

## RESEARCH ARTICLE

# Bioavailability of lutein from a lutein-enriched egg-yolk beverage and its dried re-suspended versions

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### Abstract

Drying a fresh lutein-enriched egg-yolk beverage would extend its shelf life, however, functional properties should not be affected. It was investigated whether consumption of a dried beverage containing lutein-enriched egg-yolk significantly increases serum lutein. One-hundred healthy young subjects participated in this 6-weeks randomized controlled study. Subjects consumed either a “plain” control beverage ( $n = 26$ ), a fresh lutein-enriched egg-yolk beverage ( $n = 25$ ), a dried version of this beverage ( $n = 25$ ), or a beverage composed of the dried individual components of the drink ( $n = 24$ ). The fresh and both dried versions of the lutein-enriched egg-yolk beverage were able to increase serum lutein levels after 6 weeks of consumption (lutein change:  $-38 \pm 47$  nmol/L,  $+304 \pm 113$  nmol/L,  $+148 \pm 79$  nmol/L and  $+178 \pm 83$  nmol/L for control, fresh, dried and combined dried group respectively;  $p < 0.001$ ). No significant change in serum cholesterol level was seen in the beverages containing lutein-enriched egg-yolk compared to the control drink.

### Keywords

Age-related macular degeneration, cholesterol, egg-yolk, serum lutein

### History

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### Introduction

Age-Related Macular Degeneration (AMD) is a degenerative eye disease and the leading cause of irreversible visual loss in the elderly (>60 years) in the Western world, with an estimated current prevalence of AMD from around 1.47% in those over 40 years old (Friedman et al., 2004) to as high as 3.5% in a population aged 49 and older (Wang et al., 2007). Several nutrients are related to AMD, like vitamins (vitamin A, E, C, D, B), minerals (e.g. zinc), dietary fatty acids (both omega-3 PUFA and omega-6 PUFA) and carotenoids (lutein and zeaxanthin,  $\beta$ -carotene; Zampatti et al., 2014).

Macular pigment is composed primarily of the xanthophyll carotenoids lutein and zeaxanthin. It was reported that the risk of AMD is inversely proportional to the lutein concentrations in the diet, serum and the macula lutea (Chung et al., 2004). Epidemiological evidence indicates that a higher intake of lutein and zeaxanthin is associated with a lower risk for developing AMD (Moeller et al., 2006). It is hypothesized that lutein and zeaxanthin can help in the prevention of AMD by absorbing blue light and protecting the retina from oxidative stress by neutralizing free radicals (Bernstein et al., 2001; Landrum & Bone, 2001; Landrum et al., 1997; O’connell et al., 2006). Eggs are an important source of highly bioavailable lutein. Earlier studies showed that the lipid matrix of the egg yolk is a good vehicle for the efficient absorption of dietary lutein and that it is

possible to increase serum/plasma and macular levels of lutein in humans when consuming egg-yolk (Burns-Whitmore et al., 2010; Chung et al., 2004; Clark et al., 2006; Goodrow et al., 2006; Handelman et al., 1999; Vishwanathan et al., 2009, 2010; Wenzel et al., 2006). Moreover, the bioavailability of lutein from egg-yolk is much higher than of lutein from either supplements or vegetables (Thurnham, 2007). Interestingly, it is possible to increase the lutein concentration of eggs, so called lutein-enriched eggs or macular eggs (Thielen et al., 2008) which creates opportunities to further increase lutein intake when needed, as has been suggested for subjects suffering from AMD. In a small scale pilot study with healthy subjects, the consumption of lutein-enriched eggs or a beverage containing lutein-enriched egg-yolk indeed increased plasma and macular levels of lutein when compared with consumption of non-enriched eggs (Baumgartner et al., 2013; T. Berendschot and J. Plat, unpublished observation, ClinicalTrials.gov Identifier: NCT00527553).

However, this fresh liquid lutein-enriched egg yolk beverage has a limited shelf life of two to three weeks, which can be extended by drying the fresh beverage. It was recently shown that pasteurization, spray and freeze-drying egg-yolks can affect stability of the egg yolk and may result in a changed xanthophyll concentration per gram egg-yolk (Wenzel et al., 2010). A question that remained was, whether lutein bioavailability, i.e. increase in serum lutein concentration, is affected by the different drying procedures.

