doi:10.1017/S0043933910000322

Every egg may have a targeted purpose: toward a differential approach to egg according to composition and functional effect

N. SHAPIRA

Tel Aviv University, Stanley Steyer School of Health Professions, Tel Aviv, Israel
Corresponding author: nivnet@inter.net.il

Despite the suggested health advantages of traditionally- over industrially-produced western 'regular' eggs and the flexibility of egg composition, studies on the effects of egg intake refer mostly to quantity and lack qualitative information. The possibility of lending differential nutritional enhancement and functional advantages, i.e. vs. cardiovascular disease (CVD) or for perinatal health, could impact the current egg intake debate. N-3 polyunsaturated fatty acid (PUFA)/long-chain PUFA (LCPUFA) fortification of regular eggs by feeding extruded linseed (5%) yielded 3.8-fold higher total n-3 PUFA and 2.4-fold higher docosahexaenoic acid (DHA), with 3.6-fold lower n-6:n-3 PUFA ratio ($p \leq 0.0005$). This resulted in human dietary contributions of 10-20% of the n-3 PUFA Dietary Reference Intake (DRI) and 40% for DHA. Together with antioxidants, they may be beneficial against CVD risks as associated with oxidative stress, endothelial dysfunction, dyslipidemia, and inflammatory processes, especially in diabetics. Eggs fortified via poultry feed supplementation could attain higher %DRI for pregnancy or lactation for key nutrients, i.e. DHA (≈120- 130%), vitamins A (9.0-15.2%) and E (51.6-65.3%), iodine (15.2-20.1%), and selenium (33.7-39.3%). For infants aged 1-3 years, the improvement in %DRI for vitamins, minerals, and n-3 PUFA needed during peak brain development could be even higher. Compared to increased low-density lipoprotein (LDL) oxidation as seen with intake of two regular high n-6 PUFA eggs/day, eggs with reduced n-6 PUFA (by 40%), increased n-9 monounsaturated FA (MUFA) (by 30%), reduced PUFA: MUFA ratio (by 50%), and increased antioxidants vitamin E and carotenoids (by >200%), were associated with a 30% drop in LDL oxidisability ($p<0.01$), back to levels seen with a low-egg diet (2-4 eggs/week). Because egg composition is highly feed-dependent and closely affects plasma nutrients and lipoprotein composition and physiological qualities, it has much potential for imparting both nutritional and functional benefits. Poultry feeding could be carefully tailored for egg modification to address specific risks and requirements in consumers, warranting further research regarding differential effects and corresponding quantitative recommendations for egg intake, to maximise beneficial and preventative potential.

World's Poultry Science Journal, Vol. 66, June 2010
Received for publication February 22, 2009
Accepted for publication March 9, 2010

Keywords: egg; n-3 PUFA; n-9 MUFA; antioxidants; perinatal nutrition; CVD

Introduction

Despite suggested health advantages of the traditional type egg – *i.e.* from hens raised on 'wild' foods naturally rich in key nutrients such as n-3 polyunsaturated fatty acids (PUFA) and carotenoids – over modern industrially-produced eggs high in n-6 PUFA, and flexibility of egg composition depending on feed, the current experience with 'designer' eggs has not yet provided evidence about their health effects. Some beneficial modifications may suggest it is possible to improve the nutritional and functional impact, enabling 'tailor-made' eggs to be produced, and justifying health claims according to their composition and compatibility with various populations.

Numerous epidemiological and clinical studies suggest a relationship between high n-6 PUFA intake and n-6:n-3 PUFA ratio and risk of developing chronic diseases associated with Western dietary patterns (Simopoulos, 2008). These have been observed at high rates in Western countries such as the United States, Northern Europe, and Israel (Harris *et al.*, 2009; Dubnov and Berry, 2003; Hu and Willett, 2002; Yam *et al.*, 1996), as well as other parts of the developed world.

High intake of n-6 PUFA-rich eggs has been found to induce increases in low-density lipoprotein (LDL) oxidation (Levy *et al.*, 1996; Levy *et al.*, 1997; Shapira, 2004). Oxidised LDL has been suggested to play a key role in the pathophysiology of atherosclerosis (Steinberg *et al.*, 1989; Stocker and Keaney, 2004). LDL PUFA, mostly the phospholipid (PL) and cholesteryl ester (CE) fractions, are readily oxidised in vivo and may stimulate inflammatory processes, further facilitating LDL oxidation (Kaplan and Aviram, 1999; Kratz *et al.*, 2002). LDL susceptibility to oxidation is influenced by the balance between antioxidants (*i.e.* vitamins E and A, carotenoids, and selenium; Chancharme *et al.*, 2002; Reaven and Witztum, 1996; Stocker and Keaney, 2004), oxidation-resistant constituents (*i.e.* monounsaturated fatty acids, MUFA (Aviram and Eias, 1993; Baroni *et al.*, 1999; Hargrove *et al.*, 2001; Mata *et al.*, 1997), and pro-oxidants (*i.e.* n-6 PUFA; Kratz *et al.*, 2002; Reaven and Witztum, 1996).

High intake of n-3 PUFA has been suggested as beneficial in reducing the risk of a number of human disease conditions, including type 2 diabetes mellitus, neurological disorders and cardiovascular disease (CVD) (Hu and Willett, 2002; Lewis *et al.*, 2000; Nettleton *et al.*, 2004; Seo *et al.*, 2005; Simopoulos, 2008; Weisman *et al.*, 2004). N-3 long-chain PUFA (LCPUFA), eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) in particular, compete with arachidonic acid (ARA, 20:4 n-6) in metabolic pathways, reducing its resultant pro-inflammatory icosanoids (Seo *et al.*, 2005; Weisman *et al.*, 2004). Moreover, n-3 PUFA – as well as n-9 MUFA and certain antioxidants, may protect endothelial cells, and have been inversely correlated with inflammatory effects and endothelial dysfunction associated with post-prandial lipemia/hypertriglyceridemia (Rivellese *et al.*, 2003; Hennig *et al.*, 2001; Goodfellow *et al.*, 2000; Bruckner, 1997).

Where modern industrially-produced eggs in some western countries, notably America and Israel, typically have very high content of n-6 polyunsaturated fatty acids (PUFA) relative to n-3 PUFA (n-6:n-3 PUFA ratio ≥17.4:1), traditionally-produced eggs contained significant amounts of n-3 PUFA and LCPUFA, as well as a variety of antioxidants and phytonutrients (Yannakopoulos *et al.*, 2005; Simopoulos, 1999). As egg fatty acid (FA) profiles are highly dependent upon the formulation of the feed given to laying hens (Sosin *et al.*, 2006; Yannakopoulos *et al.*, 2005; Lewis *et al.*, 2000; Nitsan *et al.*, 1999), they could be altered to provide a significant source of n-3 PUFA and LCPUFA. However, modern western high n-6 PUFA feed may competitively inhibit enzymatic transformation of n-3 PUFA to LCPUFA (James *et al.*, 2000). Recent agricultural research has shown that it is possible to modify egg composition by decreasing n-6 PUFA or increasing n-3 PUFA, n-9 MUFA, and/or antioxidants (Yannakopoulos *et al.*, 2005; Surai *et al.*, 2000; Jiang and Sim, 1993). Eggs have been found to be highly effective in fatty acid (FA) and antioxidant accretion from hen feed and delivery to consumers, as shown by increased n-3 PUFA cellular levels (Makrides *et al.*, 2002), modified n-6:n-3 PUFA ratio (Weill *et al.*, 2002), and increased blood n-9 MUFA (Shapira and Pinchasov, 2008). Consumers of modified eggs, which have enhanced n-3 PUFA or n-9 MUFA at the expense of n-6 PUFA, may lend a health advantage (Shapira and Pinchasov, 2008; Bourre and Galea, 2006; Surai *et al.*, 2000).

