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Modified Egg Composition To Reduce Low-Density Lipoprotein Oxidizability: High Monounsaturated Fatty Acids and Antioxidants versus Regular High *n*−6 Polyunsaturated Fatty Acids

NIVA SHAPIRA* AND JOSEPH PINCHASOV

Stanley Steyer School of Health Professions, Tel Aviv University, Ramat Aviv, Tel Aviv, Israel, and Animal Nutrition and Physiology, SiAP, Rehovot, Israel

Eggs high in *n*−6 PUFA, predominant in Western markets, were found to increase blood LDL oxidation, suggesting a new health concern beyond raising cholesterol. Protective composition was explored by increasing egg antioxidants and MUFA and reducing *n*−6 PUFA. Lag times to plasma LDL oxidation were significantly shortened with two eggs/day of high-PUFA compositions compared to a low-egg (2–4/week) regime, by 28.8% following "HPUFA-regular" ($p < 0.01$) and by 27.2% following antioxidant-fortified "HPUFA-HAOX" ($p < 0.01$). However, two "HMUFA-HAOX" eggs/day with reduced egg *n*−6 PUFA FA% (LA by 30.7%) and PUFA:MUFA ratio (LA:OA by 45.8%) plus increased antioxidants (vitamin E 500%, carotenoids 260%), resulting in increased plasma OA 33.3%, vitamin E 22.4%, and carotenoids 55.0% ($p < 0.01$), were associated with lag-time only 6.6% shorter than low-egg (NS). Among health-oriented egg modifications, here for the first time they reduced associated LDL oxidization, consistent with anti-inflammation and antioxidant paradigms, warranting further research on functional advantages of antioxidative egg composition.

KEYWORDS: egg; LDL; oxidation; MUFA; PUFA; cholesterol; antioxidants

INTRODUCTION

High intake of eggs with high *n*−6 polyunsaturated fatty acids (PUFA) has been found to induce increases in blood low-density lipoprotein (LDL) oxidizability (*1–3*), suggesting new health considerations for egg consumption beyond raising blood cholesterol and new questions regarding the potential of designing a more antioxidative composition.

Reviewed literature suggest that oxidized LDL may play a key role in the pathophysiology of atherosclerosis. Specifically, data indicate it is toxic to endothelial cells, decreases vasodilatation, generates foam cells and fatty streaks in blood vessel walls, stimulates proatherogenic autoantibodies in blood plasma and tissues, and may promote monocyte and T-cell recruitment and monocyte adhesion to the endothelium, endothelial expression of growth factors, and proinflammatory cytokines (*4, 5*).

LDL *n*−6 PUFA, mostly in the phospholipid (PL) and cholesteryl ester (CE) fractions, are readily oxidized in vivo and may stimulate inflammatory processes, which further facilitates LDL oxidation (*6*). Reaven and Witztum (*7*) reported that LDL linoleic acid (LA, 18:2, *n*−6 PUFA) strongly correlates with LDL oxidation, whereas LDL oleic acid [OA, 18:1 *n*−9 monounsaturated fatty acids (MUFA)]—increased by high dietary intake—is associated with increased LDL resistance and reduced oxidation (*8–11*).

* Correspondence and reprint requests: Address: 5 Kehilat Zitomir St., Tel Aviv 69405, Israel. Tel: +972-3-6497998. Fax: +972-3-6472148. E-mail: nivnet@inter.net.il.

Antioxidants such as vitamin E, which increases LDL content with dietary supplementation, may effectively reduce LDL susceptibility to oxidation in humans, and carotenoids, with a similar but lesser independent effect (*12, 13*), may protect vitamin E and thus synergistically enhance its effectiveness in a highly oxidative environment (*14*) as in high-PUFA eggs. Otherwise, individual antioxidants could be converted to prooxidants (*15*).

