ELSEVIER

Distribution of carotenoids from the yolk to the tissues of the chick embryo

Peter F. Surai*† and Brian K. Speake*

**Department of Biochemistry and Nutrition, Scottish Agricultural College, Ayr, UK; and †Department of Physiology, Biochemistry and Nutrition, Poultry Research Institute, Kharkov Region, Ukraine*

Laying hens were placed on either a control diet or a carotenoid-supplemented diet. The subsequent concentrations of carotenoids in the yolks of the newly laid fertile eggs (μg carotenoid/g fresh yolk) were 13.3 and 41.1 on the control diet and high-carotenoid diet, respectively. For the day-old chicks derived from the high-carotenoid group, the carotenoid levels in the yolk sac membrane, liver, and plasma were approximately 3 times greater than in the control group; however, the other tissues were less responsive. The proportion of lutein (% wt/wt of total carotenoids) was far lower in the liver of the chick than in the yolk. However, in the non-hepatic tissues the proportion of lutein was generally higher than in the yolk. This suggests that discrimination between different carotenoids may occur in the embryo. For the high-carotenoid group, the peroxidative susceptibility of extracts of the yolk, yolk sac membrane, and liver was decreased compared with samples from the control group. Thus, carotenoids may provide antioxidant protection to the tissues. (J. Nutr. Biochem. 9:645–651, 1998)

Keywords: carotenoid; lipid peroxidation; chick embryo

Introduction

Carotenoids are lipid-soluble pigments synthesized by plants and photosynthetic microorganisms.[1–3] In animals, an important function of dietary carotenoids is to act as precursors for the synthesis of vitamin A, although only a small proportion of the naturally occurring carotenoids exhibit this activity.[1–9] Other functions that have been ascribed to carotenoids include antioxidant activity,[10–12] the promotion of cell differentiation,[13–15] regulation of cell proliferation,[16,17] and the enhancement of immune function.[18,19] Many of these postulated actions of carotenoids may be relevant to aspects of embryonic development. In particular, the embryo will require an effective array of antioxidant components in order to protect the developing tissues from peroxidative damage.[20] It may be relevant that the antioxidant activity of carotenoids is mainly expressed at the low oxygen tensions[10–12] that prevail in embryonic tissues.[21] The effects of carotenoids in regulating cell differentiation and proliferation are also of potential significance for the embryo.[20]

The embryo of the chicken develops within a closed system, the egg, which contains all the nutrients required for the 21-day developmental process.[22] The lipid-rich yolk contains the lipid-soluble vitamins E and A, and also a range of carotenoids.[23] Current evidence suggests that, during development, the lipids and associated components are transferred from the yolk to the surrounding yolk sac membrane (YSM) by phagocytosis.[22] Within the YSM, lipids are assembled together with vitamin E, carotenoids, and apoproteins to form lipoprotein particles, which are released into the embryonic circulation.[22,24–27] Following de-lipidation by the action of lipoprotein lipase in the capillaries of the embryo's adipose tissue and muscle,[28] the lipoprotein remnants containing vitamin E and carotenoids are deposited in the embryonic liver.[27,29] Thus, high levels of vitamin E and carotenoids accumulate in the liver, particularly during the final few days of development, whereas lower amounts of these components are distributed to the other tissues.[27] The brain of the chick embryo accumulates only low levels of vitamin E[27] and no detect-

Address correspondence and reprint requests to Dr Brian K. Speake, Department of Biochemistry and Nutrition, Scottish Agricultural College, Auchincruive, Ayr KA6 5HW, UK.
This research was funded by the Scottish Office Agriculture Environment and Fisheries Department. P.F. Surai was supported by a Royal Society Research Fellowship.
Received April 2, 1998; accepted July 21, 1998.

655 Avenue of the Americas, New York, NY 10010
0955-2863/98/$19.00
PII S0955-2863(98)00068-0

able carotenoids[27] and is especially susceptible to lipid peroxidation in vitro[27] and in vivo.[30]

The profile of carotenoids present in the yolk of the newly laid egg will depend to a large extent on the types of carotenoids present in the diet of the parent hen. However, other factors in the laying hen, such as the efficiency of absorption of the various carotenoids from the intestine and the extent to which certain carotenoids are converted to vitamin A in the intestinal mucosa or the liver, are also important determinants of the carotenoid profile of the yolk.[31–37] Eggs from hens on standard grain-based diets contain lutein as the major yolk carotenoid.[27] Although β-carotene is a major carotenoid present in human plasma and tissues,[5–9] hens are very efficient converters of β-carotene to vitamin A.[31–40] Thus, little or no β-carotene accumulates in the yolk, even at high levels of supplementation.[27] At present, the prime reason for supplementing hens' diets with carotenoids is to modulate the color of the yolk.[41] However, the potential effects of maternal supplementation with carotenoids on the immune function of the newly hatched chick have also received some attention.[42,43]

There were three primary aims of the present study: (i) to evaluate the effects of enhanced levels of carotenoids in the maternal diet on the levels of carotenoids in the yolk and in the embryonic tissues; (ii) to investigate the possibility of discrimination between different carotenoids by the embryo; and (iii) to determine the effects of elevated tissue levels of carotenoids on the sensitivity of tissue extracts to lipid peroxidation in vitro.