Although recent research has suggested that consumption of up to seven eggs/week is unlikely to increase CVD risk (Djoussé and Gaziano, 2008; Hu *et al.*, 1999), correlations to negative effects may still be found in specific populations, notably diabetics (Djoussé and Gaziano, 2008; Qureshi *et al.*, 2007; Hu *et al.*, 1999), and those with increased LDL oxidation risk, such as hypercholesterolemia, general oxidative stress, abdominal obesity (Knopp and Paramsothy, 2006), impaired glucose tolerance (Schwab *et al.*, 1998), and diabetes mellitus (Dimitriadis *et al.*, 1996). As eggs have a high capacity to yield n-3 PUFA/LCPUFA, n-9 MUFA, and antioxidants in amounts that can affect blood levels in consumers (Shapira and Pinchasov, 2008; Makrides *et al.*, 2002; Jiang and Sim, 1993), such modifications may reduce the potential risk as compared to consumption of high n-6 PUFA generic eggs. The present paper details examples of how 'designer' eggs could be relevant to different health purposes and, if properly targeted and wisely consumed, may provide a significant nutritional and functional advantage, justifying differential recommendations regarding quality and quantity for various populations.

N-3 PUFA egg fortification: nutritional significance to the human diet

In light of the important benefits of adequate n-3 PUFA (especially n-3 LCPUFA) and low dietary n-6:n-3 PUFA ratio associated with various medical conditions (Hu and Willett, 2002; Lewis *et al.*, 2000; Nettleton *et al.*, 2004; Seo *et al.*, 2005; Weisman *et al.*, 2004), as well as the needs of various populations, the question regarding n-3 PUFA fortification' of eggs for a wider range of consumers is worth addressing. A controlled study comparing eggs produced with either standard feed high in n-6 PUFA (particularly linoleic acid, LA) or the same feed supplemented with alpha-linolenic acid (ALA, 18-3 n-3) from 5% extruded linseed (Weill *et al.*, 2002) showed increases in total n-3 PUFA by 3.8-fold that of control eggs (258.2 *vs.* 67.3 mg/egg), ALA by 6.4-fold (156.7 *vs.* 24.5 mg), and DHA by 2.4-fold (101.6 *vs.* 42.8 mg). Correspondingly, the ratio of total n-6:n-3 PUFA was reduced 3.6-fold from the control, LA:ALA by 5.7-fold, and LCPUFA ARA:DHA 3.0-fold (*Table 1*). N-3 PUFA increased 3.4-fold after the first week of feeding the fortified diet, then gradually to 3.7- and 4.0-fold after the third and fifth weeks, respectively, while n-6 LCPUFA ARA was slightly reduced, to 0.79 of the control.

Egg contribution as a percentage of daily recommendations (% Dietary Reference Intakes (DRI)) for humans as calculated by comparing FA contents to dietary intake of American (Ervin *et al.*, 2004) and Israeli (ICDC, 2004) populations, and to DRI (Institute of Medicine, 2002) for adults aged ≥19 years (ALA 0.6-1.2% daily kcal intake, and LCPUFA 0.06-0.12%), showed. %DRI of DHA provided in the high n-3 PUFA-modified egg may be highly relevant to western countries such as the United

States and Israel, that have high total n-6 PUFA intakes. Its DHA contribution equalled approximately 27.9% and 39.9% of the upper DRI (0.12% kcal) for American and Israeli men, respectively (*vs.* control eggs 11.8% and 16.8%), and 39.5% and 42.8% for women (*vs.* 16.7% and 17.8%, respectively), while total n-3 PUFA and ALA contributed a much lower % DRI (*Figure 1*).

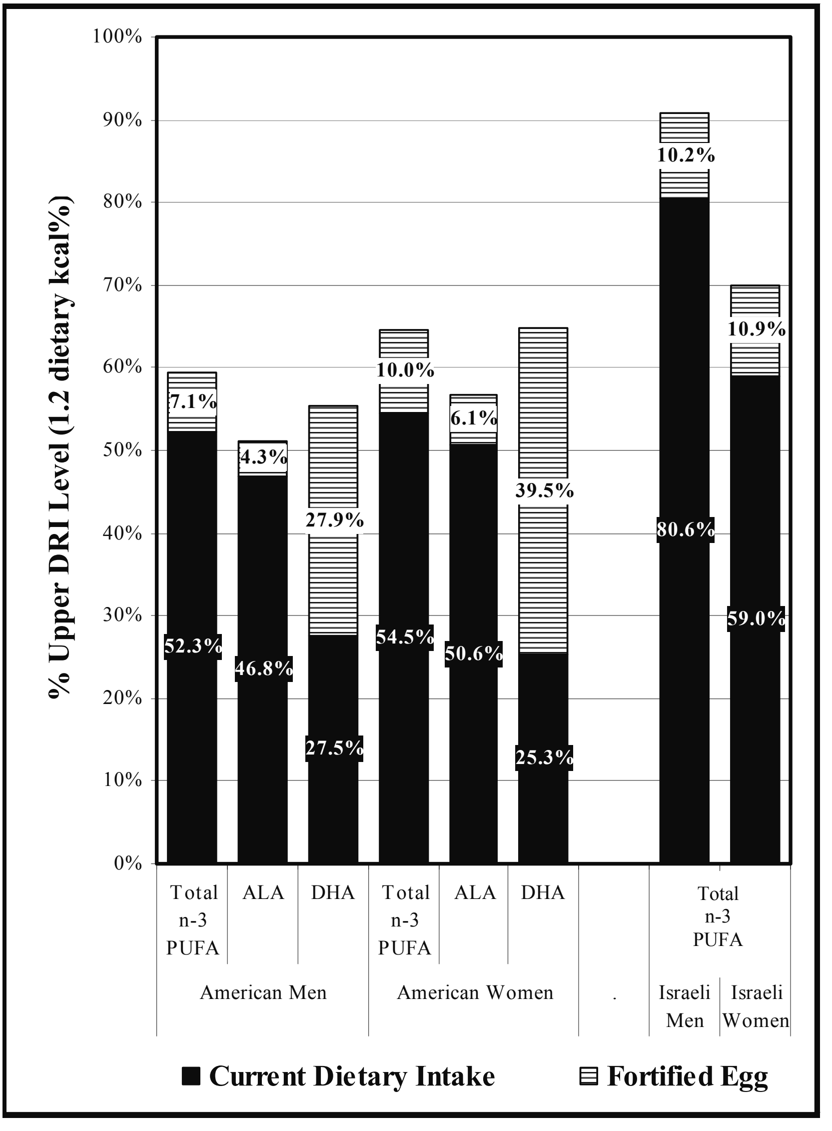

Figure 1 **N-3 PUFA in current diets and in fortified eggs as %upper DRI (kcal%) levels[a] calculated for American (total, ALA, DHA) and Israeli (total[b]) men and women.**
[a] DRI ranges: total n-3 PUFA and/or ALA=0.6-1.2 kcal%, DHA=0.06-0.12 kcal% of daily kcal intake for Americans (CDC, 2006) and Israelis (ICDC, 2003)
[b] Information for Israeli intake is limited to major FA classes

