.5 ± 0.7	0	24.4 ± 9.0	41.7 ± 3.1
5	0	1.2 ± 1.2	26.1 ± 13.9	22.4 ± 8.2

[a]Each value is the mean from four rats, expressed as μg β-carotene/g liver.
[b]Mean liver β-carotene concentration before repletion was 2.6 ± 0.9 μg/g liver.

that there was no clear difference in serum retinol concentrations of rats in the different treatment groups. As discussed above, serum retinol concentration does not correlate with liver retinol concentration (Fig. 13, using data in Table 6 and Table 8), and is not a good indicator of vitamin A nutriture of the rats.

Table 8. Serum retinol concentration (mean ± SD) of rats repleted with retinol, β-carotene or carrot[a,b]

Weeks of repletion	Sources of vitamin A			
	Nil (not repleted)	60 μg RE retinol	60 μg RE from β-carotene	60 μg RE from carrot
1	70.9 ± 15.6	79.2 ± 2.9	59.8 ± 5.9	40.6 ± 10.1
2	57.8 ± 6.4	68.8 ± 8.1	56.5 ± 5.2	40.2 ± 7.5
3	44.1 ± 9.4	47.2 ± 5.0	44.3 ± 7.3	45.6 ± 4.4
4	42.9 ± 3.7	45.8 ± 4.6	50.1 ± 11.9	41.9 ± 3.3
5	31.2 ± 5.7	43.3 ± 5.0	45.7 ± 3.0	56.0 ± 4.8

[a]Each value is the mean from four rats, expressed as μg retinol/dl serum.
[b]Mean serum retinol concentration before repletion was 63.2 ± 3.1 μg/dl serum.

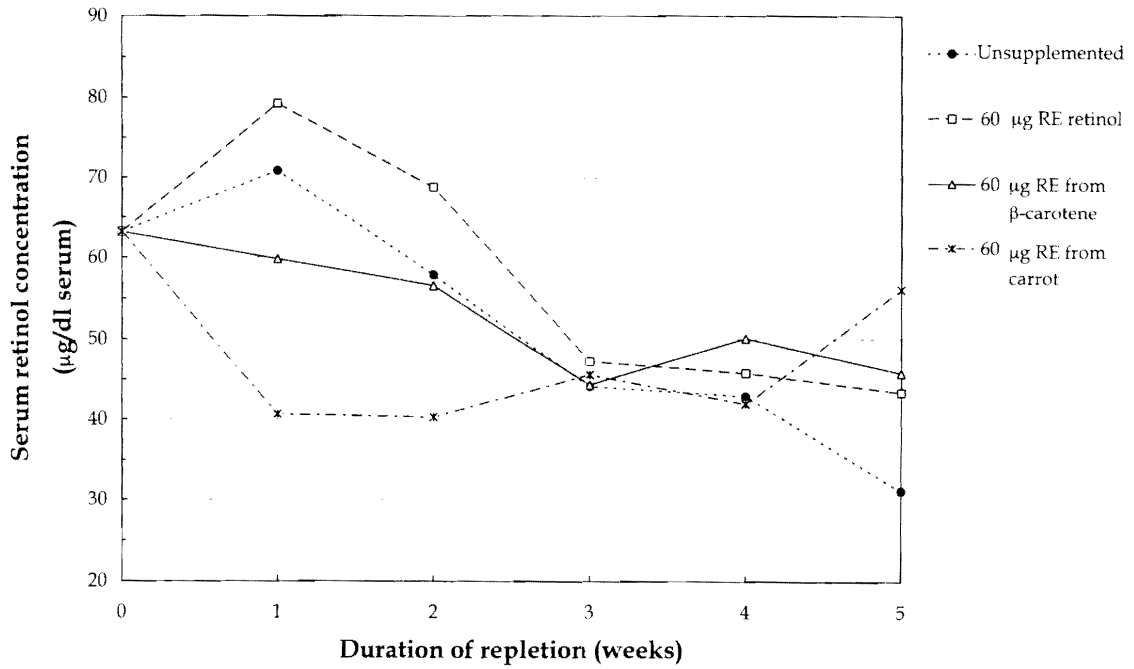

Fig. 12. Changes in serum retinol concentration of rats repleted with retinol, β-carotene or carrot.

