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Effect of Egg Yolk Feeding on the Concentration and Composition of Serum Lipoproteins in Man

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Summary

The effect of egg yolk consumption on the composition of LDL and on the concentration of HDL subclasses was studied in healthy subjects. Six volunteers consumed a diet low in cholesterol for 10 days and then daily added 6 egg yolks to their diet for another 10 days; the experiment was repeated 1 year later with the same subjects. Egg yolk consumption caused the cholesterol intake to increase by 1600 mg/day, and the fat intake by 7 energy % at the expense of carbohydrates; this increase was due almost exclusively to monounsaturated fatty acids.

Upon egg yolk feeding the mean level of serum total cholesterol rose by 13%; the bulk of this rise was due to LDL cholesterol, which increased by 21%. VLDL and IDL cholesterol decreased by 19 and 11%, and serum total triglycerides by 17%. Marked relative increases of 35 and 36% were seen in the cholesterol level of the HDL subfractions with densities of 1.055–1.075 g/ml (HDL₁) and 1.075–1.100 g/ml (HDL₂), respectively. The HDL₂/LDL cholesterol ratio increased by 16%. No change in cholesterol in HDL₃ ($d > 1.100$ g/ml) was observed. The increase in cholesterol in HDL isolated by density gradient ultracentrifugation significantly exceeded the increase in cholesterol in heparin-Mn²⁺ soluble HDL. This suggests the

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Abbreviations: VLDL = very low density lipoproteins; LDL = low density lipoproteins; IDL = intermediate density lipoproteins; HDL = high density lipoproteins; SDS = sodium dodecyl sulfate.

formation of apo E-containing HDL, i.e. HDL_c, which has HDL density but is not soluble in heparin-Mn²⁺.

The composition of the LDL particles was significantly altered; the core became enriched in esterified cholesterol at the expense of triglycerides, and the ratio of core components to surface components increased by 7%.

Key words: *Dietary cholesterol – Man – Serum lipoproteins*

Introduction

The effect of dietary cholesterol on serum levels of total cholesterol in man has been extensively studied [1]. Although differences in individual susceptibility exist [2–5], on average a moderate rise in the concentration of serum total cholesterol occurs when cholesterol intake is increased. It is less clear whether the excess cholesterol that appears in serum after cholesterol feeding is atherogenic. Although most of the increase in serum cholesterol resides in the LDL fraction [3,4,6–11], large percentual increments in the level of cholesterol in the HDL fraction after cholesterol feeding have also been reported [7–11]. The situation is further complicated by the fact that the HDL density range contains a variety of particles, some of which are associated with a lower risk for atherosclerotic heart disease, and others which are not [12]. Thus the net atherogenic potential of a cholesterol-rich diet can be evaluated only after the effects on the concentration and composition of these various lipoprotein particles have been defined. We have therefore measured the effects of egg yolk feeding on the concentration and the composition of LDL and the concentration of the various HDL subfractions in healthy subjects. Parts of this work have appeared in abstract form [4,13,14].

Methods

Subjects

Three female and 3 male members of the Department volunteered to participate. They were all apparently healthy, were normocholesterolemic, had no anaemia and had no glucosuria or proteinuria. Age and height (mean \pm SD) of the subjects in 1981 were 35 ± 6 years (range, 26–42 years) and 173 ± 8 cm (range, 163–182 cm). Body weight was 71 ± 11 kg (range, 56–81 kg) in 1981 and 69 ± 11 kg (range, 54–77 kg) in 1982. Serum cholesterol concentrations were 4.94 ± 0.70 mmol/l (range, 3.64–5.70 mmol/l) in 1981 and 4.60 ± 0.74 mmol/l (range, 3.52–5.57 mmol/l) in 1982. The experimental protocol was fully explained to the participants, who were all scientists familiar with dietary studies, and informed consent was obtained.

Experimental design

One of the original aims of the study was to investigate the reproducibility of the cholesterolemic response to dietary cholesterol [4]; for that reason it was performed

twice with the same subjects in the fall of 1981 and 1982. The experiments in 1981 and 1982 were performed in exactly the same way. The subjects consumed their habitual diets, but during the first 10 days of the study cholesterol-rich products were forbidden, whereas during the second 10 days of the study the diets were supplemented with 6 egg yolks per day. During the first 10 days the subjects were asked not to eat eggs or egg-containing products, shell fish, organ meats and butter, and to limit their intake of meat and fish to 100 g/day. During the cholesterol-rich period fresh egg yolks were supplied daily as fried or boiled whole eggs, as raw yolks homogenized with orange juice, or worked into salads and desserts. The subjects generally avoided monotony by varying between different items.