Egg cholesterol content was compared to current United States guidelines (≤300 mg/day) and to current consumption (Ervin *et al.*, 2004; ICDC, 2004). In response to cholesterol consumption, blood levels vary widely, and substitution of 'designer' for regular eggs does not provide additional dietary cholesterol changes. The 'individual risk' approach to egg consumption guidelines appears to be most relevant including with regard to amounts and type of egg (regular or modified).

The highly effective incorporation and transformation of supplemental ALA into egg yolk lipids (Nitsan *et al.*, 1999) enables fortified eggs to provide a significant dietary source of DHA. Although lower than coldwater marine fatty fish – DHA in one egg equalling approximately 25% the average daily amount that could be obtained by two 100 g servings/week of wild marine salmon – it does not present the drawback of increasing scarcity and environmental contaminants associated with marine harvesting (Foran *et al.*, 2005). The substantial increase in egg n-3 LCPUFA is in accordance with previous studies (Lewis *et al.*, 2000; Sosin *et al.*, 2006; Yannakopoulos *et al.*, 2005), demonstrating the exceptional effectiveness of the egg for transforming ALA (18:3 n-3 PUFA) to DHA (22:6 n-3 LCPUFA), even in a high n-6 PUFA feed, and without an accompanying increase in n-6 LCPUFA, primarily ARA (*Table 1*). Because conversion of ALA to DHA in humans can be quite low (0.05-4.0%) (Burdge and Calder, 2005) – and there is enzymatic competition with high levels of n-6 PUFA LA, as n-6 and n-3 undergo transformation via the same enzyme (James *et al.*, 2000), alternative sources of preformed n-3 LCPUFA are particularly important in light of their general scarcity for some populations (Endevelt and Shahar, 2004; Lewis *et al.*, 2000). Of note, modification of eggs to enhance n-3 PUFA content has not been associated with negative effects (Jiang and Sim, 1993; Lewis *et al.*, 2000; Makrides *et al.*, 2002). Feed cost analysis showed slightly greater total feed cost/egg, translating to an increase of 2.0-2.5% in total egg cost, subject to market feed prices.

N-3 PUFA-fortified egg for perinatal nutrition supplementation

Beyond the unique innate nutritional composition of hens eggs, including high quality protein, fats, and essential vitamins and minerals, including choline, iron, and selenium (*Table 2*), levels of some of these nutrients can be further enhanced to meet specific perinatal requirements (Crawford, 2006), *i.e.* for n-3 PUFA (Bourre, 2006a; 2006b; Bourre, 2005; Yannakopoulos *et al.*, 2005; Makrides *et al.*, 2002; Borod *et al.*, 1999; Jiang and Sim, 1993), which are increasingly scarce, especially marine n-3 LCPUFA (Endevelt and Shahar, 2004; Lewis *et al.*, 2000).

N-3 PUFA and LCPUFA (particularly DHA) are of primary interest for the perinatal period, as they are required in higher levels during pregnancy and lactation and are progressively depleted (Makrides and Gibson, 2000), especially in conditions of combined multiparity and dietary inadequacy (Prentice *et al.*, 1989), and are essential for optimal brain development during the peak foetal and infancy periods (Bourre, 206b; Singh, 2003). The dramatic increases in egg total n-3 PUFA and DHA attained by linseed diet fortification (Shapira *et al.*, 2008) reached up to 18-20% and 73-78% of pregnancy-lactation DRI, respectively, exemplify how significantly egg composition can be adapted to serve as a reliable and clean source for maternal, foetal, and infant needs. Fortified eggs may supply a significant amount of the daily intake recommendations in pregnancy and lactation for vitamins A (9.0-15.2%) and E (51.6-65.3%), and minerals iodine (15.2-20.1%) and selenium (33.7-39.3%). Meeting recommendations for children (1-3 years) are even more successful when supplying designer eggs in their daily food intake (Shapira, 2009).

Epidemiological studies suggest that concern regarding hypercholesterolemia may be lower during childbearing years (20-44), as it has been reported among 10.4% of American women in this age bracket (CDC, 2006). Total cholesterol intake in this group averages 241 mg/day, median 185 mg/day (Ervin *et al.*, 2004) – *vs.* DRI ≤300 mg/day – concurrent with intake of 0.7 egg/day (AEB, 2008).

Egg allergy incidence has recently been noted to equal cow's milk allergy (Bhombal *et al.*, 2006), and egg exclusion to not always eliminate allergen passage from mother to foetus. Rather, early-life exposure has unexpectedly been observed to modulate immune responses, and even be somewhat protective (Vance *et al.*, 2005). Furthermore, as high n-3 PUFA consumption during pregnancy (Sausenthaler *et al.*, 2007) and lactation (Palmer and Makrides, 2006) was recently suggested to decrease general allergy risk in human offspring, high n-3 PUFA eggs may provide an advantage relative to regular eggs in reducing rather than increasing allergy risk.

Egg modifications *vs.* CVD risk

HIGH N-3 EGG *VS.* DYSLIPIDEMIA AND ENDOTHELIAL FUNCTION

Postprandial lipemia can be used as a model representing the advantage of high n-3 PUFA eggs *vs.* CVD risk in reducing postprandial triglyceride levels, attenuating inflammatory response and protecting endothelial function (Shapira, 2008). The postprandial triglyceridemia response has been shown to be reduced by adding n-3 PUFA to either high-fat, high-MUFA, or high-saturated FA (SFA) meals (Rivellese *et al.*, 2003). This modificaton is correlated to improvements in endothelium-dependent (Goodfellow *et al.*, 2000; Okuda *et al.*, 1997) and endothelium-independent flow (Mori *et al.*, 2000). This effect may be mediated by increased membrane fluidity of endothelial cells, and promote synthesis and/or release of nitric oxide (Anderson *et al.*, 2001; Goode *et al.*, 1997). N-3 PUFA may reduce vascular inflammation, as suggested by a positive correlation with decreased inflammatory activity in cells (De Caterina *et al.*, 2000). The reduction in plasma triacylglycerides after increased n-3 LCPUFA intake has been demonstrated to have a favorable impact on LDL size (Griffin *et al.*, 2006). The egg's capacity as an effective vehicle for n-3 PUFA and antioxidant delivery suggest that fortified eggs may contribute significantly to a reduction of postprandial CVD risks, yielding a particular advantage over typical western eggs high in n-6 PUFA (Shapira, 2008), exemplifying key interrelationships between protective nutrients *vs.* risk factors (Hennig *et al.*, 2001).

