

Designer egg evaluation in a controlled trial

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Objective: To evaluate the ability of designer eggs enriched in vitamin E, lutein, selenium (Se) and docosahexaenoic acid (DHA) to deliver micronutrients to the human in a palatable and visually acceptable form.

Design: Double-blind, placebo-controlled trial, two treatment groups balanced for sex and age.

Setting: Department of Biochemistry and Nutrition, SAC, Scotland.

Subjects: Forty healthy adult volunteers completed the study. Volunteers were recruited among staff of the Scottish Agricultural College

Interventions: Volunteers consumed, for 8 weeks, either a designer egg or a normal table egg per day. Fasting blood samples were taken before and at the end of the study.

Results: Consumption of designer eggs enriched in vitamin E, lutein, Se and DHA significantly increased the levels of α -tocopherol, lutein and DHA in plasma as compared to the changes found after consumption of normal table eggs, with the largest increases found in plasma lutein (1.88-fold increase). The proportion of DHA was increased in all the main lipid classes of the plasma including triacylglycerol (2.3-fold), free fatty acids (1.6-fold), cholesteryl ester (1.4-fold) and phospholipid (1.3-fold). Egg consumption did not change Se concentration in plasma, blood pressure, total plasma lipid concentrations or the concentrations of total cholesterol and HDL-cholesterol in plasma.

Conclusion: Consumption of designer eggs enriched in vitamin E, lutein, DHA and Se as part of normal diet for 8 weeks effectively increased the blood levels of α -tocopherol, lutein and DHA.

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Descriptors: designer eggs; antioxidants; vitamin E; lutein; DHA; Se; humans

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Introduction

The possibility of manipulating the nutrient composition of eggs was considered as long ago as 1934 (Cruikshank, 1934) and modification of the polyunsaturated fatty acid composition has been pursued since the early sixties (Wheeler *et al.*, 1959). It was initially believed that egg consumption was associated with a rise in blood cholesterol levels (Yaffee *et al.*, 1991) and as a consequence was deleterious to health and life expectancy. Recent studies have shown however, that large numbers of eggs can be consumed over lengthy periods without incurring any adverse changes to plasma cholesterol or other lipid components (Farrell, 1998). Other nutrients in eggs can have their contents manipulated as well, for example, selenium (Se) (Cantor, 1997) and various vitamins and provitamins (Naber, 1993).

The 'normal' Scottish diet is deficient in many essential micronutrients. For example, among Scottish secondary

school children less than 5% achieved a Healthy Food Choice score (Wrieden and Moore, 1995). Also in the Scottish population, a high plasma cholesterol level was recorded (Tunstall-Pedoe *et al.*, 1989; McEwan *et al.*, 1998) with increasing body mass index (McEwan *et al.*, 1998). This may well account, at least in part for the relatively poor health record of those living in Scotland when compared to the other European countries (Smith *et al.* 1990).

It has been shown that inadequate intakes of n-3 polyunsaturated fatty acids, particularly docosahexaenoic acid (DHA), adversely affect cardiovascular function (Kinsella *et al.*, 1990) and, in neonates, a deficient supply of this polyunsaturate may impair the development and functioning of the retina and brain (Green & Yavin, 1998). DHA is also important for maintaining correct membrane fluidity and permeability in certain cell types (Stiliwell *et al.*, 1997). Vitamin E, Se and the carotenoids are important antioxidants and contribute significantly to the body's defences against free radical attack and hence to its ability to counteract many disease processes (Diplock *et al.*, 1998).

Our research and that of other workers have shown that in Scotland there is often an inadequate intake of these nutrients. For example, there has been a 50% decline in dietary Se intake (and an associated decline in levels of Se in the plasma) with the result that it now provides less than half our recommended daily requirement (Barclay *et al.*, 1995). Similarly low intakes of the carotenoids result from our low consumption of green vegetables (Whichelow & Prevost, 1996) and vitamin E intake in Scottish population is often lower than the Recommended Dietary Allowance (RDA) (Bodner *et al.*, 1998).

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Contributors: PFS was the chief organiser of the study, and vitamins E and A and peroxidation analyses. Main writer of the paper. AMacP was responsible for recruitment of volunteers, ethical approval, organization of blood pressure measurement, blood sampling, cholesterol and HDL-cholesterol analyses and statistical analyses. BKS carried out fatty acid analysis and data analyses and was involved in discussion. NHCS was responsible for chicken diet formulation, egg production and poultry husbandry and was involved in data analyses and discussion.

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While all these nutrients can be obtained in tablet or capsular forms from pharmacists or health food shops, it is generally held that their supply in normal dietary components is a valuable option (Diplock *et al*, 1998). In this respect antioxidant-fortified food (van het Hof *et al*, 1998; Reilly, 1998) can be considered as an important step in improvement of the diet. Based on the results of our analyses of eggs obtained from certain wild and free-range birds (Speake *et al*, 1999) which were characterized by very high concentrations of n-3 fatty acids, vitamin E and lutein, it was decided to produce an egg which had enhanced levels of these components and also of Se, a so-called 'designer egg'. Designer eggs, particularly those with enhanced levels of the n-3 series of fatty acids have been produced in many countries (Leskanich & Noble, 1997). However, to date no eggs have been produced that have complemented the n-3 PUFA's with suitable antioxidants and few studies have evaluated the effect of consuming designer eggs. This study was designed to evaluate the ability of such an egg to deliver micronutrients to the human in a palatable and visually acceptable form.