Therefore, the primary objective of this study was to investigate whether consumption of a beverage containing dried lutein-enriched egg-yolk significantly increases serum lutein

concentration in healthy volunteers. A secondary objective was to evaluate whether the increase in serum lutein concentrations from the dried re-suspended versions were comparable to the fresh lutein-enriched egg-yolk containing beverage.

## Methods

A 6-weeks randomized controlled study with a parallel design was performed, in which subjects consumed either: a “plain” control beverage, a fresh lutein-enriched egg-yolk beverage, a dried version of this beverage, or a beverage composed of the dried individual components of the drink. Measurements were performed before and after the intervention period. The study protocol was approved by the Medical Ethical Committee of Wageningen University, performed according to the principles of Good Clinical Practice (GCP), monitored by a third party and registered at ClinicalTrials.gov on July 20, 2011 as NCT01400763.

## Study population

The study population consisted of healthy male and female subjects, age between 18–35 years, BMI between 18 and 25 kg/m<sup>2</sup>. Subjects were recruited via information leaflets at the university grounds and using an e-mail database of the Department of Human Nutrition. Subjects had to be weight stable, did not use any medication (except oral contraceptives), did not smoke or suffer from any chronic gastro-intestinal disease, and were not pregnant or breastfeeding. Furthermore, they were not on a vegetarian diet and not allergic to cow milk, dairy products, eggs, or egg-rich products. Subjects were recruited via advertisements in local newspapers, and information leaflets at the university grounds. A screening visit six weeks before the start of the study was performed to check eligibility of the subjects.

A sample size calculation indicated that 18 evaluable subjects per group would be sufficient to detect a relevant change in serum lutein concentration in this group ( $\alpha = 0.05$ ,  $\beta = 0.20$  and  $\sigma$  and  $\delta$  being 0.130  $\mu\text{mol/L}$  and 0.110  $\mu\text{mol/L}$ ). In order to compensate for potential drop-outs and losing subjects to follow-up, a total of 102 subjects were included in the study, of which 100 completed the study protocol.

## Study design

Volunteers were asked to avoid lutein-rich vegetables and fruits such as spinach, broccoli, peas and some fruits, and were not allowed to use vitamin supplements during the entire study period of 8 weeks. Next to this, egg consumption was restricted to 1 egg per week.

All participants started with a two week run-in period, to get familiar with the low-lutein diet. After this run-in period, subjects were randomly assigned to either one of the four groups, stratified for gender. Blood samples were collected in the non-fasted state at three different time points during the study: before the run-in period ( $T = -2$  weeks), after the run-in period ( $T = 0$ ), and after the intervention period ( $T = 6$  weeks). Blood samples were taken in duplicate, with a lag time of two days (Figure 1), and the

same time of the day for each individual participant (between 1 and 4 pm).

The study was single-blinded, as it was clear from the product formulation whether the study product was a liquid or had to be re-suspended in the case of the powder formulation. However, both liquid and both powder versions were supplied in comparable packaging. Research assistants and laboratory technicians performing product delivery, blood collection and measurements and performing the data analyses, were not aware of the study status of the volunteer.

During the intervention period of six weeks subjects consumed one bottle of 80 ml beverage each day or an equivalent of the dried version re-suspended in 75 ml of tap water. Subjects were asked to consume the beverage daily at 9 o'clock in the morning. Blood samples were taken around four to seven hours post ingestion, thus between 1 and 4 pm. Bottles and sachets were picked up every other week. Subjects were asked to return empty packages to assess compliance.

## Beverages

Four different treatments were evaluated: a lutein-free control beverage (control), a fresh lutein-enriched egg-yolk beverage (Fresh), a dried version of this beverage (Dried) or a beverage composed of the dried individual components of the drink (Com Dried).

The fresh beverage consisted of buttermilk with 1.5 lutein-enriched egg-yolk, and some additional sweeteners, flavoring ingredients and preservatives. The control drink was made of the same ingredients but without lutein-enriched egg-yolk. Table 1 shows detailed information about the composition of the drinks. For both drinks, the ingredients were mixed and then pasteurised. Fresh drinks were prepared every two weeks.

For the dried treatment, blended egg-yolk, buttermilk and sweeteners were pasteurized and dried with an industrial scale spray dryer. Powder was blended in final formulation, adding flavoring ingredients and free-flowing agent, just before packaging.