ANTI-OXIDATIVE EGG MODIFICATION *VS.* LDL OXIDATION

The potential for modifying egg composition specifically to limit the recently identified effect of increased human LDL oxidative response following high consumption of high n-6 PUFA eggs was assessed in a controlled study (Shapira and Pinchasov, 2008). Key biochemical measures were evaluated in human subjects consuming eggs produced with either standard feed high in n-6 PUFA (LA 3.1% FA) based on corn (50.0%) and soy (31.0%) ('HPUFA-regular' – control), or feed with reduced n-6 PUFA (LA 1.4% FA) and much higher n-9 MUFA (oleic acid [OA] > 36.9% FA) and antioxidants. The latter diets were based on milo (62.1%) and a vegetal antioxidant premix enhanced with vitamin E and carotenoids ('HMUFA-HAOX'). Experimental egg cholesterol ranged from 213-230 mg/egg in all types; vitamin E ranged from 1.0-2.0 mg in HPUFA-regular to 5-10 mg in HMUFA-HAOX, and carotenoids 350-800 μg/egg, respectively.

Following consumption of two eggs/day of either type for three consecutive weeks each, consumers' LDL oxidisability was comparable to the low oxidation levels observed at baseline ('low-egg' – 2-4 HPUFA-regular eggs/week). The baseline length of lag-time to LDL oxidation was shortened with two HPUFA-regular eggs (by 28.8%, $p<0.01$), indicating increased oxidisability, whereas with two HMUFA-HAOX eggs/day lag-time was numerically 6.6% shorter, and 31.0% ($p<0.01$) longer than with two HPUFA-regular eggs/day. Similar results were observed when conjugated diene formation was measured by optical density (OD) at 234 nm (*Figure 2*).

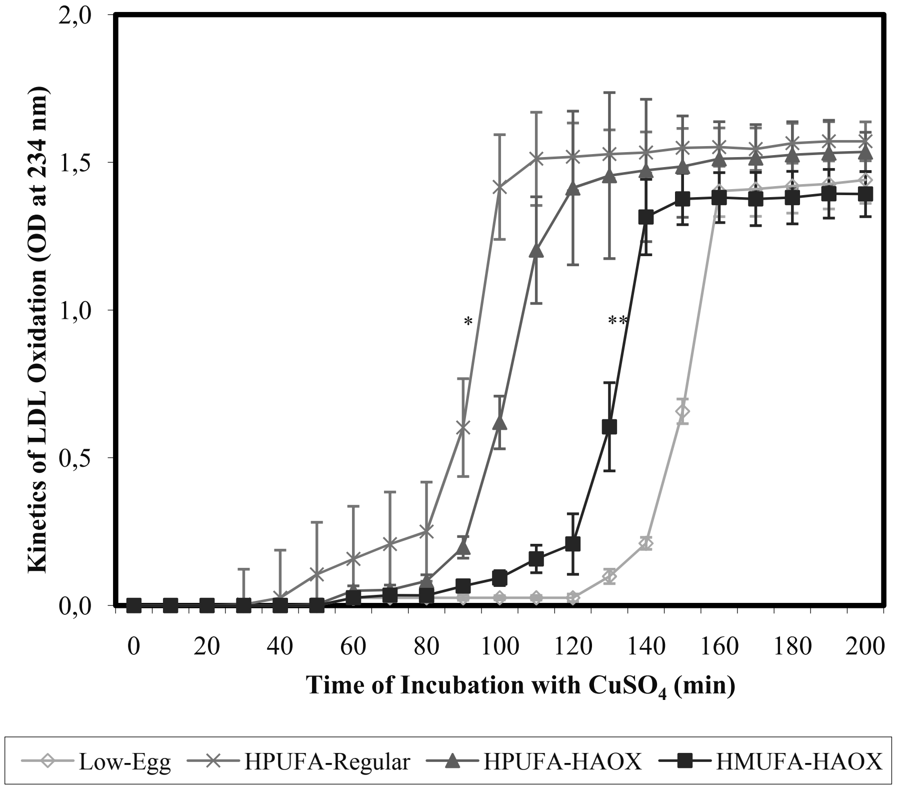

Figure 2 Kinetic analysis of LDL oxidation in blood following low-egg regime and two eggs/day of HPUFA-regular, HPUFA-HAOX, or HMUFA-HAOX (n=17).
$^{*}p<0.01$ (*vs.* low-egg); $^{**}p<0.01$ (*vs.* HPUFA-regular)

The finding that reducing egg PUFA (n-6 LA) and increasing MUFA (n-9 OA) and antioxidants (vitamin E and carotenoids) limited the increased LDL oxidisability observed with high n-6 PUFA regular egg consumption shows the potential of designing anti-oxidative eggs with synergistic compositions for reducing LDL oxidation. The anti-oxidative success of the HMUFA-HAOX egg composition was consistent with a previous study suggesting the protective potential of a diet high in MUFA and vitamin E against LDL oxidation (Reaven *et al.*, 1994). However, the unexpected larger changes in LDL oxidation response (±30%) following relatively minor dietary FA changes (egg OA, LA ±1-3 g, ±15-25% of daily intake), emphasises the close inter-relationship between egg and LDL compositions. This, in turn, may suggest unique functional potential for egg in regulating cholesterol metabolism, including potential for reducing LDL susceptibility to oxidation (Shapira and Pinchasov, 2008).

Conclusions

Beyond the egg's inherent nutritional contribution, studies reflect the flexibility of its composition, which is highly feed-dependent, and may suggest further potential for nutritional as well as functional enhancement by simple and low-cost methods of modifying feed. Close relationships between egg yolk lipids and lipoprotein composition, much beyond their nutritional significance and their physiological effects – *i.e.* on LDL oxidation – may suggest significant health potential for egg modification. These effects may ultimately impact qualitative measures and quantitative recommendations for egg composition. Health-oriented agriculture may be relevant in medical areas, including risks directly associated with egg consumption, *i.e.* increased plasma cholesterol and LDL oxidation, and for combating general risks of CVD, *i.e.* postprandial lipemia, endothelial dysfunction, and inflammation. It is also relevant for perinatal health of child and mother, including optimal brain development – where it can make a significant supplemental dietary contribution – and maternal replenishment, well-being, and functioning.