Materials and methods

Egg production

Forty laying hens (ISA Brown, 30 weeks of age at the start of the study) were housed in cage units and fed either a standard commercial diet or a supplemented diet (both were fed *ad libitum*). The supplemented diet (currently subject to patent pending) included increased levels of vitamin E (Hoffman La Roche, Switzerland), lutein (Marigold extract, Christian Hansen, Ireland), Se (Selplex, Alltech, UK) and DHA (Tuna oil, Clover Healthcare, UK) and incorporated on average 19 mg vitamin E, 209 mg DHA, 32 µg Se and 1.9 mg lutein into each egg. Each egg would therefore supply from 40 to 100% of the adult daily reference nutrient intake of the first three of these nutrients. No reference value has yet been determined for lutein, which was the predominant carotenoid in the egg, accounting for > 85% of the total with zeaxanthin being the other main component. For comparative analyses fresh free range medium eggs, class A and organically produced free range eggs, class A were purchased from Tesco Stores Ltd.

Subjects

Forty-four healthy adult volunteers (24 men and 20 women; minimum, mean and maximum ages, respectively were 26, 41.1 ± 1.5 and 59 y) were recruited, following a full explanation and informed consent, to participate in an ethically controlled trial. The volunteers did not use vitamin E, carotenoid, Se or DHA supplements and were not using medically prescribed diets or slimming regimes. Volunteers were recruited from employees of our college. Subjects were stratified by age and sex and then randomly allocated to either a designer or a commercial table egg per day in a double-blind trial. The study protocol was approved by the University of Glasgow Ethics Committee. Subjects gave their written informed consent prior to participation. Forty volunteers (20 in control and 20 in experimental group) successfully finished the trial.

Protocol

Prior to the start of the trial, all subjects were blood sampled and their blood pressures recorded. Subjects were required to consume an average of one egg per day

(raw or cooked) for a total of 8 weeks. At the end of the trial, subjects were blood sampled and their blood pressures measured.

Analytical methods

Total and HDL-cholesterol were determined by a fully enzymatic colourimetric assay (Randox Laboratories Ltd). The intra-assay CV for cholesterol assay was 2.7%. Total lipids from egg yolk and human plasma were extracted by standard procedures following homogenization in a suitable excess of chloroform/methanol (2:1) (Christie, 1982). The amount of total lipid in the samples was determined gravimetrically. Lipids were separated into their major classes by thin-layer chromatography on silica gel G using a solvent system of hexane-diethyl ether-formic acid (80:20:1). Portions of the phospholipid, triacylglycerol, cholesteryl ester and free fatty acid fractions were subjected to transimethylation (Christie *et al*, 1970) and the composition of the resultant fatty acid methyl esters was determined by gas-liquid chromatography using a CP9001 instrument (Chrompack, Middleburg, The Netherlands) fitted with a 30 m × 0.25 mm capillary column system (Carbowax, film thickness 0.25 µm; Alltech, Carnforth, UK). Integration of the peaks and subsequent data handling was performed using an EZ Chrom Data System (Scientific Software Inc., San Jose, USA) enabling the fatty acid composition and the total amount of derived fatty acid from each sample to be quantified. The identities of the peaks were verified by comparison with the retention times of standard fatty acid methyl esters. The Data System enabled the expression of the fatty acid compositions in terms of wt% and also enabled the calculation of the amount of each acyl-lipid class from which the methyl esters were derived.

Vitamins E (α and γ tocopherols) and A in the egg yolk and human plasma were determined by the HPLC-based method of McMurray *et al* (1960) as previously described (Surai *et al*, 1996). In brief the samples were saponified with ethanolic KOH in the presence of pyrogallol and the retinol and tocopherols were extracted from the mixture with hexane. The extract was dried under nitrogen, redissolved in methanol and injected into the HPLC system (Shimadzu Liquid Chromatograph, LC-10AD, Japan Spectroscopic Co. Ltd with JASCO Intelligent Spectrofluorometer 821-FP) fitted with a Spherisorb Type S30DS2, 3 µ C₁₈ reverse-phase HPLC column, 15 cm × 4.6 mm (Phase Separations Ltd, UK). Chromatography was performed using a mobile phase of methanol/water (97:3) at a flow rate of 1.1 ml/min. Fluorescence detection of vitamin A involved excitation and emission wavelengths of 330 and 480 nm respectively. The relevant wavelengths for tocopherol detection were 295 and 330 nm. Standard solutions of all-trans retinol and α-tocopherol in methanol were used for instrument calibration and tocol was used as an internal standard. The inter-assay CVs for retinol and α-tocopherol determinations were 3.9 and 4.4%, respectively.