Overall LDL susceptibility to oxidation is influenced by the balance between antioxidants (i.e., vitamins E and A, carotenoids, and selenium (*5, 7, 16*) and oxidation-resistant lipids (i.e., MUFA) (*8–11*) vs oxidation-prone (i.e., PUFA (*16, 17*). Low plasma oxidative stress and reduced LDL oxidizability were found to be associated with diets high in antioxidants and MUFA, i.e. from olive oil (*14, 18*).

Among PUFA, both *n*−3 and *n*−6 PUFA (particularly long-chain) are highly susceptible to oxidation (*6*), but where *n*−3 PUFA may reduce in vivo oxidation due to anti-inflammatory properties, metabolic products of *n*−6 PUFA are proinflammatory and high amounts may be associated with chronic disease (*19*). As *n*−6 PUFA are generally much higher in Western diets and livestock feeds (*19*), including in egg production, the potential for reducing their proportion and associated health risk was the focus of this study.

Research has repeatedly shown that it is possible to modify egg composition by decreasing saturated fatty acids (SFA) or increasing *n*−3 PUFA and/or antioxidants (*20–22*). While some

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clinical investigations have shown an association between such modifications and consumers' plasma levels of lipids and/or antioxidants (*21, 22*), no effort has been hitherto invested in modifying egg composition specifically for reducing blood LDL oxidizability.

This study aimed to evaluate the potential for modifying egg composition to limit increases in human LDL oxidative response when compared to high $n-6$ PUFA eggs, the predominant "regular" egg in Western markets, by reducing PUFA (mostly $n-6$ LA), and increasing MUFA (mostly $n-9$ OA) and key antioxidants (mostly vitamin E and carotenoids).

MATERIALS AND METHODS

Egg Production. Laying hens (Yarkon strain, aged 4–5 weeks; Poultry Breeding Union, Israel) were fed for 44 weeks with either "HPUFA-regular" (control) standard Israeli feed mixture (Koffolk, Tel Aviv, Israel; Milumor Ltd., Kiriat Byalik, Israel) based on corn (50.0%) and soy (31.0%), high in $n-6$ PUFA (3.1% LA); "HPUFA-HAOX" feed, standard corn-soy mixture enhanced with a vitamin E (α-tocopherol) and carotenoid (zeaxanthin, cryptoxanthin, violaxanthin, neoxanthin, antheraxanthin, and polyoxyxanthophylls) premix based on Biotene Total PX (a proprietary supplement including rice bran, alfalfa meal, and kelp); or "HMUFA-HAOX" feed based on milo (62.1%), with reduced $n-6$ PUFA (1.4% LA). Ingredient breakdown and nutritional composition of feeds are provided in **Table 1**. Feed intakes were similar between groups, averaging 116 g/day. Vitamin E mean intakes were 3.3 mg/day in the HPUFA-regular regime (28.6 mg/kg feed), and 18.2 and 18.8 mg/day in the HPUFA-HAOX and HMUFA-HAOX regimes, respectively (158.2 mg/kg feed). Control and test egg production was supervised by the Hebrew University of Jerusalem, Faculty of Agriculture (Rehovot, Israel), in compliance with regulations governing animal subject welfare, as reviewed by the animal care and use committees.

Study egg compositions are summarized in **Table 2**; cholesterol content ranged from 213 to 230 mg in all types, vitamin E ranged from 1.0 to 2.0 mg in HPUFA-regular and 5 to 10 mg in HAOX-types, and carotenoids ranged from 450 to 600 μg in HAOX-types. Whole egg weight did not differ significantly between eggs, HPUFA-regular ranging from 58.0 to 63.8 g, HPUFA-HAOX from 58.5 to 61.5 g, and HMUFA-HAOX from 64.7 to 64.8 g. Analyses were performed by SiAP and AminoLab Laboratories (Rehovot, Israel).