High n-3 PUFA eggs may become an increasingly important source of n-3 LCPUFA, given increasing scarcity and lack of sustainable harvesting of marine fish (Jenkins *et al.*, 2009), traditionally relied upon (Foran *et al.*, 2005), as well as contamination and associated health risks resulting in their being cautioned against during pregnancy and lactation (Oken *et al.*, 2003). Further, farmed fish increasingly contain high n-6 LCPUFA levels (Weaver *et al.*, 2008), which may unexpectedly exacerbate rather than ameliorate high n-6:n-3 PUFA dietary and tissue ratios. High-antioxidant eggs may become an essential source for effective delivery of such nutrients as lutein and carotenoids, essential for eye and skin health, and additional phytonutrients, otherwise limited in typical diets (Bourre and Galea, 2006).

Where an individual approach to the cholesterol concern seems to be most relevant – *i.e.* for cholesterol hyper-responders (Greene *et al.*, 2006), hypercholesterolemics, and diabetics (Djoussé and Gaziano, 2008; Qureshi *et al.*, 2007; Hu *et al.*, 1999), including gestational diabetics, or per reproductive stage – a differential approach to specific designer eggs may be relevant. While the anti-oxidative egg higher in n-9 MUFA and antioxidants may not be optimal for peak brain development (Shapira, 2009), it may have anti-LDL oxidation and heart benefits. Eggs with more n-3 LCPUFA and additional nutrients would be the preference for perinatal health. Moreover, results from two recent large prospective United States cohort studies (Physicians' Health Study I, 1982-2007, and Women's Health Study, 1992-2007) showed a correlation between daily or greater egg consumption and risk of developing type 2 diabetes mellitus (Djoussé *et al.*, 2009), which may suggest benefits of eggs with anti-oxidative (Shapira and Pinchasov, 2008) and high n-3 PUFA (Ohman *et al.*, 2008) compositions *vs.* measures associated with diabetes risk, further suggesting potential directions for egg design.

Research regarding benefits and practical possibilities of producing eggs designed specifically to support specific health areas, such as CVD and perinatal nutrition for brain development – produced simply and economically, and therefore having wide-ranging and popular applicability – is highly warranted. The scope of the relevance of tailored egg consumption may expand significantly in this capacity. Future optimisation of guidelines may combine qualities of egg composition with quantities for egg consumption recommendations, for the general population, specific subgroups, and individual requirements, and may affect the debate regarding the nutritional benefits of eggs in general.

References

AMERICAN EGG BOARD (AEB) (2008) US Population, egg production and consumption 2004. http://www.aeb.org/egg-industry/industry-facts/egg-production-and-consumption.

ANDERSON, R.A., JONES, C.J. and GOODFELLOW, J. (2001) Is the fatty meal a trigger for acute coronary syndromes. *Atherosclerosis* **159**: 9-15.

AVIRAM, M. and EIAS, K. (1993) Dietary olive oil reduces low-density lipoprotein uptake by macrophages and decreases the susceptibility of the lipoprotein to undergo lipid peroxidation. *Annals of Nutrition and Metabolism* **37**: 75-84.

BARONI, S.S., AMELIO, M., SANGIORGI, Z., GADDI, A. and BATTINO, M. (1999) Solid monounsaturated diet lowers LDL unsaturation trait and oxidisability in hypercholesterolemic (type IIb) patients. *Free Radical Research* **30**: 275-285.

BHOMBAL, S., BOTHWELL, M.R. and BAUER, S.M. (2006) Prevalence of elevated total IgE and food allergies in a consecutive series of ENT pediatric patients. *Otolaryngology, Head and Neck Surgery* **134**(4): 578-580.

BOROD, E., ATKINSON, R., BARCLAY, W.R. and CARLSON, S.E. (1999) Effects of third trimester consumption of eggs high in docosahexaenoic acid on docosahexaenoic acid status and pregnancy. *Lipids* **34** Suppl: S231.

BOURRE, J.M. and GALEA, F. (2006) An important source of omega-3 fatty acids, vitamins D and E, carotenoids, iodine and selenium: a new natural multi-enriched egg. *Journal of Nutrition in Health and Aging* **10**(5): 371-376.

BOURRE, J.M. (2005) New multi-enriched natural egg: exceptional nutrient content, especially in omega-3 fatty acids, vitamins, minerals and carotenoids. *Medical Nutrition* **41**: 116-134.

BOURRE, J.M. (2006a) Effects of nutrients (in food) on the structure and function of the nervous system: update on dietary requirements for brain. Part 1: micronutrients. *Journal of Nutrition in Health and Aging* **10**: 377-385.

BOURRE, J.M. (2006b) Effects of nutrients (in food) on the structure and function of the nervous system: update on dietary requirements for brain. Part 2: macronutrients. *Journal of Nutrition in Health and Aging*. **10**: 386-399.

BRUCKNER, G. (1997) Microcirculation, vitamin E and omega 3 fatty acids: an overview. *Advances in Experimental Medicine and Biology* **415**: 195-208.

BURDGE, G.C. and CALDER, P.C. (2005) Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. *Reproduction, Nutrition, Development* **45**: 581-597.

CENTERS FOR DISEASE CONTROL AND PREVENTION (CDC) (2006) *Health, United States, with chartbook on trends in the health of Americans* (Hyattsville, National Center for Health Statistics).

CHANCHARME, L., THEROND, P., NIGON, F., ZAREV, S., MALLET, A., BRUCKERT, E. and CHAPMAN, M.J. (2002) LDL particle subclasses in hypercholesterolemia. Molecular determinants of reduced lipid hydroperoxide stability. *Journal of Lipid Research* **43**: 453-462.

CRAWFORD, M.A. (2006) Docosahexaenoic acid in neural signaling systems. *Nutrition and Health* **18**(3): 263-276.

DE CATERINA, R., LIAO, J.K. and LIBBY, P. (2000) Fatty acid modulation of endothelial activation. *American Journal of Clinical Nutrition* **71**(suppl 1): 213S–223S.

DIMITRIADIS, E., GRIFFIN, M., COLLINS, P., JOHNSON, A., OWENS, D. and TOMKIN, G.H. (1996) Lipoprotein composition in NIDDM: effects of dietary oleic acid on the composition, oxidisability and function of low and high density lipoproteins. *Diabetologia* **39**: 667-76.

DJOUSSÉ, L. and GAZIANO, J.M. (2008) Egg consumption in relation to cardiovascular disease and mortality: the Physicians' Health Study. *American Journal of Clinical Nutrition* **87**(4): 964-969.

DJOUSSÉ, L., GAZIANO, J.M., BURING, J.E. and LEE, I.M. (2009) Egg consumption and risk of type 2 diabetes in men and women. *Diabetes Care* **32**(2): 295-300.

DUBNOV, G. and BERRY, M.E. (2003) Omega-6/omega-3 fatty acid ratio: the Israeli paradox. *World Reviews in Nutrition and Diet* **92**: 81-91.

ENDEVELT, R. and SHAHAR, D.R. (2004) Omega-3: the vanishing nutrient beyond cardiovascular prevention and treatment. *Israel Medical Association Journal* **6**: 2359.