Methods and materials

Dietary groups and sample collection

Hens (Ross 1 broiler-breeder strain) were maintained on a standard layer's wheat-barley-soya-based diet until they were 30 weeks old. The basal components of the standard diet contained lutein (4.5 mg/kg feed) as the only detectable natural carotenoid; however, the diet was supplemented with additional lutein (4.0 mg/kg feed) and citranaxanthin (6.5 mg/kg feed). At 30 weeks of age, the birds were divided into two dietary groups, each of 15 hens. One group continued receiving the standard diet (control group). The second group (high carotenoid) was provided with the standard diet enriched with a carotenoid mixture (Trouw Nutrition, Northwich, UK) so that the final concentrations of the various carotenoids were (mg/kg feed): lutein, 24.5; citranaxanthin, 45.0; canthaxanthin, 3.0; β-apo-8′-carotenoic acid, 24.0. This carotenoid mixture is a commercial dietary supplement that is commonly used to enhance the color of table egg yolks for consumer preference. Both diets contained vitamin A (2 mg/kg feed) and vitamin E (20 mg/kg feed). The birds were maintained on these diets for 23 days, and eggs were collected throughout this period.

The conditions of care and welfare of the hens were as approved by the SAC Animal Ethics Committee. Fertile eggs collected over a 2-week period from day 23 from hens on the two diets were incubated at 37.8°C and 60% relative humidity in a forced-draught incubator with automatic egg turning. Embryos were sampled at various stages of development. Chicks were sampled 1 day after hatching (day 22 of development); drinking water but no food was provided to the chicks on hatching. Embryos were sacrificed by anaesthetic injection (pentobarbitone, 60 mg/kg body wt; Rhone Merieux, Harlow, UK) and day-old chicks by neck dislocation, in accordance with the UK 1986 Animals (Scientific Procedures) Act. Tissue samples dissected from the embryos and chicks were washed rapidly in ice-cold 0.9% (wt/vol) NaCl solution, blotted with filter paper, weighed and stored at −80°C for up to 2 weeks prior to analysis.

Analysis of carotenoids

Yolk or tissue samples were homogenized in 1 vol of H_2O, and 2 mL of the homogenate was mixed with 6 mL of ethanol/H_2O (2:1, vol/vol). Hexane (5 mL) was then added and the mixture shaken vigorously for 5 min. The hexane phase, containing the carotenoids, was separated by centrifugation and collected. Analysis of the carotenoids was performed by HPLC using a Spherisorb type S30DS2, 5-μ C_{18}, reverse-phase column, 25 cm × 4.6 mm (Phase Separations, Clwyd, UK), with a mobile phase of acetonitrile/dichloromethane/methanol (7:2:1, vol/vol) using detection by absorbance at 445 nm. Peaks were identified by comparison with the retention times of a range of carotenoid standards (variously obtained from Sigma, Poole, UK; Fluka, Gillingham, UK; Apin, Abingdon, UK; and Hoffmann-LaRoche, Basel, Switzerland). Using a flow rate of 1.5 mL/min, the retention times (min) of lutein, canthaxanthin, citranaxanthin, and β-apo-8′-carotenoic acid were, respectively, 3.22, 3.85, 4.67, and 5.16. To quantify the amounts of these carotenoids from the absorbance at 445 nm, calibration curves were obtained for each carotenoid using the appropriate standards. Detection limits on the column were 5–10 ng.

Analysis of vitamins E and A

The vitamin E and vitamin A contents of the samples were determined as previously described.[26] In brief, the samples were saponified with ethanolic KOH in the presence of pyrogallol and then vitamins E and A were extracted from the mixture with petroleum spirit. The extract was dried under N_2, redissolved in methanol, and injected onto a Spherisorb type S30DS2, 3-μ C_{18}, reverse-phase HPLC column, 15 cm × 4.6 mm (Phase Separations, Clwyd, UK). Chromatography was performed using a mobile phase of methanol/water (97:3, vol/vol) at a flow rate of 1.1 mL/min. Fluorescence detection utilized excitation and emission wavelengths of 295 and 330 nm, respectively, for vitamin E and 330 and 480 nm, respectively, for vitamin A. Peaks of α-, δ-, and γ-tocopherol and α- and γ-tocotrienol were identified in the extracts by comparison with the retention time of standard tocopherols (Sigma, Poole, UK) and tocotrienols (Merck, Darmstadt, Germany). In all samples, α-tocopherol comprised 80–90% of the total vitamin E.

Lipid peroxidation

The total lipids were extracted from the samples by homogenization in a suitable excess of chloroform/methanol (2:1, vol/vol) according to the Folch method.[44] The chloroform extracts, which contained the lipids and also the lipid-soluble vitamins and carotenoids endogenous to the yolks or tissues, were dried under N_2 in round-bottom flasks. Sodium phosphate buffer (pH 7.4, 0.05 M) containing 1.15% (wt/vol) KCl was added to the dried lipid, and the mixture was vortex-mixed for 5 min at room temperature and then sonicated in a sonicating water bath for 5 min at 4°C. The relative proportions of lipid extract and sodium phosphate buffer were selected so as to give lipid concentrations (mg lipid/mL buffer) of 15.1–16.2 for the yolk samples, 18.2–23.4 for the YSM samples, and 5.0–6.5 for the liver samples. The precise lipid concentrations were chosen in order to ensure that, for the extracts from a particular tissue, the concentration of vitamin E in the incubation mixture was the same irrespective of dietary group. Portions (1 mL) of these mixtures were incubated at 37°C for 4 hr under air in the absence or presence of $FeSO_4$ (0.1 mM). At the end of the incubation, butylated hydroxytoluene was added to give