In 1982 the 24-h recall method was used twice per subject per dietary period to estimate food intakes during the experiment. Food intake data were converted into nutrients using the computerized Dutch food table [15]. In 1981 food intakes were not determined.

Blood sampling

Blood was drawn into vacuum tubes from an antecubital vein after an overnight fast on the last 2 days of both the low-cholesterol and high-cholesterol periods. Blood samples were also drawn after 1 and 2 days of egg yolk consumption. Blood samples were allowed to clot at room temperature for 1–2 h and the serum was prepared by low-speed centrifugation.

Analytical methods

Whole serum and lipoprotein fractions were stored at -20°C until analysis. All samples of one subject were analysed within one batch.

Total serum cholesterol was measured with the Liebermann–Burchard reagent under strictly standardized conditions [16]. Total HDL cholesterol was determined after manganese–heparin precipitation of apo B and apo E containing lipoproteins (VLDL, LDL and HDL_c) [17].

The lipoproteins were also separated by density gradient ultracentrifugation [18]; sera for ultracentrifugation were stored for a maximum of 3 days at $+4^{\circ}\text{C}$. After centrifugation the lipoprotein fractions were harvested by aspiration on the basis of the known density gradient in the centrifuge tubes [18]. The following density classes (d in g/ml) were isolated: VLDL ($d < 1.006$); IDL ($1.006 < d < 1.019$); LDL ($1.019 < d < 1.055$); HDL₁ ($1.055 < d < 1.075$); HDL₂ ($1.075 < d < 1.100$) and HDL₃ ($d > 1.100$). The density of the HDL₁ fraction overlaps with that of classical LDL ($1.019 < d < 1.063$) and HDL_{2b} ($1.063 < d < 1.100$). All subjects had negligible serum levels of sinking pre-beta lipoprotein.

Cholesterol concentrations in the lipoprotein fractions were determined enzymatically [19] (catalase method, Boehringer-Mannheim, F.R.G.) using serum calibrators [16]. The day-to-day variation coefficient for control sera was 0.9%. The mean recovery of lipoprotein cholesterol from whole serum for the 48 samples was $94 \pm 4\%$ (\pm SD).

Free cholesterol in LDL was measured by omitting cholesterol esterase in the enzymatic method described above. Serum total and LDL triglyceride concentra-

tions were determined [20]. The concentration of lipid phosphorus was measured in a lipid extract [21] of the serum or LDL fraction [22]. Protein in the LDL fraction was estimated [23], using 98–99% pure bovine serum albumin (Sigma Chemical Co., St. Louis, MO) as a standard.

The apoprotein composition of the HDL₁ and HDL₂ fractions was examined by SDS-polyacrylamide gel electrophoresis [24].

Statistics

Student's 2-tailed *t*-test was used.

Results

The consumption of 6 egg yolks per day caused the cholesterol intake to increase from 200 to 1800 mg/day (Table 1). Six egg yolks weigh about 100 g, and supply about 350 kcal (1.5 MJ) of which about 85% is derived from lipids. As a result, during the high-cholesterol period the intake of fat increased from 39% to 46% of energy, and that of carbohydrates was reduced from 44 to 37 energy % (Table 1). As the predominant fatty acid in egg yolk is oleic acid, the energy percentages derived from saturated and polyunsaturated fatty acids remained constant, whereas that from monounsaturated fatty acids increased during egg yolk feeding. After the 10 days of egg yolk consumption body weight changes were -1.3 ± 0.5 kg (\pm SD) in 1981 and $+0.3 \pm 1.5$ kg in 1982.