For the Com Dried treatment sugared lutein-enrich egg-yolk was pasteurized and dried with an industrial scale spray dryer. Powder was blended in final formulation, adding buttermilk powder and flavoring ingredients, just before packaging.

Macronutrient composition as an energy content was calculated using the Dutch food composition table (NEVO), and was slightly different between conditions (Table 1). All three egg-yolk drinks contained more energy,  $\sim 3$  times higher, due to the higher fat and protein content. However, the three egg-yolk drinks were comparable in energy and macronutrient composition. For determination of lutein and zeaxanthin levels, the samples were saponified and extracted with an organic solvent. After clean-up and concentration of the solvent fraction, lutein and zeaxanthin were analyzed with liquid chromatography and UV detection. Lutein content was different after the various drying and mixing procedures, differed between batches (Fresh) and slightly decreased during storage (both dried conditions). Therefore, lutein content was measured in every batch for the fresh drink, and at several time points throughout the study period for both

Figure 1. Study design.

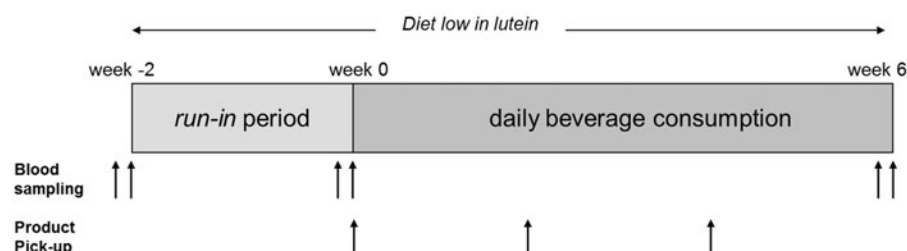


Table 1. Composition and nutritional value of the drinks per serving. \*Serving size was 80 gram for the fresh beverages (control & fresh), serving size was 23.9 gram of powder for the Dried condition (+ 75 gram tap water, 99 gram total) and 22.6 gram of powder for Com Dried (+ 75 gram tap water, 98 gram total). Nutritional value was calculated using a food composition table, #lutein and zeaxanthin were analysed with liquid chromatography and UV detection, and presented as average for the different batches (Fresh) or timepoints (Dried, Com Dried).

	Control	Fresh	Dried	Com Dried
Composition per serving*	Buttermilk, sugar, aromas, xanthan, sorbic acid, sodium benzoate, food dyes.	Buttermilk, enriched egg yolk, sugar, aroma, lactic acid, sorbic acid, sodium benzoate.	Buttermilk, enriched egg yolk, sugar, aroma, free flowing agent.	Buttermilk, enriched egg yolk, sugar, aroma, free flowing agent.
Lutein# (mg)	0	1.5 ± 0.1	0.9 ± 0.1	1.3 ± 0.1
Zeaxanthin# (mg)	0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
Energy (kcal)	41	131	140	127
Total fat (g)	0.4	9.2	9.6	8.9
– Saturated %	67.0	32.5	32.5	32.5
– Transfat %	3.1	0.3	0.3	0.3
– MUFA %	26.2	49.5	49.5	49.5
– PUFA %	3.7	17.7	17.7	17.7
Protein (g)	2.7	6.2	7.2	7.3
Carbohydrates (g)	6.6	5.8	6.1	4.4
Cholesterol (mg)	2.7	323	339	313

powders. Average lutein content of the different conditions was  $1.50 \pm 0.13$ ,  $0.93 \pm 0.06$  and  $1.26 \pm 0.08$  mg lutein per serving (per day) for the fresh beverage, the dried version and the combined dried version, respectively. The control drink did not contain any lutein.