ERVIN, R.B., WRIGHT, J.D., WANG, C. and KENNEDY-STEPHENSON, J. (2004) Advance data from vital and health statistics. Centers for Disease Control and Prevention (CDC), United States Department of Health and Human Services, National Center for Health Statistics **348**: 1-7.

FORAN, J.A., GOOD, D.H., CARPENTER, D.O., HAMILTON, M.C., KNUTH, B.A. and SCHWAGER, S.J. (2005) Quantitative analysis of the benefits and risks of consuming farmed and wild salmon. *Journal of Nutrition* **135**: 2639-2643.

GOODE, G.K., GARCIA, S. and HEAGERTY, A.M. (1997) Dietary supplementation with marine fish oil improves in vitro small artery endothelial function in hypercholesterolemic patients: a double-blind placebo controlled study. *Circulation* **96**: 2802-2807.

GOODFELLOW, J., BELLAMY, M.F., RAMSEY, M.W., JONES, C.J. and LEWIS, M.J. (2000) Dietary supplementation with marine omega-3 fatty acids improve systemic large artery endothelial function in subjects with hypercholesterolemia. *Journal of the American College of Cardiology* **36**: 265-270.

GREENE, C.M., WATERS, D., CLARK, R.M., CONTOIS, J.H. and FERNANDEZ, M.L. (2006) Plasma LDL and HDL characteristics and carotenoid content are positively influenced by egg consumption in an elderly population. *Nutrition and Metabolism (London)* **3**: 6.

GRIFFIN, M.D., SANDERS, T.A., DAVIES, I.G., MORGAN, L.M., MILLWARD, D.J., LEWIS, F., SLAUGHTER, S., COOPER, J.A., MILLER, G.J. and GRIFFIN, B.A. (2006) Effects of altering the ratio of dietary n-6 to n-3 fatty acids on insulin sensitivity, lipoprotein size, and postprandial lipemia in men and postmenopausal women aged 45-70 y: the OPTILIP Study. *American Journal of Clinical Nutrition* **84**(6): 1290-1298.

HARGROVE, R.L., ETHERTON, T.D., PEARSON, T.A., HARRISON, E.H. and KRIS-ETHERTON, P. M. (2001) Low fat and high monounsaturated fat diets decrease human low-density lipoprotein oxidative susceptibility in vitro. *Journal of Nutrition* **131**: 1758-1763.

HARRIS, W.S., MOZAFFARIAN, D., RIMM, E., KRIS-ETHERTON, P., RUDEL, L.L., APPEL, L.J., ENGLER, M,M., ENGLER, M.B. and SACKS, F. (2009) *Circulation* **119**(6): 902-907.

HENNIG, B., TOBOREK, M. and MCCLAIN, J. (2001) High-energy diets, fatty acids and endothelial cell function: implications for atherosclerosis. *Journal of the American College of Nutrition* **20**(2 Suppl): 97-105.

HU, F.B. and WILLETT, W.C. (2002) Optimal diets for prevention of coronary heart disease. *Journal of the American Medical Association* **288**: 2569-2578.

HU, F.B., STAMPFER, M.J., RIMM, E.B., MANSON, J.E., ASCHERIO, A., COLDITZ, G.A., ROSNER, B.A., SPIEGELMAN, D., SPEIZER, F.E., SACKS, F.M., HENNEKENS, C.H. and WILLETT, W.C. (1999) A prospective study of egg consumption and risk of cardiovascular disease in men and women. *Journal of the American Medical Association* **281**(15): 1387-1394.

INSTITUTES OF MEDICINE (IOM) (2002) Food and Nutrition Board. Dietary Reference Intakes. Washington D.C., National Academy Press.

ISRAEL CENTERS FOR DISEASE CONTROL (ICDC) (2003) MABAT. First Israeli National Health and Nutrition Survey 1999-2001. Part 1: General Findings, Publication No. 225.

ISRAEL CENTERS FOR DISEASE CONTROL (ICDC) (2004) MABAT. First Israeli National Health and Nutrition Survey 1999-2001. Part 2: What Israelis Eat, Publication No. 228.

JAMES, M.J., GIBSON, R.A. and CLELAND, L.G. (2000) Dietary polyunsaturated fatty acids and inflammatory mediator production. *American Journal of Clinical Nutrition* **71**(1 Suppl): 343S-348S.

JENKINS, D.J., SIEVENPIPER, J.L., PAULY, D., SUMAILA, U.R., KENDALL, C.W. and MOWAT, F. M. (2009) Are dietary recommendations for the use of fish oils sustainable? *Canadian Medical Association Journal* **180**(6): 633-637.

JIANG, Z. and SIM, J.S. (1993) Consumption of n-3 polyunsaturated fatty acid-enriched eggs and changes in plasma lipids of human subjects. *Nutrition* **9**: 513-518.

KAPLAN, M. and AVIRAM, M. (1999) Oxidized low density lipoprotein: atherogenic and proinflammatory characteristics during macrophage foam cell formation. An inhibitory role for nutritional antioxidants and serum paraoxonase. *Clinical Chemistry and Laboratory Medicine* **37**(8): 777-787.

KNOPP, R.H. and PARAMSOTHY, P. (2006) Oxidized LDL and abdominal obesity: a key to understanding the metabolic syndrome. *American Journal of Clinical Nutrition* **83**: 1-2.

KRATZ, M., CULLEN, P., KANNENBERG, F., KASSNER, A., FOBKER, M., ABUJA, P.M., ASSMANN, G.M. and WAHRBURG, U. (2002) Effects of dietary fatty acids on the composition and oxidizability of low-density lipoprotein. *European Journal of Clinical Nutrition* **56**: 72-81.

LEVY, Y., MAOR, I., PRESSER, D. and AVIRAMERICAN, M. (1996) Consumption of eggs with meals increases the susceptibility of human plasma and low-density lipoprotein to lipid peroxidation. *Annals of Nutrition Metabolism* **40**: 243-251.

LEVY, Y., SHAPIRA, N., MAOR, I., PRESER, D., MOSHE, R. and AVIRAM, M. (1997) Is it possible to reduce LDL oxidizability in normolipidemic subjects by antioxidant and fat modification of egg? *11^{th} International Symposium on Atherosclerosis*, Paris.

LEWIS, N.M., SEBURG, S. and FLANAGAN, N.L. (2000) Enriched eggs as a source of N-3 polyunsaturated fatty acids for humans. *Poultry Science* **79**: 971-974.

MAKRIDES, M. and GIBSON, R.A. (2000) Long-chain polyunsaturated fatty acid requirements during pregnancy and lactation. *American Journal of Clinical Nutrition* **71**(1 Suppl): 307S-311S.

MAKRIDES, M., HAWKES, J.S., NEUMANN, M.A. and GIBSON, R.A. (2002) Nutritional effect of including egg yolk in the weaning diet of breast- and formula-fed infants: a randomized controlled trial. *American Journal of Clinical Nutrition* **75**: 1084-1092.