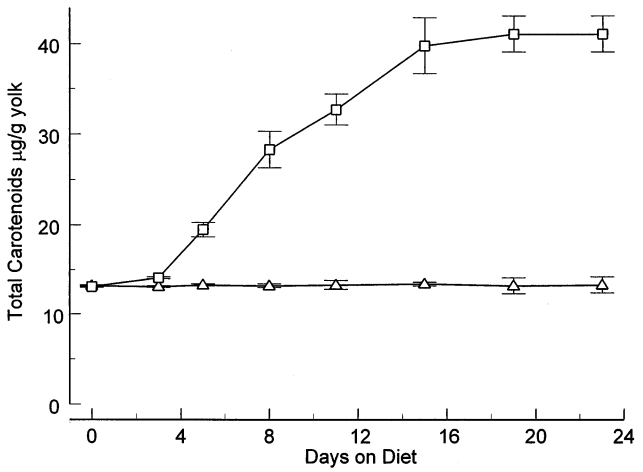

Figure 1 Time-course of the changes in yolk carotenoid concentration of freshly laid eggs after placing hens on the control (△) and high-carotenoid (❒) diets. Values are means of measurements on 5 yolks with the SE shown by the error bars.

a final concentration of 0.01% (vol/vol), and the concentration of thiobarbituric acid reactive substances (TBARS) was determined by the method of Ohkawa.[45]

Data analysis

Statistical comparisons were performed by Student's *t*-test. Values expressed as percentages were arcsine-transformed prior to statistical analysis.

Results

Dietary manipulation of yolk carotenoid content

Figure 1 illustrates the time-course of the changes in the total carotenoid content of the fresh egg yolks, which occurred when the laying hens were placed on the high-carotenoid diet. The yolk carotenoid concentration increased continuously during the period between 3 and 11 days on the high-carotenoid diet. Thereafter, the carotenoid content increased more gradually, reaching a new steady-state level by 19 to 23 days, which was 3.1 times greater than the initial concentration ($P < 0.001$). The eggs from birds that remained on the control diet maintained a constant carotenoid level throughout this period.

Developmental changes in the concentration of carotenoids in the plasma, liver, and heart of the embryo

Fertile eggs obtained from the hens over a 2-week period from the 23rd day of receiving the experimental diets were incubated and the embryos were sampled at intervals. The developmental changes in the concentrations of total carotenoids in the embryos' plasma are shown in *Figure 2A*. At day 15 of development, the plasma carotenoid concentrations in embryos derived from the high-carotenoid dietary groups were not significantly different from the control group. However, at all subsequent stages of embryonic development, the plasma concentrations from the high-

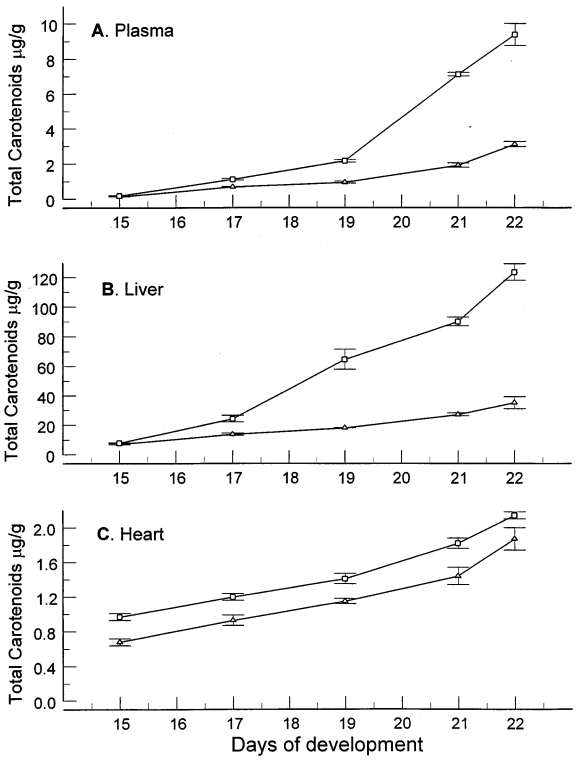

Figure 2 Changes in the concentration of carotenoids in the plasma (A), liver (B), and heart (C) of the chick embryo during development. Embryos were derived from the control (△) and high-carotenoid (❒) dietary groups. Values are means of measurements on 5 embryos with the SE indicated by the error bars.

carotenoid dietary group were significantly ($P < 0.001$) higher than in the embryos derived from the control group. In particular, for the high-carotenoid group, the concentration of carotenoids in the embryos' plasma increased dramatically between days 19 and 22 of development (day 22 represents one day after hatching). A similar but less marked increase occurred in the control group.

Changes in the concentrations of total carotenoids in the livers of the embryos during development are shown in *Figure 2B*. From day 17 onwards, the values were significantly ($P < 0.001$) higher for embryos derived from the high-carotenoid group compared with the control group. For the high-carotenoid dietary group, the rate of accumulation of carotenoids in the embryonic liver increased sharply from day 17 of development, whereas a more moderate rate of accumulation was observed for the control group.

The patterns of accumulation of total carotenoids in the embryonic heart are depicted in *Figure 2C*. From day 15 to day 22 of development, concentrations in the high-carotenoid dietary group were significantly ($P < 0.001$) higher than in the hearts from the control group, an exception being at day 22 when the values for the high-carotenoid and control groups were not significantly different. However, profile differences in the heart were distinct from those in the liver and plasma. In particular, throughout this developmental period, the heart carotenoid concentrations were only slightly higher (approximately 1.2- to 1.3-fold) in the high-carotenoid group than in the control group.