### Blood sampling and analysis

Blood was collected by venipuncture in the non-fasted state for practical reasons. Serum carotenoids and cholesterol levels are known to show a diurnal pattern, however, for each individual, blood collection was performed on the same time of the day at all occasions. Furthermore subjects were asked to consume the beverage daily at 9 o'clock in the morning. Samples were centrifuged and serum was stored at  $-80^{\circ}\text{C}$  until further analysis. Blood samples were collected between May and July of the same year, depending on the group. Immediately (within one week) after the study blood sample analyses were started and finished up to three weeks post start. The minimal blood storage time was about 1 week to maximally 10 weeks after the blood sampling. Serum carotenoids were analyzed by HPLC (Thermo Scientific Accela LC system, Thermo Fisher Scientific Inc., Waltham, MA) with EZChrom Elite version 3.2.2 SP2 software from Agilent (Agilent Technologies, Santa Clara, CA). To 500  $\mu\text{L}$  serum, 500  $\mu\text{L}$  sodium chloride (0.9 w/v% in water) and 1000  $\mu\text{L}$  ethanol (containing retinyl acetate as an internal standard) were added and mixed. Next, the mixture was extracted twice with 3.0 mL hexane. The hexane layers were pooled and evaporated to dryness under nitrogen at  $35^{\circ}\text{C}$ . The residue was dissolved in a 250  $\mu\text{L}$  mixture of methanol and butanol (60/40 v/v %), and 15  $\mu\text{L}$  was injected for HPLC analysis. Separations of lutein, zeaxanthin,  $\beta$ -cryptoxanthin,  $\alpha$ -carotene,  $\beta$ -carotene and lycopene were performed on a Vydac 201TP52 RP-column (Grace Alltech, Breda, The Netherlands) using gradient elution and monitored at 450 nm on a PDA detector. Runtime was 25 minutes per sample. All sample preparations were done under subdued yellow light. Within- and between-run CVs for lutein were 4.1% and 6.6% respectively.

Serum total and HDL cholesterol levels were measured using an enzymatic method (SHO Lab, Velp, The Netherlands); LDL cholesterol was calculated using Friedewald formula (Friedewald et al., 1972). The average of both duplicate blood samples at each time-point (week -2, 0 and 6) was calculated. The effect of the different treatments was evaluated by comparing the data at week 0 and 6.

### Data analysis

SPSS 19.0 was used to perform data analysis. All parameters are expressed as mean  $\pm$  standard deviation. Log transformation was used to obtain normal distribution.

For serum lutein levels, an outlier analysis was performed using a TREND estimation per treatment with linear regression analysis. A standardized residual  $>2.5$  or a value  $<-2.5$  was considered an "outlying value". In case of an outlying value, the individual was excluded from further analysis.

As lutein intake differed between experimental conditions, between batches and slightly over time, we calculated the cumulative lutein intake over the 6 week study period for each individual using the measured lutein content of the provided drink or powder. This enabled us to express the change in serum lutein after 6 weeks intervention relative to cumulative intake ( $\Delta$  Lutein Ratio, expressed in nmol/L/mg intake).

One-way ANOVA was used to evaluate differences in serum lutein and cholesterol between treatments, with the change in serum lutein or cholesterol over the 6-week intervention period as dependent variable and treatment as independent variable. In case of a significant treatment effect, post hoc analysis was performed to test differences between treatments, using a Bonferroni correction for multiple testing. A  $p$  value  $<0.05$  was considered significant.

### Results

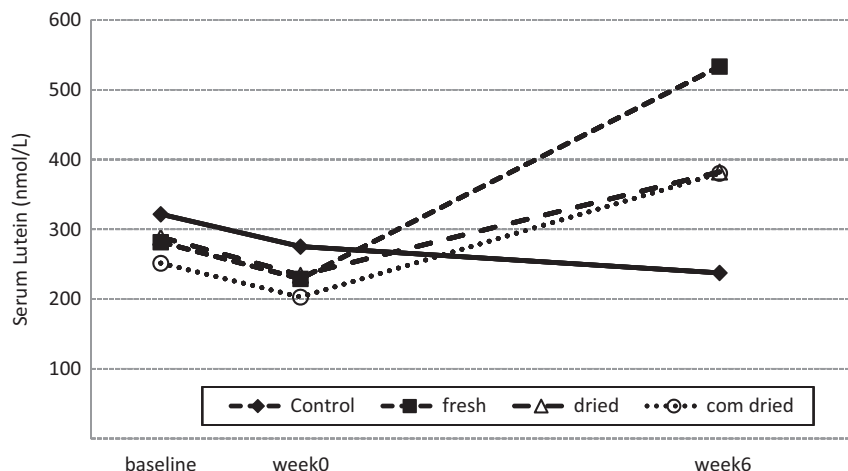
In total 102 subjects were included in the study; 2 subjects dropped out during the first week of intervention due to personal reasons. Outlier analysis revealed that 6 subjects needed to be excluded from further analysis, of which 2 from the control group, 2 from the Fresh and 2 from the Dried group. Baseline characteristics of the final study population ( $n = 94$ ) can be found in Table 2. Baseline serum lutein levels were  $322 \pm 112$  nmol/L,  $282 \pm 75$  nmol/L,  $289 \pm 79$  nmol/L and  $251 \pm 72$  nmol/L for control, fresh, dried and combined dried respectively, with the values being statistical significant different between control and combined dried. No other statistical significant differences between the groups were observed.