Lutein was separated from other carotenoids in egg yolk and human plasma and determined by HPLC, as previously described (Surai & Speake, 1998) using a mobile phase of acetonitrile-methanol (85:15) and acetonitrile-dichloromethane-methanol (70:20:10) in gradient elution as described by Granado *et al* (1998).

Plasma and egg Se was measured following production of the hydride from a continuous flow hydride generator

(PS Analytical) by means of an atomic fluorescence detector (PS Analytical) using a high powered Se lamp to initiate the Se fluorescence, as previously described (Surai *et al*, 1999). The method was validated by use of a Nycomed standard serum. The inter-assay CV for Se determination was 3.4%.

Yolk susceptibility to lipid peroxidation was determined as previously described (Surai *et al*, 1996) using incubation of egg yolk homogenate in phosphate buffer (pH 7.4) in the presence of FeSO₄ (0.1 mmol/l). The inter-assay CV was 5.6%.

For the study of vitamin stability in egg during cooking, eggs were heated in boiling water for 10 min, egg yolk was separated and vitamin E and lutein were determined as previously described

Statistical Analysis

Data were analysed by analysis of variance using the Minitab 10.5 for Windows statistical package

Results

Egg composition

The nutrient compositions of the designer and table eggs are detailed in Table 1. The carotenoid content of designer eggs was 16 times higher than in the control eggs, and different in composition from, the table eggs: in the former lutein predominated (87%) while in the latter lutein (32%) was accompanied by citranaxanthin (30%) and carotenoic acid (26%). The vitamin E concentration in the designer eggs was 20 times higher than in the control eggs. The designer eggs also provide much higher amounts of vitamin E, lutein and DHA compared to free range or organically produced free range eggs (Table 1).

Boiling of the designer eggs did not lead to any change in lutein concentration (106.2 ± 7.3 vs 103.1 ± 5.8 $\mu\text{g/g}$ yolk, $n = 10$) and no significant difference in vitamin E was observed (1090.5 ± 32.3 vs 1046.5 ± 35.8 $\mu\text{g/g}$ yolk, $n = 10$). Furthermore, the combination of antioxidant nutrients in the designer eggs increased lipid stability against

Table 1 Nutrient composition of the control eggs and the designer eggs

Amount per egg	Table eggs	Designer eggs
DHA (mg)	32.41 ± 1.11	$208.61 \pm 8.44^{***}$
Vitamin A (mg)	0.11 ± 0.01	0.12 ± 0.01
α -Tocopherol (mg)	0.72 ± 0.06	$19.33 \pm 1.02^{***}$
γ -Tocopherol (mg)	0.09 ± 0.01	0.08 ± 0.01
Lutein (mg)	0.12 ± 0.01	$1.91 \pm 0.14^{***}$
Se (μg)	4.22 ± 0.48	$32.44 \pm 3.16^{***}$

Values are means \pm s.e. ($n = 20$).

Significance of difference, from control eggs: $***P < 0.001$.

A free range medium table egg (Tesco Stores Ltd) provides 0.09 mg vitamin A, 0.61 mg α -tocopherol, 0.14 mg γ -tocopherol and 0.20 mg lutein and 45.6 mg DHA. A free range organically produced medium egg (Stonegate farmers, Tesco Stores Ltd) provides 0.08 mg vitamin A, 0.78 mg α -tocopherol, 0.32 mg γ -tocopherol and 0.05 mg lutein and 51.4 mg DHA ($n = 10$).

peroxidation. In an *in vitro* experiment, the formation of malondialdehyde (MDA) in yolk homogenates as a result of Fe²⁺-stimulated peroxidation, was 2.2 ± 0.1 $\mu\text{g/g}$ yolk ($n = 5$) in the table eggs and 0.6 ± 0.04 $\mu\text{g/g}$ yolk ($n = 5$) in the designer eggs ($P < 0.001$). The amount of DHA per egg was 6.4 times higher in the designer eggs than in the controls.

Effect of egg consumption on blood pressure and on the lipids, vitamins E and A, lutein and Se of the plasma

The initial and final blood pressures (systolic and diastolic) did not differ significantly between treatments although both treatments showed a slight decline in systolic pressure over the course of the study (Table 2). Total and HDL cholesterol levels did not differ significantly either between the treatments or from the beginning to the end of the study (Table 2). In the volunteers consuming the designer eggs, plasma concentrations of vitamin E increased from 25.63 ± 0.94 to 30.47 ± 1.08 $\mu\text{mol/l}$ ($P = 0.0012$, Table 3). In the control group there was no significant change in concentration (25.98 ± 1.11 and 24.95 ± 0.98 $\mu\text{mol/l}$) between the initial and final sampling. Therefore there was a significant ($P = 0.0006$) increase in α -tocopherol

Table 2 Mean initial and final blood pressure values and serum total and HDL-cholesterol concentrations of the control and experimental groups

Parameter	Control group		Experimental group	
	Beginning	End	Beginning	End
Systolic blood pressure (mmHg)	132.1 ± 4.0	128.2 ± 3.9	130.33 ± 4.2	126.47 ± 3.3
Diastolic blood pressure (mmHg)	78.9 ± 2.7	80.0 ± 2.4	77.67 ± 1.9	76.57 ± 2.0
Total plasma cholesterol (mmol/l)	5.41 ± 0.3	5.51 ± 0.2	5.30 ± 0.2	5.67 ± 0.2
HDL Cholesterol (mmol/l)	1.15 ± 0.1	1.12 ± 0.1	1.22 ± 0.1	1.25 ± 0.1

Values are means \pm s.e. ($n = 20$).

