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Effects of lutein-enriched egg yolk in buttermilk or skimmed milk on serum lipids & lipoproteins of mildly hypercholesterolemic subjects

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Abstract *Background and aims:* Earlier studies in our group suggested that traditionally prepared buttermilk influences cholesterol metabolism. We therefore designed a study to evaluate whether traditionally prepared buttermilk lowers serum low-density lipoprotein cholesterol (LDL-C) and/or prevents the LDL-C raising effect of egg yolks.
Methods and results: Mildly hypercholesterolemic subjects were randomly allocated to one of four diet groups consuming daily at lunch 80 ml skimmed milk with ($n = 23$) or without ($n = 25$) lutein-enriched egg yolk (28 g from 1.5 eggs providing 323 mg cholesterol) or traditionally prepared buttermilk with ($n = 23$) or without ($n = 21$) lutein-enriched egg yolk during a 12 week period. Fasting blood samples were taken to measure concentrations of serum lipids, (apo)lipoproteins, liver and kidney function markers, and plasma lutein, zeaxanthin and high-sensitive C-reactive protein (hsCRP). Egg yolk consumption significantly increased serum total cholesterol (total-C) ($p = 0.035$) and LDL-C concentrations ($p = 0.022$). Buttermilk did not change the effects of egg yolk on serum lipids and (apo)lipoproteins. There was a trend towards significant lower total-C ($p = 0.077$), but not LDL-C ($p = 0.204$) concentrations in the buttermilk groups. Plasma lutein and zeaxanthin concentrations increased significantly ($p < 0.001$) in the egg yolk groups.
Conclusion: In mildly hypercholesterolemic subjects, daily consumption of traditionally prepared buttermilk for 12 weeks did not lower serum total-C or LDL-C concentrations, nor did it prevent the serum total-C and LDL-C raising effect of daily egg yolk consumption.
Registration number: This study is registered at www.clinicaltrials.gov as NCT01566305.

Introduction

Eggs are foods with a high nutritional value. Not only because egg proteins have a high biological value, but also because eggs contain many important micronutrients such as folate, vitamins A, B12, D and K, and carotenoids like lutein [1]. However, eggs are also a concentrated source of cholesterol. It is known that dietary cholesterol increases serum total cholesterol (total-C) and low-density lipoprotein cholesterol (LDL-C) concentrations, and it is generally accepted that elevated serum LDL-C concentrations are causally related to cardiovascular disease (CVD) risk. In

Abbreviations: ALAT, alanine transaminase; ALP, alkaline phosphatase; AMD, age-related macular degeneration; ANOVA, analysis of variance; Apo A1, apolipoprotein A1; Apo B100, apolipoprotein B100; ASAT, aspartate aminotransferase; BMI, body mass index; CVD, cardiovascular disease; FFQ, food frequency questionnaire; Gamma GT, γ-glutamyl transpeptidase; HDL cholesterol, high-density lipoprotein cholesterol; hs CRP, high-senstive C-reactive protein; LAB, lactic acid bacteria; LDL cholesterol, low-density lipoprotein cholesterol; MFGM, milk fat globule membrane; TAG, triacylglycerol; VLDL, very-low-density lipoproteins.