The baseline serum cholesterol values of the 6 subjects measured after 10 days on the low-cholesterol diet, ranged from 3.45 to 4.98 mmol/l (133–193 mg/dl). The addition of 6 egg yolks per day to the diet for 10 days caused a rise in serum cholesterol within a few days, the mean increase being 0% after 1 day and about 5% after 2 days [4]. After 10 days the mean increase in the concentration of serum cholesterol was 11% in 1981 and 14% in 1982 (Table 2). The cholesterolemic

TABLE 1
COMPOSITION OF THE LOW- AND HIGH-CHOLESTEROL DIETS IN 1982
Results are expressed as means \pm SD for 5 subjects.

| | Low cholesterol | High cholesterol |
|--|--------------------|---------------------|
| Energy (kcal/day) | 2260 \pm 574 | 2689 \pm 961 |
| (MJ/day) | 9.4 \pm 2.4 | 11.2 \pm 4.0 |
| Total fat (energy %) | 39 \pm 5 | 46 \pm 6 |
| Saturated fatty acids (energy %) | 16 \pm 1 | 16 \pm 3 |
| Monounsaturated fatty acids (energy %) | 13 \pm 3 | 18 \pm 2 |
| Polyunsaturated fatty acids (energy %) | 7 \pm 2 | 8 \pm 2 |
| Carbohydrates (energy %) | 44 \pm 5 | 37 \pm 4 |
| Protein (energy %) | 14 \pm 1 | 14 \pm 2 |
| Alcohol (energy %) | 4 \pm 3 | 4 \pm 2 |
| Cholesterol (mg/day) | 207 \pm 76 | 1803 \pm 155 |

response after 10 days was quite variable between subjects, the extremes being -3 and $+27\%$ in 1981 and $+3$ and $+26\%$ in 1982.

The coefficient of variation (CV, relative standard deviation) of the observed cholesterolemic response was about 10% (Table 2). The week-to-week within-person CV of the absolute level of serum cholesterol on a constant diet is also about 10% [25]. This leads to a predicted CV for the response of $10 \times \sqrt{2} = 14\%$, and slightly lower predicted CV when multiple blood samples are obtained. Thus most of the observed variation in response between persons can be accounted for by random fluctuations of the serum cholesterol level within subjects. However, the latter variations will cancel each other when group mean responses are used.

Table 2 also shows effects of egg yolk consumption on the mean concentration of cholesterol in serum lipoprotein fractions. The mean concentration of cholesterol in the VLDL and IDL fractions fell after egg yolk consumption, but this effect did not reach statistical significance. In the experiment of 1981, we also found a significant fall in serum triglycerides from 0.86 ± 0.18 mmol/l (76.1 ± 15.9 mg/dl) to 0.71 ± 0.10 mmol/l (62.8 ± 8.9 mg/dl); the change was $-17 \pm 12\%$ ($P < 0.05$, $n = 6$). Increases were found in the cholesterol content of the LDL, HDL₁ and HDL₂ density fractions. The mean concentration of cholesterol in the HDL₃ fraction was not affected. A small increase was seen in total heparin-manganese soluble HDL cholesterol measured independently. In absolute terms, the effect of cholesterol feeding was most marked in the LDL fraction, and it accounted for about 90% of the increase in serum total cholesterol. In relative terms however, the increments in

TABLE 2

BASELINE SERUM TOTAL CHOLESTEROL AND LIPOPROTEIN-CHOLESTEROL LEVELS (mmol/l OF SERUM) AND CHANGES (%) AFTER DAILY CONSUMPTION OF 6 EGG YOLKS FOR 10 DAYS

Conversion factor: cholesterol, 1 mmol/l = 38.7 mg/dl.

| | Baseline ^a | Change ^b (%) | |
|--|-----------------------|-------------------------|--------------|
| | | 1981 (n = 6) | 1982 (n = 6) |
| Total cholesterol | 4.33 | +11 ± 11 | +14 ± 10 * |
| VLDL ($d < 1.006$) | 0.24 | -20 ± 35 | -17 ± 39 |
| IDL ($1.006 < d < 1.019$) | 0.15 | -16 ± 25 | -5 ± 32 |
| LDL ($1.019 < d < 1.055$) | 2.32 | +18 ± 20 | +24 ± 17 * |
| HDL ₁ ($1.055 < d < 1.075$) | 0.19 | +26 ± 25 | +43 ± 28 * |
| HDL ₂ ($1.075 < d < 1.100$) | 0.44 | +23 ± 16 * | +49 ± 26 * |
| HDL ₃ ($d > 1.100$) | 0.96 | +0 ± 10 | +3 ± 7 |
| HDL ₂ + HDL ₃ | 1.40 | +8 ± 10 | +14 ± 6 * |
| HDL (heparin-Mn) | 1.42 | +8 ± 11 | +5 ± 9 |
| HDL ₂ /LDL ratio | 0.20 | +8 ± 30 | +24 ± 7 * |
| HDL (heparin-Mn)/LDL ratio | 0.62 | -6 ± 26 | -14 ± 7 * |
| HDL ₁ /HDL ₂ ratio | 0.44 | +2 ± 11 | -4 ± 8 |

^a Baseline values represent the means of days -1 and 0 in 1981 and 1982.