Table 1 Concentration of total carotenoids (μg/g fresh wt) in the initial yolk and in the tissues of day-old chicks derived from hens on the control and the high-carotenoid diets

	Diet	
	Control	High-carotenoid
Parent's feed	15.0 ± 0.1	96.5 ± 0.2*
Initial yolk	13.3 ± 0.9	41.1 ± 2.1*
Day-old chick		
YSM	43.7 ± 3.3	122.4 ± 5.2*
Liver	35.1 ± 4.2	123.6 ± 5.7*
Heart	1.9 ± 0.1	2.1 ± 0.1†
Kidney	2.2 ± 0.1	4.2 ± 0.4**
Lung	2.4 ± 0.1	3.0 ± 0.3†
Thigh muscle	1.4 ± 0.2	1.8 ± 0.1†
Plasma	3.1 ± 0.2	9.4 ± 0.6*

YSM = yolk sac membrane. Values are means ± SE of measurements on 5 yolks or on tissues from 5 chicks or on 5 replicate feed samples. Comparison with control group: *$P < 0.001$; **$P < 0.002$; †not significant.

Table 2 Carotenoid profile of the initial yolk and of tissues of the day-old chicks derived from hens on the control diet

	% (wt/wt) of total carotenoids		Ratio
	Lutein	Citranaxanthin	lutein/citranaxanthin
Parent's feed	51.5 ± 0.2*	39.9 ± 0.2***	1.29 ± 0.05**
Initial yolk	61.6 ± 2.0	29.4 ± 1.5	2.15 ± 0.18
Day-old chick			
YSM	62.0 ± 0.6§	28.7 ± 1.0§	2.18 ± 0.09§
Liver	32.0 ± 0.3*	40.2 ± 1.4***	0.80 ± 0.02*
Heart	73.8 ± 0.8***	23.5 ± 0.7**	3.15 ± 0.05***
Kidney	60.7 ± 1.4§	13.0 ± 0.9*	4.90 ± 0.42***
Lung	61.9 ± 0.6§	16.0 ± 0.8*	3.89 ± 0.18***
Thigh muscle	66.7 ± 2.0§	20.7 ± 1.4**	3.40 ± 0.32***
Plasma	70.1 ± 1.6‡	21.8 ± 0.7**	3.26 ± 0.17†

Significantly lower than initial yolk: *$P < 0.001$; **$P < 0.002$; significantly higher than initial yolk: ***$P < 0.001$; †$P < 0.002$; ‡$P < 0.02$; §not significant. Ratios of the concentrations (μg/g) of lutein and citranaxanthin in the samples are shown. Other details as for Table 1.

The effects of the differences in dietary supply of carotenoids to the hens on the concentration of carotenoids in the initial yolk and subsequently in the tissues of the day-old chick (i.e., day 22 of development) are summarized in *Table 1*. In the case of the chicks derived from the high-carotenoid hens, the concentrations of carotenoids in the YSM, liver, and plasma were significantly greater than in the chicks from the control group. Moreover, the scale of these differences was similar to that between the initial yolks from the high-carotenoid and control groups (approximately 3-fold). By contrast, the levels of carotenoids in the heart, thigh muscle, and lung of the chicks were not significantly different in the high-carotenoid group compared with controls. Under both parental dietary regimes, the accumulation of carotenoids was far higher in the liver than in the other tissues of the chick. High concentrations of carotenoids were also present in the YSM of the chick.

Dietary regulation of the carotenoid profiles of the yolk and the neonatal tissues

The relationships among the profiles of carotenoids exhibited by the hens' feed, the yolk, and the tissues of the day-old chick for the control and high-carotenoid dietary groups are depicted in *Table 2* and *Table 3*. These samples contained varying proportions of two unidentified peaks, with retention times of 4.24 and 12.7 min, which together comprised between 4% and 28% of the total carotenoid present. Based on the standards available, it was confirmed that these unidentified peaks were not due to cryptoxanthin, lycopene, β-carotene, or α-carotene.

The samples from the control group contained lutein and citranaxanthin but no detectable β-apo-8′-carotenoic acid or canthaxanthin (*Table 2*). The proportions of lutein and citranaxanthin in the YSM of the chick were not significantly different from the proportions initially present in the yolk. However, the proportion of lutein in the liver of the chick was markedly lower than in the yolk, whereas the proportion of citranaxanthin in the liver was significantly higher than in the yolk. The profiles for the non-hepatic tissues were very different from that of the liver. Thus, the proportions of lutein in the heart and plasma of the chick were significantly higher than in the yolk, whereas the proportions in the kidney, lung, and thigh muscle did not differ significantly from the yolk value. Moreover, the proportions of citranaxanthin in the heart, kidney, lung, thigh muscle, and plasma were significantly lower than in the yolk.

The samples from the high-carotenoid group (*Table 3*) contained lutein, citranaxanthin, and β-apo-8′-carotenoic acid. Canthaxanthin was detected in the yolk and in the chicks' YSM, liver, and heart. The relative proportions of the identified carotenoids were generally similar when comparing the yolk with the YSM. Again, the proportion of lutein in the chick liver was far lower than in the yolk. Also, the proportions of lutein in the heart, lung, thigh muscle, and plasma of the chick were significantly greater than originally present in the yolk. By contrast, the proportions of citranaxanthin and β-apo-8′-carotenoic acid in the non-hepatic tissues of the chick were significantly lower than in the original yolk.