Study Population. Participants were healthy, nonsmoking adults ($n = 17$; 14 women, 3 men), aged 30–50 years, BMI 23–26 kg/m^2, with high health awareness and low regular egg consumption (2–4/week). Eligibility was based on nutritional, lifestyle, and metabolic screening. Exclusion criteria were hyperlipidemia, history of disease influencing lipid oxidative/antioxidative metabolism, smoking, pregnancy, use of medications (including hormones or anticholesterol) or antioxidant supplements, or lack of regular physical exercise (<30 min three times/week).

Participants consumed a standard "Israeli"-type diet, at baseline averaging 1738.0 ± 517.9 kcal/day (29% from fat, 53% from carbohydrate, 18% from protein), and Israeli average PUFA:SFA ratio (0.92) and PUFA:MUFA ratio (0.77) (*23*). During the low-egg (two to four/week) period, they consumed approximately 146 mg/day cholesterol, and during the intervention period, normal dietary intake plus two study eggs/day averaged 1867.6 ± 556.5 kcal/day (32.9% from fat, 50% from carbohydrate, 17.1% from protein) and 587.0 ± 13.5 mg/day cholesterol.

Compliance. All participants gave informed, written consent to the study protocol, which was approved by the Helsinki Committee at Rambam Medical Center, Haifa, Israel.

Written self-reports of dietary intake were confirmed in individual interviews to establish study intervention compliance.

Experimental Protocol and Egg Regimes. Participants maintained their normal lifestyle and diets, with the exception of differing egg regimes for consecutive periods of three weeks: (1) "low-egg", intake of two to four commercially available eggs/week; (2) two "HPUFA-regular" eggs/day, similar to commercially available; (3) two "HPUFA-HAOX" eggs/day; and (4) two "HMUFA-HAOX" eggs/day. Eggs were numbered and distributed to participants weekly and consumed separately, one at lunch and one at dinner, and total consumption was confirmed by counts.

Table 1. Composition and Nutritional Analysis of Laying Hen Feeds in HPUFA-Regular, HPUFA-HAOX, and HMUFA-HAOX Regimes

	composition, g/kg		
ingredient	HPUFA-regular	HPUFA-HAOX	HMUFA-HAOX
corn	500	500	–
soybean meal 44	310	310	–
barley		30	30
milo	–	–	621
soybean meal 48	–	–	160
corn gluten meal	–	–	70
wheat bran	–	–	27
soapstock oil	40	–	–
canola oil	–	40	10
vitamin and mineral premix (control)	10	–	–
vitamin and mineral premix (fortified)	–	10	10
synthetic lysine	–	–	0.187
DCP	16	16	11
salt	3.3	3.3	3
DL-methionine	0.4	0.4	0.514

	nutritional analysis		
component	HPUFA-regular	HPUFA-HAOX	HMUFA-HAOX
metabolizable energy, kcal/kg	2800	2800	2800
protein, g/kg	183	183	180
arginine, g/kg	12.4	12.4	13
lysine, g/kg	10.6	10.6	10.5
methionine, g/kg	3.9	3.9	4
methionine + cysteine, g/kg	6.7	6.7	7.1
calcium, g/kg	37	37	38
phosphorus, g/kg	4	4	3.1
vitamin E, mg/kg	28.6	158.2	158.2
fat, wt %	6	6	3
% total fatty acid			
lauric 12:0	0.3	0.3	–
myristic 14:0	0.5	0.3	0.2
palmitic 16:0	12.2	8.5	11.2
palmitoleic 16:1	0.4	0.1	–
stearic 18:0	5.9	3	1.5
oleic 18:1	30.7	37.6	36.9
linoleic 18:2	41:1	41:1	40.8
linolenic 18:3	2.9	6.7	4.8
arachidonic 20:4	1	0.2	0.3
lingoceric 24:0	2.2	0.4	0.9

Individual dietary intake was assessed at baseline and following each study regime using a semiquantitative food-frequency questionnaire validated by the Clinical Nutrition Department at Rambam Medical Center in Haifa, Israel. Nutritional analysis was performed by Dietitian (version 8.3) software, designed and validated by Rambam Medical Center, based on Magic eDeveloper (Magic Software Enterprises, Ltd., Israel), using Israeli food composition tables (Ministry of Health, Jerusalem, Israel).