Table 3 Initial and final plasma antioxidant concentrations of the control and experimental groups

Parameter	Control		Experimental	
	Before	After	Before	After
α -Tocopherol ($\mu\text{mol/l}$)	25.98 ± 1.1	24.95 ± 1.0	25.63 ± 1.0	$30.47 \pm 1.0^*$
γ -Tocopherol ($\mu\text{mol/l}$)	2.10 ± 0.2	2.20 ± 0.1	2.02 ± 0.2	1.98 ± 0.2
Lutein ($\mu\text{mol/l}$)	0.21 ± 0.0	0.21 ± 0.0	0.24 ± 0.0	$0.45 \pm 0.0^{**}$
Vitamin A ($\mu\text{mol/l}$)	2.31 ± 0.1	2.17 ± 0.1	2.13 ± 0.1	2.15 ± 0.1
Se ($\mu\text{g/l}$)	82.78 ± 3.2	79.67 ± 2.5	84.07 ± 2.0	84.54 ± 3.8

Values are means \pm s.e. ($n = 20$).

Significance of designer egg effect: $*P = 0.0012$; $**P < 0.001$.

concentration in plasma of volunteers consuming the designer eggs compared to the placebo group.

The plasma α -tocopherol:cholesterol ratio increased from 4.90 to 5.43 $\mu\text{mol}/\text{mmol}$ ($P=0.015$) during designer egg consumption and was significantly ($P<0.001$) higher compared to that in the placebo group (4.55 $\mu\text{mol}/\text{mmol}$). γ -Tocopherol concentrations did not differ significantly between treatments or with time. No within- or between-treatment effects were recorded in plasma vitamin A concentrations. However the levels of plasma lutein increased significantly in the volunteers consuming the designer eggs (from 0.24 ± 0.02 to 0.45 ± 0.03 $\mu\text{mol}/\text{l}$, $P<0.001$). The difference was also highly significant ($P<0.001$) compared to the placebo group. The levels of lutein in the plasmas of those consuming the control eggs remained unchanged (0.21 ± 0.02 and 0.21 ± 0.03 $\mu\text{mol}/\text{l}$; Table 3). No significant changes in plasma concentrations of Se were observed in either treatment group (Table 3).

The concentration of total lipid in the plasma was not affected by either of the treatments (Table 4). Cholesteryl ester was the major acyl-containing lipid class, followed by phospholipid and triacylglycerol, with free fatty acid as a minor fraction. The proportions of these lipid classes were not affected by either of the dietary treatments. The lipid class with the highest proportion of DHA (approx. 4% of fatty acids) was phospholipid, and the proportion of DHA in phospholipid was significantly ($P<0.001$) increased as a result of the consumption of the designer eggs (Table 5). This difference was also significant ($P=0.022$) compared to the placebo group. The proportion of DHA in plasma triglyceride was less than 1% of fatty acids at the beginning of the trial but was increased more than 2-fold ($P<0.001$) after 8 weeks of eating the designer eggs (Table 6). In the control group there was also a trend (non-significant) towards an increased DHA proportion and as a result there was no significant difference between DHA proportion in triacylglycerol fraction of the plasma of the control and experimental groups. DHA was a very minor fatty acid of the cholesteryl ester (less than 0.7% of fatty acids and this proportion was significantly ($P<0.001$) increased in the experimental group (Table 7). The difference was also significant ($P=0.01$) compared to the placebo group. The proportion of DHA in the free fatty acid fraction of the plasma was between 1 and 2% with a significant ($P<0.001$) increase in this proportion in the designer egg group (Table 8), but this difference did not reach a significant level compared to the placebo group. No significant changes in the proportion of DHA in any lipid class were observed as a result of consuming control eggs. Linoleic acid (18:2n-6) was the major polyunsaturate of all the lipid classes, and high proportions of arachidonic acid (20:4n-6) were evident in the phospholipid and cho-

Table 5 Initial and final fatty acid composition of plasma phospholipids (as percentage of total fatty acids) of the control and experimental groups