To illustrate further the possibility of differential distribution of the various carotenoids among the developing tissues, we compared the ratios of the amounts of lutein/citranaxanthin in the yolk and in the different tissues (*Table 2* and *Table 3*). A similar pattern was evident for both the control and high-carotenoid groups. First, the ratio of these two carotenoids did not differ significantly between the yolk and the YSM. Second, the ratio of lutein/citranaxanthin was markedly lower in the liver of the day-old chick than in the initial yolk. Third, this ratio was significantly higher in the heart, kidney, lung, thigh muscle, and plasma of the chick than in the initial yolk. For example, in the high-carotenoid group, this ratio was 4.8 times higher in the chick's plasma than it was in the yolk.

Table 3 Carotenoid profile of the initial yolk and of the tissues of the day-old chicks derived from hens on the high-carotenoid diet

	% (wt/wt) of total carotenoids				Ratio Lutein/Citranaxanthin
	Lutein	Citranaxanthin	Carotenoic	Canthaxanthin	
Parent's feed	23.1 ± 0.1*	42.4 ± 0.2†	22.6 ± 0.1*	2.8 ± 0.1††	0.54 ± 0.03*
Initial yolk	29.5 ± 0.3	34.1 ± 1.1	29.5 ± 0.2	2.2 ± 0.1	0.87 ± 0.02
Day-old chick					
YSM	26.2 ± 0.9***	28.8 ± 0.6***	25.8 ± 0.8**	2.2 ± 0.1§	0.92 ± 0.05§
Liver	11.5 ± 0.4*	30.4 ± 0.9ns	32.6 ± 0.8††	3.5 ± 0.1†	0.39 ± 0.02*
Heart	43.0 ± 2.8‡	24.9 ± 0.8*	15.9 ± 0.6*	4.2 ± 0.2†	1.77 ± 0.16†
Kidney	35.7 ± 2.3§	23.0 ± 0.8*	13.3 ± 0.4*	—	1.55 ± 0.07†
Lung	53.7 ± 1.5†	16.9 ± 0.7*	10.5 ± 0.5*	—	3.25 ± 0.20†
Thigh muscle	33.3 ± 0.8‡	28.8 ± 1.7ns	18.6 ± 1.0*	—	1.18 ± 0.07‡
Plasma	54.3 ± 1.4†	13.2 ± 0.5*	—	—	4.18 ± 0.24†

Significantly lower than initial yolk: *$P < 0.001$; **$P < 0.002$; ***$P < 0.02$; significantly higher than initial yolk: †$P < 0.001$; ‡$P < 0.002$; ††$P < 0.02$; §not significant. Ratios of the concentrations (μg/g) of lutein and citranaxanthin are shown. Other details as for Table 1.

Effect of dietary carotenoids on the levels of vitamins E and A in the yolk and the neonatal tissues

Concentrations of vitamin E in the initial yolk and in the YSM of the day-old chick were not significantly affected by the level of carotenoids in the hens' diet (*Table 4*). However, the concentration of vitamin E in the chick's liver was significantly greater in the high-carotenoid group than in the control group. Levels of vitamin A in the initial yolk and in the neonatal YSM and liver were not increased by the high-carotenoid diet. However, vitamin A concentrations in the neonatal livers of the high-carotenoid dietary group were significantly greater than in the livers of the control group.

Effect of tissue carotenoid content on the susceptibility of tissue extracts to lipid peroxidation

The extent of lipid peroxidation during the incubation of tissue extracts, as estimated by the formation of TBARS, was significantly affected by the level of tissue-derived carotenoids in the mixtures. Thus, the rates of both spontaneous and Fe-stimulated peroxidation in extracts of yolk and of neonatal YSM and liver were significantly reduced in samples from the high-carotenoid group compared with the control group (*Table 5*).

Table 4 Concentrations of vitamins E and A in the initial yolks and in the day-old chick's yolk sac membrane and liver

	Dietary group	
	Control	High-carotenoid
Vitamin E (μg/g)		
Initial yolk	76.5 ± 1.3	78.5 ± 1.2
YSM	283.1 ± 5.0	298.9 ± 7.6
Liver	337.6 ± 6.2	366.8 ± 6.2**
Vitamin A (μg/g)		
Initial yolk	4.7 ± 0.1	4.7 ± 0.1
YSM	4.5 ± 0.1	5.0 ± 0.2
Liver	7.9 ± 0.1	9.3 ± 0.1*

Means ± SE of measurements on 5 yolks or on tissue from 5 chicks. Comparison with control group: *$P < 0.001$; **$P < 0.01$.

Discussion

Effect of the level of carotenoids in the parental diet on the carotenoid content of the yolk and the neonatal tissues

The concentration of total carotenoids in the yolk responded rapidly to the changes in the level of carotenoid provision to the hen. Thus, 23 days after the entry of the hens into the high-carotenoid dietary regime, a new steady state was established in which the yolk carotenoid levels were about 3 times higher than in the control. Studies in various mammalian species have suggested that different carotenoids may compete with each other for absorption and metabolism.[8] It is, therefore, possible that in the present study, the combinations of carotenoids present in the hens' diets may have resulted in interactions in which the bioavailability of each carotenoid was affected by the presence of the others. In fact, there was some evidence (*Table 2* and *Table 3*) for the preferential incorporation of dietary lutein into the yolk as the level of lutein as a percentage of total carotenoids tended to be greater in the yolk than in the diet.