Fasting blood samples were taken once following each dietary treatment period, 12 h after the supper meal. Blood was drawn into tubes with 1 mmol/L sodium ethylenediaminetetraacetate (Na_2EDTA) and stored at 4 °C under nitrogen until analysis (*24*); analyses were performed for all samples at the same time.

Biochemical and Hematological Analyses. *Fatty Acid (FA) Composition.* Egg fat and blood plasma were extracted by the Folch method (*25*) and saponified in 2.7 mmol/L potassium hydroxide at 37 °C for 5 h. FA of plasma and eggs were esterified in methanol containing 0.36 mmol/L hydrochloric acid and 0.38 mol/L 2,2 dimethoxypropane at 70 °C for 35 min and analyzed by gas–liquid chromatography (GLC) (*26*)) using a Hewlett-Packard model 5890

Table 2. Size and Composition of HPUFA-Regular[a], HPUFA-HAOX, and HMUFA-HAOX Eggs

	HPUFA-regular	HPUFA-HAOX	HMUFA-HAOX
weight, g (without shell)	63.8 (57.8)	64.7 (57.9)	64.8 (58.7)
yolk, g	16.9	15.2	17.2
fat, g	5.2	5.4	5.3
cholesterol, mg	212.5	220.4	229.9
carotenoids, μg	193.9	471.3	560.3
vitamin E, mg	1.1	6.6	9.3
% total fatty acid (mg/g yolk)			
lauric 12:0	–	–	–
myristic 14:0	0.3 (0.9)	0.3 (1.1)	0.4 (1.2)
palmitic 16:0	30.1 (92.6)	29.9 (106.2)	27.2 (83.8)
palmitoleic 16:1	0.8 (2.5)	0.8 (2.8)	4.5 (13.9)
stearic 18:0	10.5 (33.6)	10.7 (38.0)	5.5 (16.9)
oleic 18:1	37.4 (115.1)	34.1 (121.1)	47.3 (145.8)
linoleic 18:2	17.9 (55.1)	21.5 (76.4)	12.4 (38.2)
linolenic 18:3	0.4 (1.2)	0.4 (1.4)	0.6 (1.8)
arachidonic 20:4	0.3 (0.9)	0.3 (1.1)	0.3 (0.9)
lingoceric 24:0	0.2 (0.6)	0.2 (0.7)	0.8 (2.5)
linoleic:oleic ratio	0.48:1	0.63:1	0.26:1

[a] Used in both low-egg and HPUFA-regular study regimes.

series II gas chromatograph (Valbron, Germany), under the following conditions: splitless for the first 1 min and then in split mode with a 1:15 ratio; the oven was programmed from 150 to 290 °C at a rate of 10 °C/min and then held at 290 °C for 20 min; helium flow was 33.7 cm/s and flow in the column was 0.8 mL/min, with a constant pressure of 7.2×10^4 Pa (*24*).

Standard Plasma Biochemistry. Glucose concentrations were determined by the glucose dehydrogenase method (Hoffmann-La Roche, Basel, Switzerland, catalog no. 07–3671–6); blood urea nitrogen (BUN) by an enzymatic hydrolysis method of urea by urease and determination of absorbance at 340 nm (Raichem, San Diego, CA, USA, catalog no. 82042); and serum total cholesterol, LDL, very low-density lipoprotein (VLDL), high-density lipoprotein (HDL) cholesterol, and triacylglyceride (TG) concentrations by enzymatic kits (Raichem, catalog nos. 80015, 85510, and 80008).