Fatty acid	Control		Experimental	
	Before	After	Before	After
16:0	26.8 \pm 0.3	26.5 \pm 0.3	26.7 \pm 0.2	26.7 \pm 0.4
16:1	0.9 \pm 0.0	0.8 \pm 0.1	0.9 \pm 0.0	0.8 \pm 0.0
18:0	13.8 \pm 0.3	13.8 \pm 0.2	13.9 \pm 0.2	13.4 \pm 0.2
18:1n-9	11.8 \pm 0.3	11.1 \pm 0.3	12.1 \pm 0.2	11.6 \pm 0.3
18:1n-7	2.0 \pm 0.1	1.9 \pm 0.1	1.9 \pm 0.1	1.7 \pm 0.0
18:2n-6	21.8 \pm 0.7	22.1 \pm 0.7	22.6 \pm 0.5	22.7 \pm 0.7
20:3n-6	3.3 \pm 0.2	3.3 \pm 0.1	3.7 \pm 0.2	3.5 \pm 0.2
20:4n-6	9.9 \pm 0.4	10.9 \pm 0.4	9.1 \pm 0.3	9.2 \pm 0.4
20:5n-3	1.6 \pm 0.2	1.5 \pm 0.2	1.4 \pm 0.2	1.6 \pm 0.2
22:5n-3	1.2 \pm 0.0	1.1 \pm 0.0	1.1 \pm 0.1	1.0 \pm 0.0
22:6n-3	4.0 \pm 0.3	4.2 \pm 0.2	3.8 \pm 0.2	4.9 \pm 0.2**

Values are means \pm s.e. ($n=20$).

Significance of designer egg effect: ** $P<0.001$.

Table 6 Initial and final fatty acid composition of plasma triacylglycerol (as percentage of total fatty acids) of the control and experimental groups

Fatty acid	Control		Experimental	
	Before	After	Before	After
14:0	1.9 \pm 0.2	1.7 \pm 0.2	2.3 \pm 0.2	2.2 \pm 0.2
16:0	26.1 \pm 0.6	24.6 \pm 0.8	26.1 \pm 0.7	25.5 \pm 0.7
16:1	4.4 \pm 0.3	3.6 \pm 0.3	4.3 \pm 0.3	3.6 \pm 0.2
18:0	4.4 \pm 0.5	4.3 \pm 0.2	4.8 \pm 0.2	4.4 \pm 0.2
18:1n-9	37.5 \pm 0.7	37.2 \pm 0.7	38.0 \pm 0.9	36.9 \pm 0.5
18:1n-7	2.5 \pm 0.4	2.6 \pm 0.1	2.5 \pm 0.2	2.4 \pm 0.1
18:2n-6	15.2 \pm 0.9	17.5 \pm 1.2	14.6 \pm 0.6	16.4 \pm 0.7
18:3n-3	1.3 \pm 0.3	1.2 \pm 0.3	1.3 \pm 0.1	1.3 \pm 0.1
20:4n-6	1.4 \pm 0.1	1.6 \pm 0.1	1.2 \pm 0.1	1.3 \pm 0.1
20:5n-3	0.4 \pm 0.1	0.5 \pm 0.1	0.4 \pm 0.1	0.5 \pm 0.1
22:5n-3	0.5 \pm 0.0	0.6 \pm 0.0	0.5 \pm 0.0	0.5 \pm 0.1
22:6n-3	0.9 \pm 0.1	1.2 \pm 0.1	0.7 \pm 0.1	1.6 \pm 0.2**

Values are means \pm s.e. ($n=20$).

Significance of designer egg effect: ** $P<0.001$.

lesteryl ester fractions. No significant changes in the proportions of any fatty acids other than DHA were observed in any of the lipid classes as a result of egg consumption

Discussion Egg composition

By manipulating the feed of laying hens it was possible to enhance the levels of Se, vitamin E, lutein and DHA in the egg by 7.7, 26.8, 15.9 and 6.4 fold, respectively. A single designer egg contained 50% of the RDA of Se, 100% of the RDA of long chain n-3 PUFA's, and 130% of the RDA of

Table 4 Initial and final plasma acyl-lipid content and composition for the control and experimental groups

Fraction	Control		Experimental	
	Before	After	Before	After
Total lipids (mg/ml)	7.2 \pm 0.3	6.8 \pm 0.3	7.3 \pm 0.2	7.2 \pm 0.3
Lipid classes (% of total lipid)				
Cholesteryl esters	39.9 \pm 4.4	40.4 \pm 0.7	39.6 \pm 1.0	39.8 \pm 0.8
Phospholipids	28.9 \pm 0.6	29.3 \pm 0.6	28.7 \pm 0.8	29.9 \pm 0.7
Triacylglycerol	19.6 \pm 0.8	11.9 \pm 0.9	20.1 \pm 1.3	18.3 \pm 1.2
FFA	3.2 \pm 0.3	3.3 \pm 0.3	3.1 \pm 0.2	2.7 \pm 0.3

Values are means \pm s.e. ($n=20$)

Table 7 Initial and final fatty acid composition of plasma cholesterol esters (as percentage of total fatty acids) of the control and experimental groups

Fatty acid	Control		Experimental	
	Before	After	Before	After
16:0	10.6±0.2	10.8±0.2	10.5±0.2	10.8±0.1
16:1	3.6±0.3	3.5±0.3	3.4±0.3	3.3±0.2
18:0	0.9±0.0	0.9±0.0	0.9±0.0	0.9±0.0
18:1n-9	19.0±0.6	18.6±0.6	19.0±0.4	18.9±0.4
18:1n-7	1.5±0.1	1.3±0.0	1.4±0.0	1.2±0.0
18:2n-6	51.6±1.2	51.8±1.2	52.5±0.7	52.7±0.8
18:3n-3	0.8±0.2	0.7±0.0	0.8±0.1	0.8±0.1
20:4n-6	6.7±0.3	7.3±0.6	6.2±0.3	6.1±0.3
20:5n-3	1.4±0.1	1.3±0.2	1.3±0.1	1.3±0.1
22:6n-3	0.6±0.1	0.7±0.0	0.6±0.0	0.8±0.0**

Values are means±s.e. (*n* = 20).