MATA, P., VARELA, O., ALONSO, R., LAHOZ, C., DE OYA, M. and BADIMON, L. (1997) Monounsaturated and polyunsaturated n-6 fatty acid-enriched diets modify LDL oxidation and decrease human coronary smooth muscle cell DNA synthesis. *Arteriosclerosis, Thrombosis, and Vascular Biology* **17**: 2088-2095.

MORI, T.A., WATTS, G.F., BURKE, V., HILME, E., PUDDEY, I.B. and BEILIN, J. (2000) Differential effects of eicosapentaenoic acid and docosahexaenoic acid on vascular reactivity of the forearm microcirculation in hyperlipidemic, overweight men. *Circulation* **102**: 1264-1269.

NETTLETON, J.A. ON BEHALF OF THE ALASKA SEAFOOD MARKETING INSTITUTE (ASMI) (2004) Increasing the consumption of long-chain ome-3 polyunsaturated fatty acids by Americans. Testimony to the Joint USDA/HHS 2005 Dietary Guidelines Advisory Committee.

NITSAN, Z., MOKADY, S. and SUKENIK, A. (1999) Enrichment of poultry products with omega3 fatty acids by dietary supplementation with the alga *Nannochloropsis* and mantur oil. *Journal of Agriculture and Food Chemistry* **47**: 5127-5132.

OHMAN, M., AKERFELDT, T,. NILSSON, I., ROSEN, C., HANSSON, L.O., CARLSSON, M. and LARSSON, A. (2008) *Upsalla Journal of Medical Science* **113**(3): 315-323.

OKEN, E., KLEINMAN, K.P., BERLAND, W.E., SIMON, S.R., RICH-EDWARDS J.W. and GILLMAN, M.W. (2003) Decline in fish consumption among pregnant women after a national mercury advisory. *Obstetrics and Gynecology* **102**(2): 346-351.

OKUDA, Y, KAWASHIMA, K., SAWADA, T., TSURUMARU, K., ASANO, M., SUZUKI, S., SOMA, M., NAKAJIMA, T. and YAMASHITA, K. (1997) Eicosapentanoic acid enhances nitric oxide production by cultured human endothelial cells. *Biochemical and Biophysical Research Communications* **232**: 487-491.

PALMER, D.J. and MAKRIDES, M. (2006) Diet of lactating women and allergic reactions in their infants. *Current Opinion in Clinical Nutrition and Metabolic Care* **9**(3): 284-288.

PRENTICE, A., JARJOU, L.M., DRURY, P.J., DEWIT, O. and CRAWFORD, M.A. (1989) Breastmilk fatty acids of rural Gambian mothers: effects of diet and maternal parity. *Journal of Pediatric Gastroenterology and Nutrition* **8**(4):486-490.

QURESHI, A.I., SURI, F.K., AHMED, S., NASA, A. DIVANI, A.A. and KIRMANI, J.F. (2007) Regular egg consumption does not increase the risk of stroke and cardiovascular disease. *Medical Science Monitor* 2007 **13**: CR1-CR8.

REAVEN, P.D., GRASSE, B.J. and TRIBBLE, D.L. (1994) Effects of linoleate-enriched and oleate-enriched diets in combination with alpha-tocopherol on the susceptibility of LDL and LDL subfractions to oxidative modification in humans. *Arteriosclerosis, Thrombosis, and Vascular Biology* **14**: 557-566.

REAVEN, P.D. and WITZTUM, J.L. (1996) Oxidized low-density lipoproteins in atherogenesis: role of dietary modification. *Annual Reviews in Nutrition* **16**: 51-71.

RIVELLESE, A.A., MAFFETTONE, A., VESSBY, B., UUSITUPA, M., HERMANSEN, K., BERGLUND, L., LOUHERANTA, A., MEYER, B.J. and RICCARDI, G.E. (2003) Effects of dietary saturated, monounsaturated and n-3 fatty acids on fasting lipoproteins, LDL size and post-prandial lipid metabolism in healthy subjects. *Atherosclerosis* **167**(1): 149-158

SAUSENTHALER, S., KOLETZKO, S., SCHAAF, B., LEHMANN, I., BORTE, M., HERBARTH, O., VON BERG, A., WICHMANN, H.E. and HEINRICH, J. for the LISA STUDY GROUP (2007) Maternal diet during pregnancy in relation to eczema and allergic sensitization in the offspring at 2 y of age. *American Journal of Clinical Nutrition* **85**(2): 530-537.

SCHWAB, U.S., SARKKINEN, E.S., LICHTENSTEIN, A.H., LI, Z., ORDOVAS, J.M., SCHAEFER, E. J. and UUSITUPA, M.I. (1998) The effect of quality and amount of dietary fat on the susceptibility of low-density lipoprotein to oxidation in subjects with impaired glucose tolerance. *European Journal of Clinical Nutrition* **52**: 452-458.

SEO, T., BLANER, W.S. and DECKELBAUM, R.J. (2005) Omega-3 fatty acids: molecular approaches to optimal biological outcomes. *Current Opinion in Lipidology* **16**: 11-18.

SHAPIRA, N. (2009) Modified egg as a nutritional supplement during peak brain development: a new target for fortification. *Nutrition and Health* **20**(2): 107-118.

SHAPIRA, N. (2008) Egg composition *vs.* CVD risk: from wild-traditional to designer eggs *vs.* lipid, endothelial, and inflammatory hypotheses. II.5. In: *Wild type food in health promotion and disease prevention: the Columbus concept* (Totowa, Humana Press).

SHAPIRA, N. and PINCHASOV, J. (2008) Modified egg composition to reduce low-density lipoprotein oxidizability: high monounsaturated fatty acids and antioxidants versus regular high n-6 polyunsaturated fatty acids. *Journal of Agriculture and Food Chemistry* **56**(10): 3688-3693.

SHAPIRA, N., WEILL, P. and LOEWENBACH, R. (2008) Egg fortification with n-3 polyunsaturated fatty acids (PUFA): nutritional benefits versus high n-6 PUFA western diets, and consumer acceptance. *Israel Medical Association Journal* **10**(4): 262-265.

SHAPIRA, N. (2004) Mediterranean diet in the food chain: eggs *vs.* LDL oxidation, in: LAMBROU-PHILIPSON, C. & KONSTANTINIDIS, K. (Eds) *Conference Publication: Mediet 2004, Traditional Mediterranean Diet: Past, Present and Future* (Athens, Heliotopos).

SIMOPOULOS, A.P. (1999) New products from the agri-food industry: the return of n-3 fatty acids into the food supply. *Lipids* **34** Suppl: S297-S301.

SIMOPOULOS, A.P. (2008) The omega-6/omega-3 fatty acid ratio, genetic variation, and cardiovascular disease. *Asia Pacific Journal of Clinical Nutrition* **17** Suppl 1: 131-134.

SINGH, M. (2003) Nutrition, brain and environment: how to have smarter babies? *Indian Pediatrics* **40**(3): 213-220.

SOSIN, E., BOROWIEC, F., STRZETELSKI, J. and SMULIKOWSKA, S. (2006) The effect of feeding regular or low a-linolenic acid linseed on the fatty acid composition of egg yolks. *Journal of Animal Feed Science* **15**: 641-650.