Vitamins E and A and Carotenoids. Plasma concentrations of vitamin E, vitamin A, and carotenoids were determined using standard HPLC (high-performance liquid chromatography) according to methods applied by Reaven et al. (*12*).

LDL Oxidation. LDL resistance to oxidation was indicated by lag-time to initiation of oxidation, as measured by elevation of malondialdehyde (MDA) equivalents (nmol/mg LDL protein). Kinetics of LDL oxidation were measured by optical density (OD) of conjugated diene formation.

Blood in 1 mmol/L Na_2EDTA was centrifuged at 200× for 10 min at room temperature. Four milliliters of plasma from each blood sample were dialyzed against 0.01 M phosphate-buffered saline (PBS) overnight at 4 °C. LDL was separated from the plasma by discontinuous density-gradient ultracentrifugation, in media consisting of a saline solution of different densities, placed over a plasma solution of 1.250 g/mL (with potassium bromide) (*24*). LDL was then dialyzed against 1 mmol/L Na_2EDTA. Two milliliters of the dialyzed plasma was incubated for 3 h at 37 °C without (control) or with (100 mmol/L) 2,2-azobis 2-amidinopropane hydrochloride (AAPH) (Polysciences, Warington, PA) and then maintained at 4 °C and analyzed for oxidation state within 2 h using the thiobarbituric acid reactive substances (TBARS) assay to measure MDA equivalents (*27*). LDL was diluted in PBS to 0.2 mg of protein/mL [determined by Folin phenol reagent (*28*)] and dialyzed overnight against PBS at 4 °C to remove the Na_2EDTA. The LDL was then incubated in the presence of 10 μmol/L of $CuSO_4$ at 37 °C for 3 h. Oxidation was analyzed by conjugated diene formation, as determined by the increase in absorbance (OD) at 234 nm and terminated by refrigeration and the addition of 1 mmol/L Na_2EDTA.

Statistical Analysis. Sample size was calculated to detect differences in LDL oxidation response between egg compositions, based on a previous study (*3*), with $\alpha = 0.05$ and $\beta = 0.2$, using PASS 6.0 (NCSS, Kaysville, UT).

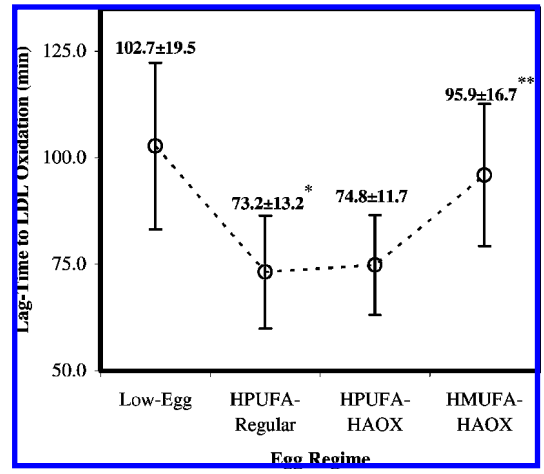

Figure 1. Lag-time to plasma LDL oxidation following low-egg (2–4/week) and two eggs/day HPUFA-regular, HPUFA-HAOX, or HMUFA-HAOX regimes ($n = 17$). *, $p < 0.01$ (vs low-egg); **, $p < 0.01$ (vs HPUFA-regular).

Blood LDL oxidation, FA, antioxidants, and other blood chemistry measures were compared among egg regimens by repeated measures ANOVA and further analyzed by Bonferroni test for multiple comparisons (*29*). ANOVA was applied to detect differences first among the three high-PUFA egg regimes (low-egg, HPUFA-regular, and HPUFA-HAOX) and then for all four egg regimes (including HMUFA-HAOX).

Plasma antioxidant and OA levels were further compared by contrasting combined regimes as follows: (a) without vs with added antioxidants (low-egg plus two/day HPUFA-regular eggs vs HPUFA-HAOX plus HMUFA-HAOX two/day egg regimes), and (b) HPUFA vs HMUFA eggs (low-egg plus two/day HPUFA egg regimes vs two/day HMUFA-HAOX eggs). Statistical analyses were carried out using SPSS 14.0 (Chicago, IL).