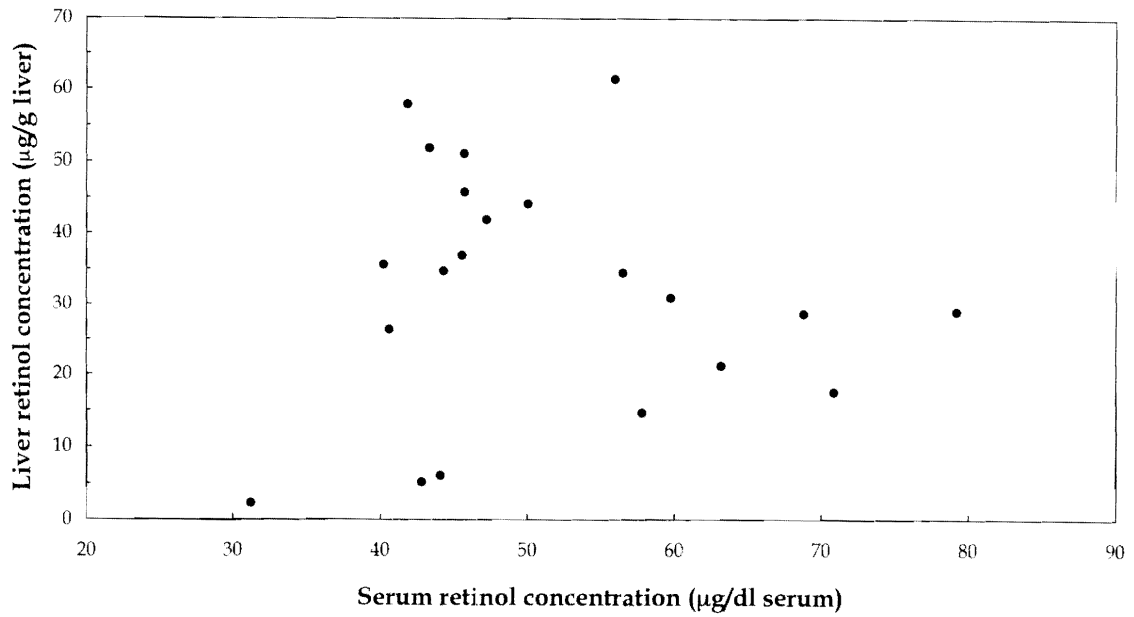

Fig. 13. Scatter diagram of serum retinol vs liver retinol concentrations of experimental rats in the depletion-repletion study.

CONCLUSIONS

The bioavailability of carotenoids in carrot and *kangkong* was investigated by two different procedures, namely rat supplementation and rat depletion–repletion methods. The biological utilization of the carotenoids was monitored weekly by measuring the serum and the liver retinol and carotenoid concentrations of the animals. Both procedures showed that the bioavailability of the major carotenoids in carrot (α- and β-carotene) and *kangkong* (β-carotene) was high, as evidenced by the accumulation of retinol in the liver of the experimental rats, in relation to crystalline retinol concentrate.

The provitamin A activity of α- and β-carotenes in the vegetables approximates the commonly used factors of 1/12 and 1/6 that of retinol, respectively.

There was no correlation between liver and serum retinol concentrations, indicating that serum levels of retinol are not good indicators of vitamin A status. The experimental rats were found to be able to accumulate carotenoids in the liver when fed increased amounts of the carotenoids. The type of carotenoids accumulated is reflective of the dietary intake. On the other hand, serum carotenoid levels were low in all experimental groups. Serum retinol and carotenoid concentrations are, therefore, unreliable indicators of the bioavailability of carotenoids from plant sources.

Both experimental procedures may be used for the study of bioavailability of carotenoids in plant sources. The supplementation study, however, takes a shorter time to carry out and would be advantageous. The 4-week supplementation period was quite sufficient to obtain clearly observable differences. If it is desirable to reduce time and cost of analysis, a 2-week supplementation would suffice, and liver retinol and carotenoid concentrations could be determined before and after supplementation.

Studies on the bioavailability of carotenoids in plant sources are few and far between in the literature. Further studies on other vegetables need to be carried out. One of the problems to be overcome in conducting these studies would be to provide the rats with as small an amount of vegetable as possible, in order to avoid bulk and interference with normal diet intake. This requirement would limit the types of vegetable that could be studied, as only vegetables with high carotenoid concentration would be suitable. The other problem relates to the acceptability of vegetables, as some tropical green-leafy varieties possess odours unacceptable to the rats.

Data on carotenoid bioavailability using human subjects are even more scarce. Two recent studies are those of Dimitrov *et al.* (1988) and Brown *et al.* (1989). The former studied pure β-carotene, whilst the latter compared carotenoids in vegetables and pure β-carotene. Both studies employed measurement of serum carotenoid concentration, and both groups of investigators pointed out that there were large individual variations in response. Especially for human subjects, whose diets are highly variable, the response in serum carotenoid concentrations could be greatly modified by various items in the diet, making valid interpretation and conclusion difficult.

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