Significance of designer egg effect: ***P* = 0.001.

Table 8 Initial and final free fatty acid composition of plasma (as percentage of total fatty acids) of the control and experimental groups

Fatty acid	Control		Experimental	
	Before	After	Before	After
14:0	1.0±0.2	0.7±0.1	1.4±0.2	1.1±0.2
16:0	19.1±0.6	19.1±0.7	20.4±0.8	20.1±1.2
16:1	3.6±0.2	2.3±0.2	2.7±0.2	2.3±0.2
18:0	10.0±0.4	11.1±0.4	10.2±0.3	10.0±0.7
18:1n-9	38.7±1.4	39.5±0.1	38.2±0.7	36.1±1.9
18:1n-7	2.5±0.2	2.5±0.1	2.6±0.1	2.3±0.1
18:2n-6	13.3±0.5	13.3±0.6	12.8±0.4	12.5±0.8
18:3n-3	1.8±0.2	1.4±0.1	1.7±0.1	1.4±0.2
20:4n-6	1.2±0.1	1.1±0.1	1.0±0.1	0.9±0.1
22:5n-6	1.1±0.3	1.3±0.3	1.5±0.3	1.4±0.4
22:5n-3	0.5±0.1	0.6±0.1	0.4±0.0	0.5±0.0
22:6n-3	1.6±0.1	1.7±0.1	1.2±0.1	1.9±0.1**

Values are means±s.e. (*n* = 20).

Significance of designer egg effect: ***P* < 0.001.

vitamin E. It also supplied 1.91 mg lutein for which no reference nutrient intake has yet been established.

The major lutein sources in the human diet are green vegetables and fruits, including spinach, squash, grapes (Sommerburg *et al*, 1998), broccoli, parsley, peas etc. (Hart & Scott, 1995). Carotenoid consumption and their serum profile vary substantially depending on the origin of the studied population. For example France presents the highest levels of serum lutein and β -carotene and Spain shows the lowest level of β -carotene, along with the highest levels of β -cryptoxanthin (Olmedilla *et al*, 1997). American women consume approximately 6 mg of total carotenoids per day (Chung *et al*, 1993), the average daily intake of major carotenoids in the Spanish population is 3.5 mg/day (Olmedilla *et al*, 1997) and in Germany total carotenoid intake amounts to 5.33 mg/day (Pelz *et al*, 1998). Daily consumption of lutein+zeaxanthin in American elderly subjects is 2.7 mg for men and 3.09 mg for women (Tucker *et al*, 1999). According to National Health Interview Surveys, the intake of lutein declined among different categories of people in the USA between 1987 and 1992 (Nebeling *et al*, 1997) and there were significant seasonal differences in plasma carotenoid concentrations in the UK, reflecting a significantly higher intake of lutein during the spring compared with summer and autumn (Scott *et al*, 1996). In this respect the inclusion of a single designer egg per day in the human diet can almost double lutein con-

sumption and can be considered as a reliable source of lutein without seasonal variation.

It is likely that increasing attention will be paid in future to the level of lutein in the diet given that it has been reported to prevent macular degeneration in the elderly, to have a protective effect against cancer and cardiovascular diseases and to increase immune competence (Mayne, 1996; Kritchevsky, 1999). Many of these effects will relate to its role as a biological antioxidant (Chopra *et al*, 1993).

The major vitamin E sources in the diet are various plant oils. In the UK, the average daily vitamin E intake is 11.7 mg in men and 8.6 mg in women and similar consumption is reported in other countries, including the USA (Weber *et al*, 1997), which is in line with the RDA. Therefore it has been concluded that, according to the RDA, the intake of antioxidants is adequate in healthy subjects (Diplock *et al*, 1998). Nevertheless, there are several categories of people whose vitamin E consumption is lower than the RDA. For example it is generally believed that elderly people have inadequate vitamin E consumption (Cid-Ruzafa *et al*, 1999) and Rudman *et al* (1995) showed that more than 60% of institutionalised elderly people consume less than 50% of the ideal dietary intake of vitamin E. Furthermore, there are many studies suggesting that intake of vitamin E in amounts much higher than the RDA are associated with reduced risk of various diseases (Bendich *et al*, 1997; Diplock *et al*, 1998; Chan, 1998) and with enhancement of certain immune responses (Meydani, 1995). On the other hand, De Waart *et al*, (1997) were not able to show a beneficial effect of 100mg vitamin E intake during 3 months on the overall immune responsiveness of elderly subjects. In this respect, Lachance (1996) has shown optimal daily vitamin E intakes to be 23 mg. Thus, an inclusion of designer eggs into the human diet (3–4 eggs per week) will increase vitamin E consumption up to this desirable level of 23 mg per day.