Potential carryover effects of consecutive egg regimes are discussed.

RESULTS

Lag times to plasma LDL oxidation as measured by MDA elevation (**Figure 1**) with two eggs/day of either HPUFA-type were significantly shortened compared to the low-egg (2–4/week) regime, by 28.8% following HPUFA-regular ($p < 0.01$) and by 27.2% following HPUFA-HAOX ($p < 0.01$), with no statistical difference between regular and high antioxidant egg regimes. However, lag-time following two/day HMUFA-HAOX eggs remained essentially unchanged from low-egg intake, shortened by only 6.6% (NS).

Similarly, LDL oxidation as measured by conjugated diene concentration (**Figure 2**) was initiated much earlier following either two egg/day HPUFA-type regime compared to low-egg intake ($p < 0.01$), but not with the HMUFA-HAOX regime (NS). Again, no statistically significant difference was observed between two/day HPUFA-regular and HPUFA-HAOX egg regimes.

Total cholesterol, LDL, and LDL:HDL ratio were all increased with consumption of two eggs/day of all three types, HPUFA-regular, HPUFA-HAOX, and HMUFA-HAOX: total cholesterol by 12.5, 18.8, and 8.3%, respectively; LDL by 17.2, 27.6, and 17.2%; and LDL:HDL ratio by 33.3, 23.8, and 14.3%. Total cholesterol increases were significant with HPUFA-regular and HPUFA-HAOX ($p < 0.01$), but not with HMUFA-HAOX eggs. HDL was relatively lower (14%) only following the HPUFA-regular regime ($p < 0.01$). Other biochemical measures remained essentially unchanged throughout the study period (liver function tests, VLDL, TG, creatinine), save for blood

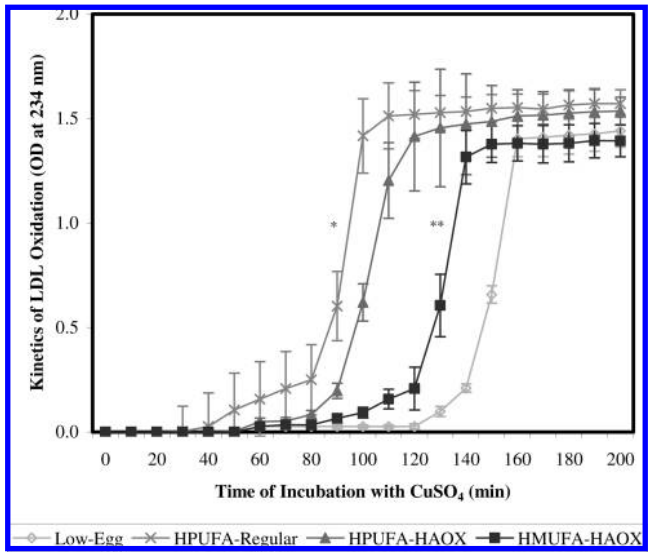

Figure 2. Kinetics of plasma LDL oxidation following low-egg (2–4/week) and two eggs/day HPUFA-regular, HPUFA-HAOX, or HMUFA-HAOX regimes ($n = 17$). *, $p < 0.01$ (vs low-egg); **, $p < 0.01$ (vs HPUFA-regular).