Compliance to the dietary guidelines, i.e. following a low-lutein diet, was evaluated by assessing the decline in serum lutein concentration during the 2-week run-in period. Eight-eight subjects indeed showed a decline in serum lutein; average decline was comparable between groups ( $-46.5$ ,  $-52.5$ ,  $-54.6$ , and  $-48.5$  nmol/L for control, fresh, dried and combined

Table 2. Baseline subject characteristics (mean  $\pm$  SD). BW, body weight; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; TG, triglycerides; S-lutein, serum lutein. S-zeaxanthin, serum zeaxanthin. \*ANOVA, post hoc analysis  $p < 0.05$  versus control.

	Control	Fresh	Dried	Com Dried
N (M/F)	24 (4/20)	23 (5/18)	23 (4/19)	24 (8/16)
Age (yr)	21.6 $\pm$ 3.1	21.2 $\pm$ 3.4	21.8 $\pm$ 3.2	21.7 $\pm$ 3.6
BW (kg)	64.4 $\pm$ 7.6	65.8 $\pm$ 7.8	64.7 $\pm$ 9.4	68.3 $\pm$ 8.4
BMI (kg/m <sup>2</sup> )	21.9 $\pm$ 1.8	22.1 $\pm$ 1.6	21.8 $\pm$ 2.0	22.5 $\pm$ 1.8
TC (mmol/L)	4.70 $\pm$ 0.87	4.85 $\pm$ 0.77	4.62 $\pm$ 0.60	4.61 $\pm$ 0.65
LDL-C (mmol/L)	2.53 $\pm$ 0.75	2.66 $\pm$ 0.73	2.49 $\pm$ 0.61	2.53 $\pm$ 0.62
HDL-C (mmol/L)	1.54 $\pm$ 0.29	1.52 $\pm$ 0.26	1.51 $\pm$ 0.37	1.45 $\pm$ 0.31
TG (mmol/L)	1.24 $\pm$ 0.44	1.33 $\pm$ 0.48	1.28 $\pm$ 0.51	1.22 $\pm$ 0.56
S-lutein (nmol/L)	322 $\pm$ 112	282 $\pm$ 75	289 $\pm$ 79	251 $\pm$ 72*
S-zeaxanthin (nmol/L)	91 $\pm$ 37	87 $\pm$ 35	77 $\pm$ 30	74 $\pm$ 28

Figure 2. Serum lutein levels at baseline, and at the start and after 6 weeks of intervention for the lutein-free lip control beverage (control), the fresh lutein-enriched egg-yolk beverage (Fresh), the dried version of this beverage (Dried) or the beverage composed of the dried individual components of the drink (Com Dried).



dried respectively). No differences in serum lutein concentration was observed at week 0, the start of the intervention.

### Serum lutein

Serum lutein levels continued to decline in the control group during the 6-week intervention period (change:  $-38 \pm 47$  nmol/L), while consumption of all lutein-enriched egg-yolk containing beverages resulted in an increase in serum lutein levels (Figure 2). On average serum lutein increase was  $304 \pm 113$  nmol/L in the fresh group,  $148 \pm 79$  nmol/L in the dried group and  $178 \pm 83$  nmol/L in the combined dried group. The change in serum lutein over the 6-week intervention period was significantly different compared to control for all experimental conditions (Figure 3; post hoc analysis:  $p < 0.001$ ). The fresh beverage resulted in a larger increase in serum lutein levels after 6 weeks compared to both dried versions (Figure 3; post hoc analysis:  $p < 0.001$ ).

As lutein content was not the same in all beverages, serum lutein change was also expressed as ratio of cumulative lutein intake over the 6 week period (Figure 4). Although the increase was still larger in the fresh beverage, only the difference between Fresh and Com Dried remained significant (post hoc analysis:  $p = 0.03$ ).