The typical 'Western' diet is considered to be imbalanced, providing high levels of n-6 polyunsaturated fatty acids (PUFA) and low levels of n-3 PUFAs, and this is associated with increased incidences of certain diseases (Shahidi & Wanasundara, 1998). Fish is the major source of DHA in the human diet and the intake of this PUFA has decreased for the last few years (Sargent, 1997). Therefore there is a recommendation to people who do not eat fish to obtain 200 mg of very long chain n-3 PUFA daily from other sources (De Deckere *et al*, 1998). The designer eggs used in this experiment can deliver this amount of DHA and are thus an alternative additional source of very long chain PUFA in the human diet.

Selenium, a key component of a number of functional selenoproteins required for normal health, is provided in our diet from bread and cereals, fish, poultry and meat (Reilly, 1998). There are indications that Se supplementation can decrease the incidence of cancer (Clark *et al*, 1996) as well as improve semen quality in subfertile men and the chance of successful conception (Scott *et al*, 1998). The level of Se provided with a designer egg (32.4 μ g) was much lower than those used in most Se supplementation trials (100–200 μ g/day), but represents a substantial improvement to the Scottish diet, bringing Se intake up to the UK Reference Nutrient Intakes (75 and 60 μ g/day for men and women respectively, Department of Health, 1991). Hence, designer eggs enriched by Se could be considered as an important source of Se in the diet along with other Se-fortified food products (Reilly, 1998).

Nutrient stability

Our results indicate that two major antioxidant constituents of the egg, vitamin E and lutein, are stable during egg boiling. It has also been shown that there is no alteration in fatty acid profile of eggs enriched with n-3 PUFAs during cooking (Van Elswyk *et al*, 1992) or during storage for 7 weeks at 25°C (Oku *et al*, 1996).

On the other hand, n-3 enriched eggs are characterized by increased susceptibility to oxidation (Cherian *et al*, 1996) which can cause problems during egg storage and cooking. Enrichment of egg yolk in vitamin E is thought to be an effective means to resolve this problem (Cherian *et al*, 1996). In the designer eggs in this experiment, a combination of high levels of two antioxidants, vitamin E and lutein, significantly decreased MDA formation as a result of Fe-stimulated lipid peroxidation, in spite of the high content of the highly unsaturated DHA in hens' eggs. Similarly, egg enrichment by vitamin E and carotenoids decreased cholesterol oxidation in egg lipids exposed to nitrogen oxide (Lai *et al*, 1996a) or during egg powder preparation (Lai *et al*, 1996b). Vitamin E enrichment of the egg yolk prevents carotenoids from oxidation as well (Lai *et al*, 1996b). Thus it seems likely that the combination of two antioxidants, namely vitamin E and lutein, may improve the storability of the designer eggs compared to normal table eggs, even in the presence of enhanced levels of DHA.

Blood pressure and total and HDL-cholesterol

There was no significant effect of egg consumption on any of these parameters and none of them changed significantly over the 8 weeks duration of the trial. What small changes did occur were generally in a favorable direction, eg systolic blood pressure (BP) decreased by 4 mm Hg and HDL-cholesterol increased slightly to 1.25 mmol/l in the supplemented group. The decrease in systolic BP was the same as that achieved by Farrell (1998), but in his case the difference achieved significance possibly because of a larger number of subjects ($n=56$). In another study, a high intake of omega-3 enriched eggs (four eggs per day for 4 weeks) lowered systolic and diastolic blood pressure and triglyceride concentration (Oh *et al*, 1991).

A great number of clinical and the epidemiological studies demonstrate that dietary cholesterol is not the major determinant of plasma cholesterol level in healthy individuals and raise a question regarding the justification of recommending restrictions on egg consumption (McNamara, 1997). In an earlier study it was also concluded that differences in egg consumption were unrelated to blood cholesterol level or to coronary heart disease incidence (Dawber *et al*, 1982). This is in agreement with another study in humans suggesting that a moderate egg intake should not be rigorously restricted in healthy individuals (Schnohr *et al*, 1994). More conclusive results have come from a recent study which involved 37,850 men and 80,082 women with the suggestion that consumption of up to one egg per day is unlikely to have substantial overall impact on the risk of cardiovascular disease or stroke among healthy men and women (Hu *et al*, 1999).

Plasma vitamins

Consumption of designer eggs significantly increased the plasma vitamin E concentration (to $30.47 \pm 1.08 \mu\text{mol/l}$) over that of the control group ($25.63 \pm 0.94 \mu\text{mol/l}$) and was effective in all treated subjects. The ratio vitamin

E:cholesterol was also significantly increased, reaching a desirable level of $5.43 \mu\text{mol/mmol}$. It is interesting that the optimal value for α -tocopherol:cholesterol was estimated to be $> 5.1 \mu\text{mol/mmol}$ (Gey, 1995). A similar response in plasma vitamin E was found after 10-week consumption of antioxidant-enriched margarine providing 31 mg vitamin E/day (van het Hof *et al*, 1998). Vitamin E concentrations in the control and experimental group were in the physiological range (Weber *et al*, 1997). Even though the baseline for vitamin E was higher than that in other recent studies (van het Hof *et al*, 1998). We can speculate that if the vitamin E baseline had been lower, the response to designer egg consumption may have been even higher. Plasma γ -tocopherol and vitamin A concentrations were unaffected by treatment.