Table 3. Plasma Chemistry and FA following Low-Egg (2–4/week) and Two Eggs/day HPUFA-Regular, HPUFA-HAOX, or HMUFA-HAOX Regimes ($n = 17$)

measure	low-egg week 0	HPUFA-regular week 3	HPUFA-HAOX week 6	HMUFA-HAOX week 9
total cholesterol, mmol/L	4.8 ± 0.6	5.4 ± 1.0[b]	5.7 ± 1.1[b]	5.2 ± 1.1
HDL-cholesterol, mmol/L	1.4 ± 0.2	1.2 ± 0.2[b]	1.4 ± 0.4[c]	1.4 ± 0.2[d]
LDL-cholesterol, mmol/L	2.9 ± 0.5	3.4 ± 0.6[b]	3.7 ± 0.5[b]	3.4 ± 0.5
VLDL[a]-cholesterol, mmol/L	0.6 ± 0.3	0.5 ± 0.3	0.6 ± 0.3	0.6 ± 0.2
triacylglycerides, mmol/L	1.2 ± 0.5	1.1 ± 0.5	1.3 ± 0.4	1.3 ± 0.4
vitamin E, μmol/L	23.2 ± 0.2	23.7 ± 4.4	33.9 ± 9.1[c]	29.0 ± 6.0[c]
vitamin A, μmol/L	1.9 ± 0.4	2.0 ± 0.5	2.7 ± 0.6[c]	2.3 ± 0.7[c]
carotenoids, μmol/L	2.1 ± 0.4	2.0 ± 0.5	3.0 ± 1.1[c]	3.1 ± 0.8[c]
glucose, mmol/L	4.7 ± 0.4	5.4 ± 0.4[b]	4.7 ± 0.4[d]	4.7 ± 0.4[d]
BUN, mmol/L	4.3 ± 1.4	5.7 ± 0.7[b]	5.4 ± 1.4	5.0 ± 1.4
key FA % total plasma FA				
palmitic 16:0	19 ± 2	21 ± 3	20 ± 2	19 ± 1
stearic 18:0	8 ± 1	10 ± 1	10 ± 2	8 ± 2
oleic 18:1	11 ± 2	12 ± 2	9 ± 2	16 ± 3[e]
linoleic 18:2	29 ± 4	24 ± 9	25 ± 5	24 ± 5
arachidonic 20:4	10 ± 2	10 ± 3	11 ± 2	9 ± 2

[a] VLDL = Very low-density lipoprotein Values are represented as mean ± SD. [b] $p < 0.01$ (vs low-egg). [c] $p < 0.01$ (vs HPUFA-regular). [d] $p < 0.05$ (vs HPUFA-regular). [e] $p < 0.01$ (vs HPUFA-HAOX).

glucose and BUN levels, which increased significantly only following HPUFA-regular eggs (**Table 3**).

Compared to the two regimes with HPUFA commercial composition—low-egg (2–4/week) and HPUFA-regular (two/day)—plasma concentrations of vitamin E following the HPUFA-HAOX regime increased by 46.1 and 43.0% ($p < 0.01$) and following HMUFA-HAOX by 25.0 and 22.4% ($p < 0.01$), respectively; plasma carotenoids increased following HPUFA-HAOX by 42.9 and 50.0% ($p \gtrsim 0.01$) and with HMUFA-HAOX by 47.6 and 55.0% ($p < 0.01$).

Plasma OA increased significantly only following HMUFA-HAOX eggs compared to the other regimes (by 45.5% vs. low-egg, $p < 0.01$); other FA were not significantly affected (**Table 3**).