Differences in the concentrations of total carotenoids in

Table 5 Susceptibility to lipid peroxidation in vitro

	TBARS (ng/hr/mg lipid)	
	Control	High-carotenoid
Fe^{2+} absent		
Initial yolk	3.5 ± 0.1	2.5 ± 0.1*
YSM	3.5 ± 0.1	2.7 ± 0.1*
Liver	10.6 ± 0.3	9.3 ± 0.2**
Fe^{2+} present		
Initial yolk	32.7 ± 0.8	25.5 ± 1.3**
YSM	8.4 ± 0.2	6.6 ± 0.4**
Liver	25.1 ± 0.8	20.9 ± 0.9**

Means ± SE of results from 5 yolk or tissue extracts. Comparison with control group: *$P < 0.001$; **$P < 0.01$.

the yolks were at least partly reflected in the carotenoid levels measured in the tissues of the day-old chicks, which developed within the eggs obtained from the two dietary groups. With regard to the chicks from the high-carotenoid group, carotenoid levels in the YSM, liver, and plasma were significantly greater than in the control group to an extent (3-fold), which reflected the difference in the carotenoid levels in the yolks. However, carotenoid concentrations achieved by the heart, lung, and thigh muscle of the chicks were not significantly greater in the high-carotenoid group than in the controls. This may be partly due to the high lipid content of tissues such as the liver[29] and YSM,[24] which may permit the accumulation of large amounts of lipid-soluble carotenoids. By contrast, tissues with a more modest lipid content at the time of hatching may be limited in their capacity to accumulate carotenoids, even when the supply of carotenoids from the yolk is increased. The higher responsiveness of the YSM, plasma, and liver of the chick to the elevated levels of carotenoids in the initial yolk may also reflect the transport mechanisms functioning in the embryo, particularly with regard to the hepatic uptake of carotenoid-rich lipoprotein remnants.[27]

The vitamin A content of the liver of the day-old chick was significantly higher in the high-carotenoid group by comparison with the control group. In order to serve as a precursor for vitamin A, a carotenoid molecule requires at least one unsubstituted β-ionone ring with an attached polyene side chain.[1–4] Thus, β-carotene, which fulfills these structural requirements, is a very effective precursor of vitamin A in organisms that express the necessary enzyme(s).[1–4,8,9] With regard to the carotenoids used in the present study, the ring systems of lutein and canthaxanthin, respectively, contain hydroxyl and carbonyl substitutions and are therefore ineffective as vitamin A precursors. However, citranaxanthin and β-apo-8′-carotenoic acid do possess pro-vitamin A activity, corresponding to about 50% and 25%, respectively, of the pro-vitamin A potential of β-carotene.[4,41] The observation that the chick's liver, but not the initial yolk, contained a greater concentration of vitamin A in the high-carotenoid group than in the controls suggests that the embryo may have some ability to convert appropriate carotenoids to vitamin A. The level of vitamin E in the liver of the day-old chicks was also significantly elevated in the high-carotenoid group. This may possibly reflect the antioxidant properties of carotenoids, preventing depletion of vitamin E levels during periods of high oxidative stress such as the hatching process. Carotenoid supplementations in humans,[8] rats,[8] and chickens[46] have variously been reported to decrease, increase, or have no effect on plasma vitamin E levels.

Discrimination between different carotenoids by the embryo

For both the control and the high-carotenoid dietary groups, the relative proportions of the various carotenoids present in the initial yolk were very similar to those displayed by the YSM of the day-old chicks. Thus, there is no evidence for any selective uptake of particular carotenoids from the yolk by the YSM during embryonic development. This is consistent with the view that lipid components are transferred from the yolk to the YSM by nonspecific phagocytosis.[22] However, a major finding of the present study was that the proportion of lutein present in the liver of the day-old chick was far lower than in the yolk and YSM, whereas the proportions of the other types of carotenoids in the chick liver were higher than in the yolk and YSM.

By contrast, the proportions of lutein in the non-hepatic tissues of the day-old chick tended to be higher than in the yolk, with commensurate decreases in the proportions of citranaxanthin and β-apo-8′-carotenoic acid in these tissues. Comparison of the lutein/citranaxanthin ratio between the different tissues provided a further illustration of the selective utilization of the various carotenoids by the embryo. In the case of both the control and the high-carotenoid groups, this ratio was the same for the yolk and the YSM, was significantly lower in the liver of the chick than in the initial yolk, and was significantly higher in the chick's heart, kidney, lung, thigh muscle, and plasma than in the yolk.