Changes in serum zeaxanthin levels followed a same pattern as observed for lutein. After 6 weeks of intervention zeaxanthin levels were  $74 \pm 35$ ,  $135 \pm 34$ ,  $114 \pm 40$  and  $116 \pm 38$  nmol/L for Control, Fresh, Dried and Com Dried respectively, with all three egg-yolk conditions being significantly different from the control treatment ( $p < 0.05$ ). The change in serum zeaxanthin over the 6-week intervention period was significantly different compared to control for all experimental conditions ( $-4.8 \pm 17$ ,  $+62 \pm 29$ ,

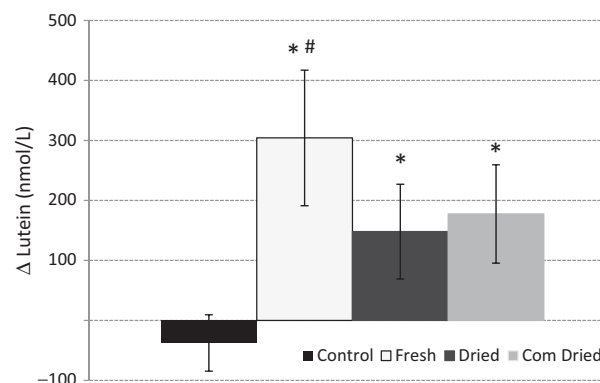


Figure 3. Change in serum lutein after 6 weeks intervention for the lutein-free control beverage (control), the fresh lutein-enriched egg-yolk beverage (Fresh), the dried version of this beverage (Dried) or the beverage composed of the dried individual components of the drink (Com Dried). \* =  $p < 0.001$  versus control, # =  $p < 0.001$  versus dried and combined dried.

$+38 \pm 30$  and  $+45 \pm 27$  nmol/L for Control, Fresh, Dried and Com Dried respectively;  $p < 0.05$ ). The fresh beverage resulted in a larger increase in serum zeaxanthin levels after 6 weeks compared to the Dried version, not the Com Dried ( $p < 0.05$ ).

### Serum cholesterol

No differences in baseline cholesterol levels were observed between groups (Table 2). Change in serum cholesterol levels are depicted in Figure 5. Changes in total and LDL serum cholesterol levels after intervention were not significantly different between



treatments (ANOVA:  $p=0.052$  and  $p=0.33$  for total and LDL cholesterol respectively), while changes in HDL cholesterol were different between treatments (ANOVA:  $p=0.048$ ). *Post hoc* analysis revealed that the change in HDL tended to be different between control and fresh (*post hoc* analysis:  $p=0.09$ ) and between control and combined dried (*post hoc* analysis:  $p=0.10$ ).

Discussion

The objective of our study was to investigate whether consumption of a lutein-enriched egg-yolk containing beverage and its dried and re-suspended versions increased serum lutein concentration in healthy volunteers. Drying will extend shelf live, however, functional properties should not be affected. All lutein-enriched egg-yolk containing beverages – both dried versions and the fresh product – were able to significantly increase serum lutein and zeaxanthin levels after 6 weeks intervention compared to the control drink. The increase in serum lutein levels was not different between the fresh lutein-enriched egg-yolk beverage and the dried version of this fresh beverage, while the beverage composed of the dried individual components resulted in a

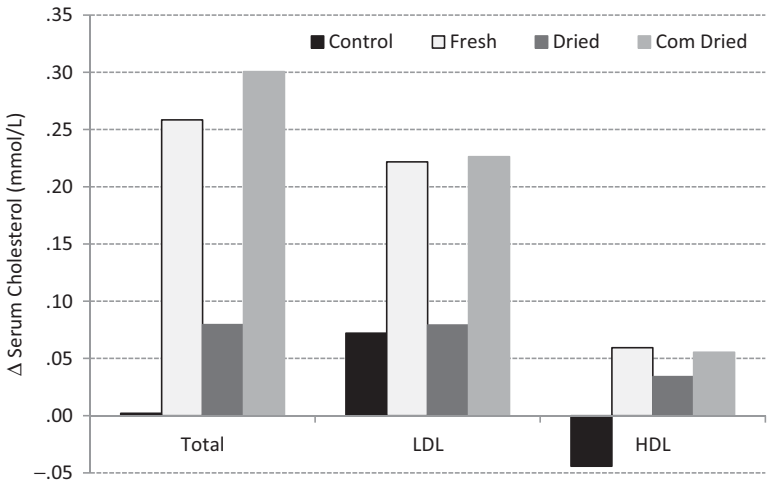
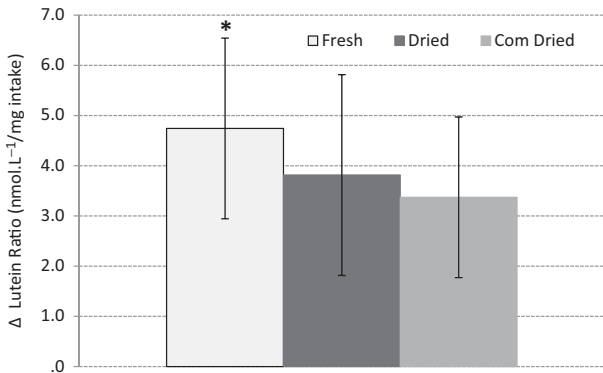
smaller increase in serum lutein levels compared to the fresh lutein-enriched egg-yolk containing beverage.