DISCUSSION

The finding that reducing PUFA ($n-6$ LA) and increasing MUFA ($n-9$ OA) plus antioxidants (vitamin E and carotenoids) reduced LDL oxidation—from the heightened levels following two eggs/day of the commercial HPUFA type, nearly to the levels associated with low-egg intake of 2–4/week (**Figure 1**)—suggests that an egg could be designed to limit egg-induced LDL oxidative response. Reduced PUFA:MUFA ratio appears to be the lead protective factor, since increases in antioxidants alone while the HPUFA composition (HPUFA-HAOX) was maintained did not significantly improve oxidative indicators (**Figures 1** and **2**), despite significant increases in plasma antioxidants (**Table 3**). The insufficiency of a high-antioxidant egg was not entirely surprising, considering the larger amounts of vitamin E needed to reduce LDL oxidation (*30*) and limited evidence of its beneficial effects against cardiac events or mortality (*31*), as well as inconsistent results with β-carotene (*13–15*). However, the unexpected efficacy of the HMUFA-HAOX composition could also have been partially associated with a much higher vitamin E:PUFA ratio (mean 14.2:1), approximately 12 times that found in HPUFA-regular eggs (1.2:1) and 2.5 times that in HPUFA-HAOX eggs (5.7:1). This may have resulted not only from the direct effect of reduced PUFA content but also from reduced vitamin E oxidation, further enhancing the protective effect of vitamin E in the blood. Carotenoids may have played an additive role in protecting vitamin E in both HAOX-type eggs (*13, 14*).

The apparent synergistic antioxidative property in the HMUFA-HAOX egg, led by HMUFA, is consistent with a previous study suggesting the protective potential of a high-MUFA, high-vitamin E combination diet against small LDL particle oxidation (*32*). A close relationship of LDL composition with diet was previously shown by a corresponding increase in CE PUFA:MUFA ratio (to 4.66:1) following a high $n-6$ PUFA diet, and a decrease (to 1.68:1) following a high-MUFA diet (*33*). However, here we saw proportionately large changes in LDL oxidation of ± 30% following relatively minor dietary FA changes in egg OA and/or LA of ± 1–3 g (±15–25% total daily intake), in contrast to blood cholesterol increases of <20% following an egg-associated dietary cholesterol increase of >200%. This may suggest a uniquely close relationship between LDL composition and egg FA and antioxidants, which is an accordance with LDL being a major vehicle for both FA and fat-soluble antioxidants. The higher increases in plasma total cholesterol with both HPUFA-regular and HPUFA-HAOX regimens (+12.2 and +18.8% above low-egg, respectively) compared to a smaller increase following the HMUFA-HAOX regime (+8.3%), despite similar cholesterol intakes, may suggest an additional benefit of antioxidative egg composition, especially for cholesterol hyper-responders.

While carryover effect is a possible limitation of the present study design with consecutive interventions, the 3-week experimental regimes may be expected to provide sufficient time to discern the influence of each treatment. Previous research showed that plasma vitamin E levels already peak at approximately 2 weeks of supplementation in most healthy adults and return to baseline within 1 week following discontinuation, with changes in lag-time to LDL oxidation being highly correlated (*13*). Further, a highly responsive increase in plasma OA and longer lag time to oxidation with the final HMUFA-HAOX regime may suggest a rapid and substantial antioxidative

impact of the latter despite the preceding six consecutive weeks of high HPUFA egg intake.

Beyond direct protection against LDL oxidation, increased egg antioxidative composition appeared to be associated with improved plasma glucose, BUN, and HDL. All are factors associated with the metabolic syndrome and thus may be highly relevant to diabetics and obese individuals. Increased HDL, which is known to be associated with paraoxonase enzymes and hydrolyzation of lipid peroxides, may indirectly further contribute to reduced LDL oxidizability (*15*). Such multipronged protection may be particularly important for egg composition given plasma LDL increases with high egg intake, as observed in this study.

The present results strongly suggest that, under conditions of continuous high egg consumption, eggs with reduced $n-6$ PUFA and enhanced $n-9$ MUFA and antioxidant content may provide better protection against LDL oxidizability compared to high-PUFA composition [particularly $n-6$, whereas egg $n-3$ PUFA may yield their own beneficial mechanisms for in vivo protection (*19*)]. Where most specialty eggs are modified either to lower cholesterol and/or SFA or increase $n-3$ PUFA, antioxidants, and other nutrients, the goal of reducing LDL oxidizability by modifying egg composition, explored here for the first time, may warrant further research. The latter may contribute to optimization of qualitative and quantitative guidelines for egg production and consumption, for the general population and for specific subgroups and individual risks.

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