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2001, Weggemans et al. [2] calculated in their meta-analysis - based on 17 RCTs with a follow-up period ranging from 3 to 20 weeks and a cholesterol intake ranging from 167 to 897 mg/d - that each 100 mg increase in dietary cholesterol intake increased serum total-C and LDL-C concentrations with respectively 0.056 mmol/l and 0.050 mmol/l. This observation contributed to the continuous, still ongoing discussion on the necessity to limit egg intake as part of a healthy diet. Although several prospective cohort studies did not demonstrate a relation between egg intake and cardiovascular risk [3–5], a recent meta-analysis [6] showed a positive association between egg consumption and CVD risk. There are specific conditions such as age-related macular degeneration (AMD) in which an increased consumption of eggs could be of benefit. Egg yolks are, like green vegetables and corn [7], a valuable source of lutein in our diet. Lutein from egg yolks is however characterized by a higher bioavailability, as the change in serum concentrations reached by 1 mg lutein in egg yolks is comparable to the change obtained by a supplement containing 5 mg lutein [8]. In an earlier pilot study in which healthy volunteers were supplied with lutein-enriched eggs, we observed a significant increase in serum lutein concentrations in the group consuming the lutein-enriched eggs versus the control group. The lutein-enriched egg yolks were provided either as complete boiled eggs or as egg yolks incorporated into a buttermilk beverage. Both food matrices resulted in comparable increases in serum lutein concentrations [8]. Interestingly, the expected increase in serum LDL-C concentrations after consumption of the boiled eggs was more pronounced as compared to the non-significant increase after consumption of the egg-yolk enriched buttermilk beverage [9]. We therefore speculated that buttermilk itself or specific buttermilk ingredients might influence (intestinal) cholesterol metabolism. Within this context, it is important to mention that we used traditionally prepared buttermilk, which has a different nutritional composition as compared to cultured buttermilk. Traditionally prepared buttermilk refers to the liquid left over after churning butter from cream. Suggestions that compounds present in traditionally prepared buttermilk, such as sphingolipids [10] and lactic acid bacteria (LAB) [11] might influence serum lipoprotein concentrations have been raised earlier. Therefore, the objective of the present study was to evaluate whether traditionally prepared buttermilk can lower serum LDL-C concentrations and/or can prevent the serum LDL-C raising effects of daily egg yolk consumption.

Methods

Study design

The study had a randomized, placebo-controlled 2 × 2 factorial design and included 110 apparently healthy men and women. The total study duration was 14 weeks of which the first two weeks were a run-in period in which subjects could become acquainted with the study procedures; i.e. drinking daily 80 ml of a milk beverage at lunchtime, eating according to the Dutch dietary guidelines (35 en% fat of which 10 en% saturated fat, and 50–55 en% carbohydrates) and not eating any additional whole eggs (food products prepared with eggs like cake or quiche were allowed as long as the intake of these products did not vary throughout the study period). At the start and at regular intervals during the study, a research dietician explained to and discussed the Dutch dietary guidelines with the subjects.

During the run-in period a skimmed milk beverage without added egg yolks was supplied. For the next 12 weeks experimental period, all subjects were randomly allocated to one of four diet groups stratified by age, sex and body mass index (BMI); the first group received a skimmed milk beverage, the second group a buttermilk beverage, the third group a skimmed milk beverage containing lutein-enriched egg yolks, and the last group a buttermilk beverage containing lutein-enriched egg yolks. The skimmed milk beverage in the experimental period was identical to the beverage consumed in the run-in period and served as control. During the entire study period, subjects recorded in a diary signs of illness, medication used and protocol deviations. Subjects were asked not to change their level of physical activity, use of alcohol and oral contraceptives throughout the study. In week 0 at day 0 (start), in week 2 at days 11 and 14 (end run-in period), in week 8 at day 56 and in week 14 at days 95 and 98 (end experimental period), fasting blood samples were taken. Venipunctures were performed as much as possible by the same technician and at the same time of day. Body weight was measured at day 0 and day 98 with subjects wearing casual (street) clothes without shoes and accessories. Food intake over the previous month was assessed by a validated food frequency questionnaire (FFQ) at the end of the run-in period and experimental period. The Dutch food composition table was used to calculate energy and nutrient intakes based on these FFQs. Study beverages were supplied every two weeks and had to be stored in a refrigerator. Compliance was measured by checking the study diaries at every visit. Compliance in the groups that received the egg-yolk beverages during the experimental period could in addition be determined by measuring plasma lutein concentrations.

Study population

Before entering the study, subjects attended two screening visits to check whether they fulfilled the in- and exclusion criteria. These criteria are described in the supplementary data. Subjects provided their informed consent for the study. The medical ethics committee of the University Hospital Maastricht and Maastricht University approved the study protocol.

Experimental products

The skimmed and buttermilk beverages containing the lutein-enriched egg yolks contained 28 g egg yolk (~1.5 eggs), which provided approximately 323 mg cholesterol