The lipid matrix of the egg-yolk is a suitable vehicle for the efficient absorption of dietary lutein and the consumption of eggs or lutein-enriched egg-yolks can increase plasma and macular levels of lutein (Clark et al., 2006, Chung et al., 2004; Handelman et al., 1999, Goodrow et al., 2006, Vishwanathan et al., 2009, 2010; Wenzel et al., 2006). In our study, we could extend this observation to lutein-enriched egg-yolk containing beverages, as all three experimental conditions increased lutein and zeaxanthin levels after 6 weeks of supplementation. The bioavailability of lutein from egg-yolk is much higher than that of lutein from either supplements or vegetables. The plasma response to egg lutein is on average at least 0.3  $\mu\text{mol/l}$  per mg of daily lutein intake, compared to  $\sim 0.1 \mu\text{mol/l}$  for pure lutein supplementation, and even lower for vegetables,  $\sim 0.05 \mu\text{mol/l}$  per mg lutein (see (Thurnham, 2007)). The serum lutein response in our study turned out to be  $\sim 0.2 \mu\text{mol/l}$  per mg egg-lutein for the fresh beverage (304 nmol/L increase over 6 week period with daily intake of 1.50 mg). Responses for the two dried conditions were 0.16  $\mu\text{mol/l}$  and 0.14  $\mu\text{mol/l}$  per mg egg-lutein for the dried version of the fresh beverage and the beverage composed of the dried individual components of the drink, respectively. These numbers indicate a somewhat lower bioavailability compared to the more recent egg studies (Burns-Whitmore et al., 2010; Goodrow et al., 2006; Wenzel et al., 2006), but still a three times higher value compared to supplements and vegetables. Consequently, eggs and egg-based foods such as the egg beverage and its dried version are a particular useful source of lutein and other xanthophyll pigments.

In both dried conditions, the daily lutein intake turned out to be lower compared to the fresh condition (1.50 mg/day for fresh; 0.93 and 1.26 mg/day for dried and combined dried). Both, the drying procedure itself, and the storage of the dried material could have affected this lower lutein intake in the dried versions. A recent study evaluating the influence of spray and freeze drying on carotenoid content in egg yolk observed that drying led to higher carotenoid content (Wenzel et al., 2010). However, storage in the dark resulted in a loss of carotenoid content, with the greatest losses occurring in the first weeks (Wenzel et al., 2010). In our study we assessed lutein content in the dried products at regular intervals, and also observed a slight decrease over time.

Figure 4. Change in serum lutein after 6 weeks intervention as expressed relative to cumulative intake ( $\Delta$  Lutein Ratio,  $\text{nmol L}^{-1}/\text{mg intake}$ ) for the fresh lutein-enriched egg-yolk beverage (Fresh), the dried version of this beverage (Dried) or the beverage composed of the dried individual components of the drink (Com Dried).  $\ast=p<0.05$  versus combined dried.

Figure 5. Change in serum total, LDL and HDL cholesterol after 6 weeks intervention for the lutein-free control beverage (Control), the fresh lutein-enriched egg-yolk beverage (Fresh), the dried version of this beverage (Dried) or the beverage composed of the dried individual components of the drink (Com Dried).