The baseline plasma lutein concentrations in this study were similar to those published by Mayne *et al* (1998) but somewhat lower compared to other studies (Granado *et al*, 1998; Paetau *et al*, 1998), and were highly significantly increased in the treated group ($0.45 \pm 0.03 \mu\text{mol/l}$) compared to the controls ($0.24 \pm 0.02 \mu\text{mol/l}$).

Taking into account that vitamin E in the egg yolk is found in the easily digestible α -tocopherol form (Surai, 1999), and the highly positive response to vitamin E and lutein supplementation in this study, it seems likely that the bioavailability of these nutrients from cooked egg yolk is quite high. For example, lutein concentration in the plasma increased 2-fold (similar to our case) after consumption of a more than 5 times greater amount of lutein (11.3 mg) from spinach powder (Muller *et al*, 1999), but the experiment was shorter (2 weeks) compared to 8 weeks of egg consumption in this study. Egg yolk contains about 6 g of lipids including saturated, mono- and polyunsaturated fatty acids (Speake *et al*, 1998) and may be ideal for providing the necessary amount of lipids for mixed micelle formation in the lumen of the human intestine, an important step in lutein and vitamin E absorption (Parker *et al*, 1999; Surai, 1999).

Plasma selenium

The lack of response to the additional Se supplied by the designer egg was surprising as there was scope for at least a 50% increase in concentration from the baseline value of $80 \mu\text{g/l}$. Such increases have been achieved in other studies, although admittedly with a higher level of supplementation ($100 \mu\text{g Se}$) given as a tablet (MacPherson & Bacso, 1995; Scott *et al*, 1998). The increased provision from the designer egg ($28.2 \mu\text{g Se}$) over the control was evidently insufficient to have a significant effect on plasma Se or alternatively its bioavailability must have been too low. Nevertheless, there is evidence that consumption of eggs enriched with Se by 4 times more than in our case during 3 months significantly increased serum and hair Se levels (Yu *et al*, 1996). Similarly, in Chinese children from the area endemic for Keshan disease, Se content in hair increased due to consumption of Se-enriched eggs during 3 y but sodium selenite was more effective (Yu *et al*, 1998). More work is needed to determine the bioavailability of Se from egg and egg products.

Fatty acids

The concentrations of total lipid in the plasma were in the normal physiological range (Akins *et al*, 1989). Eight weeks of intake of control or designer eggs did not alter either total lipid concentration or the proportions of the

lipid classes. The consumption of DHA-enriched eggs resulted specifically in significantly higher proportions of DHA in each of the human plasma lipid fractions, with no significant changes in any of the other fatty acid components. In plasma phospholipids, DHA was increased 1.3-fold from 3.7 ± 0.2 to $4.9 \pm 0.2\%$ of fatty acids by the designer egg consumption. Increases in the proportion of DHA in the other fractions ranged from 1.4-fold in cholesterol ester to 2.3-fold in the plasma triacylglycerol and 1.6-fold in free fatty acid. The increases in DHA proportion affected all treated subjects with the exception of one subject who had had the highest baseline values. It may be suggested that the enhancement of plasma DHA concentrations by eating designer eggs could result in beneficial effects on various health-related parameters such as cardiovascular function, inflammation and immunocompetence (Sanders, 1993; Uauy *et al.*, 1999).

Functional or designer foods and their roles in human diet have received substantial attention in recent years (Mazza, 1998; Reilly, 1998). For example, dairy products and other processed foods, including mayonnaise, margarine and dressings) containing DHA (Takahata *et al.*, 1998) as well as n-3 enriched eggs (Van Elswyk, 1997; Leskanich & Noble, 1997) are already on the market in different countries. Antioxidant-fortified margarine is shown to be effective in the delivery of vitamins E and C as well as α - and β -carotene to humans (van het Hof *et al.*, 1998). Clearly, the egg has a great unexplored potential in terms of improvement of human diet, as indicated by this study. Recently a range of bioactive peptides in the egg has been characterized with antihypertensive, phagocytosis-stimulating and opioid properties which may be beneficial for humans (Kato, 1998). In addition, egg consumption may be also of interest in regulating aspects of glucose metabolism (Pelletier *et al.*, 1996). These and other options of egg use in the human diet need further investigation.

Conclusion

The data indicate that a designer egg enriched in vitamin E, lutein and DHA can be not only a good nutritional product but also a good vector for the delivery of three essential nutrients vital for human health. A crucial feature of these designer eggs is the synergistic combination of n-3 fatty acids with major antioxidants, vitamin E and lutein, as an important approach to the improvement of the human diet. These eggs will not be able to replace vegetable and fruits as a major source of natural antioxidants and fish products as a source of DHA but can substantially improve the diet, especially in countries like Scotland, significantly contributing to the daily intake of vitamin E, lutein, DHA and Se.

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