STEINBERG, D., PARTHASARATHY, S., CREW, T.E., KHOO, J.C. and WITZTUM, J.L. (1989) Beyond cholesterol: modifications of low-density lipoprotein that increase its atherogenecity. *New England Journal of Medicine* **320**: 915-24.

STOCKER, R. and KEANEY, J.F. JR. (2004) Role of oxidative modifications in atherosclerosis. *Physiology Reviews* **84**: 1381-1478.

SURAI, P.F., MACPHERSON, A., SPEAKE, B.K. and SPARKS, N. (2000) Designer egg evaluation in a controlled trial. *European Journal of Clinical Nutrition* **54**: 298-305.

VANCE, G.H.S., LEWIS, S.A., GRIMSHAW, K.E.C., WOOD, P.J., BRIGGS, R.A., THORNTON, C.A. and WARNER, J.O. (2005) Exposure of the fetus and infant to hens' egg ovalbumin via the placenta and breast milk in relation to maternal intake of dietary egg. *Clinical and Experimental Allergy* **35**(10): 1318-1326.

WEAVER, K.L., IVESTER, P., CHILTON, J.A., WILSON, M.D., PANDEY, P. and CHILTON, F.H. (2008) The content of favorable and unfavorable polyunsaturated fatty acids found in commonly eaten fish. *Journal of the American Diet Association* **108**(7): 1178-1185.

WEILL, P., SCHMITT, B., CHESNEAU, G., DANIEL, N., SAFRAOU, F. and LEGRAND, P. (2002) Effects of introducing linseed in livestock diet on blood fatty acid composition of consumers of animal products. *Annals of Nutrition and Metabolism* **46**: 182-191

WEISMAN, D., MOTRO, M., SCHWAMMENTHAL, E., FISMAN, E.Z. and TENENBAUM, A., TANNE, D. and ADLER, Y. (2004) Efficacy of omega-3 fatty acid supplementation in primary and secondary prevention of coronary heart disease. *Israel Medical Association Journal* **6**: 227-232.

YAM, D., ELIRAZ, A. and BERRY, E.M. (1996) Diet and disease – the Israeli paradox: possible dangers of a high omega 6 polyunsaturated fatty acid diet. *Israel Journal of Medical Science* **32**: 1134-1143.

YANNAKOPOULOS, A., TSERVENI-GOUSI, A. and CHRISTAKI, E. (2005) Enhanced egg production in practice: the case of bio-omega-3 egg. *International Journal of Poultry Science* **4**: 531-535.

Table 1 PUFA profile of control and n-3 PUFA-fortified eggs (5% ELS), 5-week average.

	Total PUFA	**LA** 18:2 n-6	**ARA** 20:4 n-6	**Total** n-6 PUFA	**ALA** 18:3 n-3	**DHA** 22:6 n-3	**Total** n-3 PUFA	**Total** n-6:n-3 ratio	**LA:ALA** ratio	**ARA:DHA** ratio
Egg	% FA									
Control	21.1±0.6	16.5±0.4	1.9±0.1	19.6±0.5	0.4±0.0	0.7±0.1	1.2±0.1	16.3±0.4	41.3±1.1	2.7±0.2
Fortified	25.2±0.7	18.3±0.5[b]	1.5±0.1[b]	20.5±0.5[c]	2.6±0.2[a]	1.7±0.2[a]	4.5±0.3[a]	4.5±0.3[a]	7.2±0.6[a]	0.9±0.1

[a] $P < 0.0005$; [b] $P < 0.005$; [c] $P < 0.05$ *vs.* control

Table 2 Egg (regular-fortified) range of contents of selected nutrients required during peak brain development and their relative contribution (%DRI).

	Nutritional Component 1 egg (≈65 gm)														
	PUFA							Vitamins						Minerals	
	Total PUFA mg [a]	LA) 18:2 n-6 (mg	AA) 20:4 n-6 (mg	Total n-6 mg	ALA) 18:3 n-3 (mg	EPA) 20:5 n-3 (mg [c]	DHA) 22:6 n-3 (mg [c]	Total n-3 mg	Folate mcg	Choline mg	A (retinol) mcg	D (-calciferol) mcg	E (tocopherol) mg	Iodine mcg	Selenium mcg
Egg Content (range of values[b])	910.0-**1950.0**	746.2-**1142.1**	52.7-**92.3**	838.5-975.0	21.5-**715.0**	2.6-**74.8**	24.1-**168.1** [d]	48.1-**975.0**	14.3-**156.0**	163.2-178.8	91.0-117.0	0.7-1.0	0.7-**9.8**	28.6-97.5	18.2-23.6
%DRI *(recommended intake)*															
Pregnancy *(19-50 y)*	— —	5.7-8.8% *(13,000)*	— —	6.5-7.5 *(13,000)*	1.5-**51.7**% (1400)	1.9-**53.4**% *(140)*	17.2-**120.1**% *(140)*	3.4-**69.6%** *(1400)*	2.4-**26.0**% *(600)*	36.3-39.7% *(450)*	11.8-15.2% *(770)*	14.0-20.0% *(5)*	4.7-**65.3**% *(15)*	13.0-**44.3**% *(220)*	30.3-39.3% *(60)*
Lactation *(19-50 y)*	— —	5.7-8.8% *(13,000)*	— —	6.5-7.5 *(13,000)*	1.7-**55.0**% *(1300)*	2.0-**57.5**% *(130)*	18.5-**129.3**% *(130)*	3.7-**75.0%** *(1300)*	2.9-**31.2**% *(500)*	29.7-32.5% *(550)*	7.0-9.0% *(1300)*	14.0-20.0% *(5)*	3.7-**51.6**% *(19)*	9.9-**33.6**% *(290)*	26.0-33.7% *(70)*
Infancy *(1-3 y)*	— —	10.7-16.3% *(7000)*	— —	12.0-13.9 *(7000)*	3.1-**102.1**% *(700)*	3.7-**106.9**% *(70)*	34.4-**240.1**% *(70)*	6.9-**139.3%** *(700)*	9.5-**104.0**% *(150)*	81.6-89.4% *(200)*	30.3-39.0% *(300)*	14.0-20.0% *(5)*	11.7-**163.3**% *(6)*	31.8-**108.3**% *(90)*	91.0-118.0% *(20)*

Numbers in bold: ≥50% higher content or lower ratio in fortified *vs.* regular egg
Percentages underlined: ≥50% of DRI
[a]Range for egg n-6:n-3 PUFA ratios = 1.0-17.4:1; range for egg n-6:n-3 LCPUFA ratios = 0.3-3.5:1
[b]Range of representative fortified eggs from Greece, France, Belgium, Israel, and the United States
[c]EPA/DHA requirement calculated according to combined DRI of 10% ALA intake
[d]Mostly 100-120 mg/egg DHA in eggs fortified with a land-based n-3 PUFA source (*i.e.* linseed)