The mechanism of this apparent discrimination between the different carotenoids in the chick embryo is not clear. One possibility is that lutein may transfer more readily from the YSM-derived lipoproteins to high-density lipoprotein in the embryo's plasma, whereas the other carotenoids may exhibit a greater tendency to be delivered to the liver as components of the lipoprotein remnants. Studies on the absorption and transport of carotenoids by the mammalian intestine have suggested that the more polar carotenoids, such as lutein, tend to partition into the amphipathic surface layer of the chylomicrons and are therefore more likely to be transferred to high-density lipoprotein prior to the uptake of the chylomicron remnants by the liver.[6–9,47,48]

Carotenoids as antioxidants: Susceptibility of extracts of the chick tissues to lipid peroxidation

Carotenoids are efficient quenchers of singlet oxygen and are also effective scavengers of free radicals. However, the antioxidant activity observed is highly dependent on the type of carotenoid, the nature of the environment, the oxygen tension, and interaction with other antioxidants.[9–12,49] The elevated carotenoid levels in the yolk and chick tissue samples from the high-carotenoid dietary group were associated with significantly reduced susceptibilities to in vitro peroxidation. These results suggest that carotenoids are able to function effectively as antioxidants during incubation of these extracts, even in the presence of atmospheric oxygen concentrations. Because all the extracts were rich in vitamin E, it is feasible that these antioxidant effects may be brought about by synergistic interactions between carotenoids and vitamin E, as observed in other systems.[49] Whether the antioxidant protection afforded by carotenoids in the sample extracts in vitro is also relevant to the prevention of lipid peroxidation in vivo during the development of the embryo awaits further study.

Acknowledgments

We are grateful to the Scottish Office Agriculture and Fisheries Department and to the Royal Society for financial support and to Hoffmann-LaRoche for generous gifts of cryptoxanthin and lycopene standards.