	Control	Fresh	Dried	Com Dried	ANOVA
Total	0.00 ± 0.38	0.26 ± 0.37	0.08 ± 0.39	0.30 ± 0.53	P=0.052
LDL	0.07 ± 0.33	0.22 ± 0.34	0.08 ± 0.37	0.23 ± 0.48	P=0.33
HDL	-0.04 ± 0.15	0.06 ± 0.13	0.03 ± 0.15	0.06 ± 0.15	P=0.048

The serum lutein responsiveness can be affected by several factors other than the food matrix (fresh eggs versus dried eggs). The level of – habitual – intake is important, with a reduced responsiveness at higher intakes (Surai et al., 2000; Wenzel et al., 2006), and with higher baseline values from a vegetable and fruit-rich diet (Yeum et al., 1996). In addition, a high-fat diet is associated with a greater increase in lutein compared with a reduced-fat diet (Chung et al., 2004), due to a facilitated absorption. All our experimental lutein-containing beverages had a comparable fat content and the dietary fat intake was not assessed separately. Also the duration of the intervention is important, with reaching an equilibrium in circulating lutein after approximately 3 weeks of supplementation (Thurnham, 2007), indicating that our intervention of 6 weeks, was sufficient to evaluate the maximal serum lutein response. Finally, we controlled lutein intake by restricting consumption of certain products and vitamin supplements.

Lutein is hypothesized to be of help in the prevention of AMD, by absorbing blue light and protecting the retina from oxidative stress by neutralizing free radicals (Bernstein et al., 2001; Landrum et al., 1997; Landrum & Bone, 2001; O'connell et al., 2006). We here observed an increased serum lutein content after 6 weeks consumption of egg-yolk containing beverages, equivalent to ~1.5 eggs a day. An increase in serum lutein and/or zeaxanthin was repeatedly shown to be associated with increased macular pigment optical density, both in studies considering supplements (Aleman et al., 2001; Bone et al., 2003; Hammond et al., 1997) as well as eggs (Burns-Whitmore et al., 2010).

Whether lutein supplementation reduces the risk of AMD was heavily debated for decades. The large age-related eye disease study (AREDS) demonstrated that supplementation with antioxidant vitamins and minerals reduced the risk of AMD (Age-Related Eye Disease Study Research, 2001). The first results of the subsequent AREDS2 were recently published. It was concluded that lutein+zeaxanthin reduced the risk of progression to advanced AMD, and that lutein+zeaxanthin could be an appropriate carotenoid substitute in the AREDS formulation (Age-Related Eye Disease Study 2 Research, 2013). However, so far, the FDA still concluded that no relationship between the intake of lutein or zeaxanthin and the risk of AMD could be drawn (Trumbo & Ellwood, 2006).

Our study population consisted of healthy young volunteers, which is not the typical AMD population. However, the benefits of egg (yolk) consumption for blood lutein levels have been demonstrated in a variety of populations: younger subjects (Wenzel et al., 2006), elderly subjects (Goodrow et al., 2006; Handelman et al., 1999), statin users (Vishwanathan et al., 2009) and lacto-ovo vegetarians (Burns-Whitmore et al., 2010). Meagher et al. (2013) included both healthy subjects and subjects with AMD, and observed that response data were comparable when a supplement composed of lutein, zeaxanthin and meso-zeaxanthin was provided.

Egg-yolk is a source of dietary cholesterol, which can increase LDL cholesterol (Weggemans et al., 2001), and, therefore, intake should be limited according to several dietary guidelines. Several studies concluded that a higher consumption of eggs is not associated with increased risk of coronary heart disease or stroke (Hu et al., 1999; Rong et al., 2013). In addition, an increase in serum total and LDL cholesterol was observed when whole eggs were consumed, whereas the increase was more moderate when egg yolks were incorporated into a buttermilk drink (Baumgartner et al., 2013). In our study, the consumption of 1.5 egg-yolk incorporated in a buttermilk drink did not result in a significant change in serum cholesterol level, although a tendency was seen for an increase in total and HDL cholesterol

in the fresh lutein-enriched egg-yolk containing beverage and the beverage composed of the dried individual components.

## Conclusions

All lutein-enriched egg-yolk containing beverages, the fresh and both dried versions, significantly increased serum lutein levels in a healthy population. The fresh beverage increased serum lutein levels to a greater extent compared to the beverage composed of the dried individual components, while no significant difference was seen in the effect on serum lutein levels between the fresh lutein-enriched egg-yolk beverage, and the dried version of this beverage. Finally, change in serum cholesterol level was not significantly different between the lutein-enriched egg-yolk containing beverages and the control condition.

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## Declaration of interest

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