References

1 Goodwin, T.W. (1986). Metabolism, nutrition and function of carotenoids. *Annu. Rev. Nutr.* **6,** 273–297
2 Olson, J.A. (1989). Biological actions of carotenoids. *J. Nutr.* **119,** 94–95
3 Olson, J.A. (1996). Biochemistry of vitamin A and carotenoids. In *Vitamin A Deficiency: Health, Survival and Vision* (A. Sommer and K.P. West, eds.), pp 221–250, Oxford University Press, New York, NY, USA
4 Tee, E-S. (1992). Carotenoids and retinoids in human nutrition. *Crit. Rev. Food Sci. Nutr.* **31,** 103–163
5 Olson, J.A. (1994). Absorption, transport and metabolism of carotenoids in humans. *Pure Appl. Chem.* **66,** 1011–1016
6 Van Vliet, T. (1996). Absorption of β-carotene and other carotenoids in human and animal models. *Eur. J. Clin. Nutr.* **50,** S32–S37
7 Parker, R.S. (1996). Absorption, metabolism and transport of carotenoids. *FASEB J.* **10,** 542–551
8 Furr, H.C. and Clark, R.M. (1997). Intestinal absorption and tissue distribution of carotenoids. *J. Nutr. Biochem.* **8,** 364–377
9 Rock, C.R. (1997). Carotenoids: biology and treatment. *Pharmacol. Ther.* **75,** 185–197
10 Burton, G.W. (1989). Antioxidant actions of carotenoids. *J. Nutr.* **119,** 109–111
11 Krinsky, N.I. (1993). Actions of carotenoids in biological systems. *Annu. Rev. Nutr.* **13,** 561–587
12 Rice-Evans, C.A., Sampson, J., Bramley, P.M., and Holloway, D.E. (1997). Why do we expect carotenoids to be antioxidants in vivo? *Free Rad. Res.* **26,** 381–398
13 Zhang, L.X., Cooney, R.V., and Bertram, J.S. (1991). Carotenoids enhance gap junction communication and inhibit lipid peroxidation in C3H/1OT1/2 cells: relationship to the cancer chemopreventative action. *Carcinogenesis* **12,** 2109–2114
14 Zhang, L.X., Cooney, R.V., and Bertram, J.S. (1992). Carotenoids up-regulate connexin 43 gene expression independent of pro-vitamin A or antioxidant properties. *Cancer Res.* **52,** 5707–5712
15 Rock, C.L., Kushuski, R.A., Galvez, M.M., and Ethier, S.P. (1995). Carotenoids induce morphological changes in human mammary epithelial cell cultures. *Nutr. Cancer* **23,** 319–333
16 Krinsky, N.I. (1992). Effects of carotenoids in cellular and animal systems. *Am. J. Clin. Nutr.* **53,** 238S–246S
17 Bertram, J.S. and Borthiewicz, H. (1995). Dietary carotenoids inhibit neoplastic transformation and modulate gene expression in mouse and human cells. *Am. J. Clin. Nutr.* **62,** 1327–1336
18 Bendich, A. (1991). Beta-carotene and the immune response. *Proc. Nutr. Soc.* **50,** 263–274
19 Prabhala, R.H., Garewal, H.S., Hicks, M.J., Sampliner, R.E., and Watson, R.R. (1991). The effects of 13-*cis* retinoic acid and beta-carotene on cellular immunity in humans. *Cancer* **67,** 1556–1560
20 Allen, R.G. (1991). Oxygen reactive species and antioxidant responses during development: the metabolic paradox of cellular differentiation. *Proc. Soc. Exp. Biol. Med.* **196,** 117–129
21 Ar, A. and Mover, H. (1994). Oxygen tensions in developing embryos: system inefficiency or system requirement? *Isr. J. Zool.* **40,** 307–326
22 Noble, R.C. and Cocchi, M. (1990). Lipid metabolism and the neonatal chicken. *Prog. Lipid Res.* **29,** 107–140
23 Griffin, H.D., Perry, M.M., and Gilbert, A.B. (1984). Yolk formation. In *Physiology and Biochemistry of the Domestic Fowl, Vol 5* (B.M. Freeman, ed.), pp. 345–380, Academic Press, London, UK
24 Shand, J.H., West, D.W., McCartney, R.J., Noble, R.C., and Speake, B.K. (1993). The esterification of cholesterol in the yolk sac membrane of the chick embryo. *Lipids* **28,** 621–625
25 Maldjian, A., Farkas, K., Noble, R.C., Cocchi, M., and Speake, B.K. (1995). The transfer of docosahexaenoic acid from the yolk to the tissues of the chick embryo. *Biochim. Biophys. Acta* **1258,** 81–89
26 Gaal, T., Mezes, M., Noble, R.C., Dixon, J., and Speake, B.K. (1995). Development of antioxidant capacity in tissues of the chick embryo. *Comp. Biochem. Physiol.* **112B,** 711–756
27 Surai, P.F., Noble, R.C., and Speake, B.K. (1996). Tissue-specific differences in antioxidant distribution and susceptibility to lipid peroxidation during development of the chick embryo. *Biochim. Biophys. Acta* **1304,** 1–10
28 Speake, B.K., Noble, R.C., and McCartney, R.J. (1993). Tissue-specific changes in lipid composition and lipoprotein lipase activity during development of the chick embryo. *Biochim. Biophys. Acta* **1165,** 263–270
29 Shand, J.H., West, D.W., Noble, R.C., and Speake, B.K. (1994). The esterification of cholesterol in the liver of the chick embryo. *Biochim. Biophys. Acta* **1213,** 224–230
30 Budowski, P., Leighfield, M.J., and Crawford, M.A. (1987). Nutritional encephalomalacia in the chick: an exposure of the vulnerable period for cerebellar development and the possible need for both ω6- and ω3-fatty acids. *Br. J. Nutr.* **58,** 511–520
31 Poor, C.L., Miller, S.D., Fahey, G.C., Easter, R.A., and Erdman, J.W. (1987). Animal models for carotenoid utilisation studies: evaluation of the chick and the pig. *Nutr. Rep. Int.* **36,** 229–234
32 Tyczowski, J.K., and Hamilton, P.B. (1986). Evidence for differential absorption, of zeacarotene, cryptoxanthin and lutein in young broiler chicks. *Poult. Sci.* **65,** 1137–1140
33 Ben-Amotz, A., Mokady, S., Edelstein, S., and Avron, M. (1989). Bioavailability of a natural isomer mixture as compared with synthetic all-trans beta-carotene in rats and chicks. *J. Nutr.* **119,** 1013–1019
34 Mokady, S., Avron, M., and Ben-Amotz, A. (1990). Accumulation in chick livers of 9-*cis* versus all-trans-beta-carotene. *J. Nutr.* **120,** 889–899
35 Gomez, R., Alonso, A., and Martin, M. (1978). Carotenoid absorption in chick intestine. *Rev. Esp. Fisiol.* **34,** 257–259
36 Tyczkowski, J.K., and Hamilton, P.B. (1986). Absorption, transport and deposition in chickens of lutein diester, a carotenoid extracted from marigold (*Tagetes erecta*) petals. *Poult. Sci.* **65,** 1526–1531
37 Damron, B.L., Goodson, S.R., Harms, R.H., Janly, D.M., and Wilson, H.R. (1984). β-Carotene supplementation of laying hen diets. *Br. Poult. Sci.* **25,** 349–352
38 Schaffer, J.L., Tyczkowski, J., Parkhurst, C.R., and Hamilton, P.B. (1988). Carotenoid composition of serum and egg yolk of hens fed diets varying in carotenoid composition. *Poult. Sci.* **67,** 608–614
39 Maurisch, W.L. and Bauernfiend, J.C. (1981). Oxycarotenoids in poultry feed. In: *Vitamin A Precursors* (J.C. Bauernfiend, ed.), pp. 319–462, Academic Press, New York, NY, USA
40 Mayne, S.T. and Parker, R.S. (1986). Subcellular distribution of β-carotene in chick liver. *Lipids* **21,** 164–169
41 Hencken, H. (1992). Chemical and physiological behaviour of feed carotenoids and their effects on pigmentation. *Poult. Sci.* **71,** 711–717
42 Haq, A.U., Bailey, C.A., and Chinnah, A.D. (1995). Neonatal immune response and growth performance of chicks hatched from single comb white Leghorn breeders fed diets supplemented with β-carotene, canthaxanthin or lutein. *Poult. Sci.* **74,** 844–851
43 Haq, A.U., Bailey, C.A., and Chinnah, A. (1996). Effect of β-carotene, canthaxanthin, lutein and vitamin E on neonatal immunity of chicks when supplemented in the broiler breeder diets. *Poult. Sci.* **75,** 1092–1097
44 Folch, J., Lees, M., and Sloane-Stanley, G.H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226,** 497–509
45 Ohkawa, H., Onishi, N., and Yagi, K. (1979). Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* **95,** 351–358
46 Woodall, A.A., Britton, G., and Jackson, M.L. (1996). Dietary supplementation with carotenoids: effects on α-tocopherol levels and susceptibility of tissues to oxidative stress. *Br. J. Nutr.* **76,** 307–317
47 Parker, R.S. (1997). Bioavailability of carotenoids. *Eur. J. Clin. Nutr.* **51,** S86–S90
48 Bierer, T.L., Merchen, N.R., and Erdman, J.W. (1995). Comparative absorption and transport of five common carotenoids in pre-ruminant calves. *J. Nutr.* **125,** 1569–1577
49 Edge, R., McGarvey, D.J., and Truscott, T.G. (1997). The carotenoids as antioxidants—a review. *J. Photochem. Photobiol.* **41B,** 189–200