^b Changes are the mean responses (\pm SD) after days 9 and 10.

* Change significantly different from zero at $P < 0.05$.

TABLE 3

BASELINE COMPOSITION OF LOW DENSITY LIPOPROTEINS, AND CHANGES (%) AFTER DAILY CONSUMPTION OF 6 EGG YOLKS FOR 10 DAYS IN 1981

Conversion factor: cholesterol, 1 mmol/l = 38.7 mg/dl.

| | (n = 6) | |
|---|-----------------------|-------------------------|
| | Baseline ^a | Change ^b (%) |
| Total LDL cholesterol (mmol/l serum) | 2.31 ± 0.32 | +18 ± 20 |
| LDL triglycerides (mmol/l serum) | 0.19 ± 0.04 | -11 ± 15 |
| LDL phospholipids (mmol/l serum) | 0.70 ± 0.08 | -6 ± 20 |
| LDL protein (g/l serum) | 0.52 ± 0.07 | +10 ± 20 |
| Ratio of free to esterified cholesterol | 0.46 ± 0.04 | -4 ± 8 |
| Ratio of esterified cholesterol to triglycerides | 8.5 ± 2.0 | +38 ± 23 * |
| Ratio of core components to surface components (g/g) ^c | 0.93 ± 0.06 | +7 ± 6 * |

^a Baseline values represent the mean ± SD of days -1 and 0.

^b Changes are the mean responses (±SD) after days 9 and 10.

^c The ratio of core components (esterified cholesterol, M_r 651; triglycerides, M_r 886) to surface components (free cholesterol, M_r 387; phospholipids, M_r 698; protein) was calculated on a weight basis.

* Change significantly different from zero at *P* < 0.05.

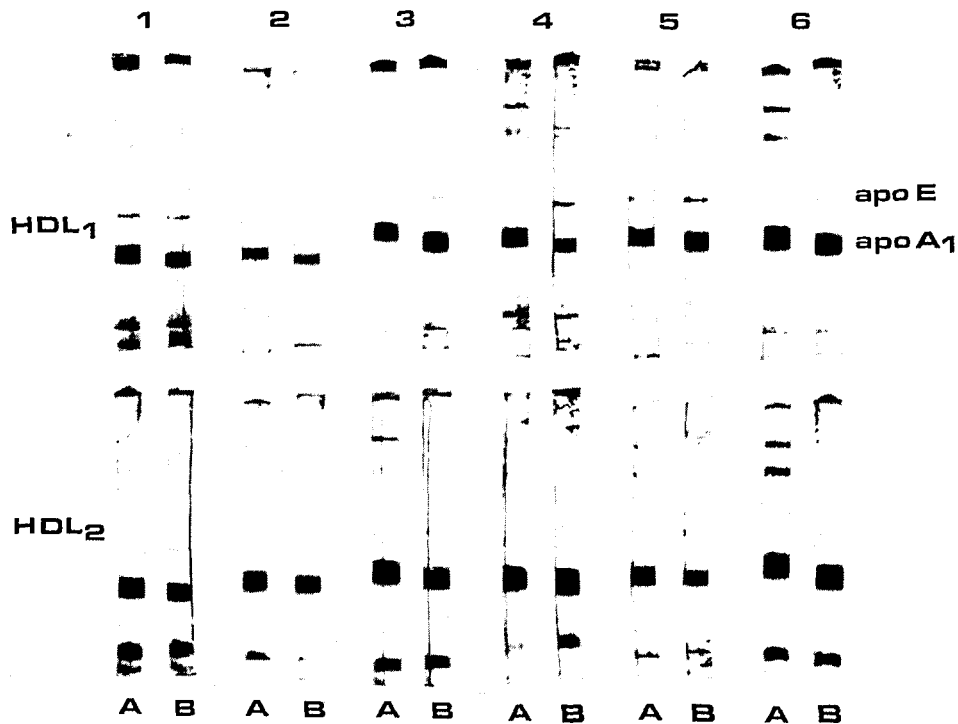


Fig. 1. SDS-gel electrophoresis patterns of apoproteins (10 µg of protein) in 10% polyacrylamide gels. HDL₁ and HDL₂ lipoproteins were isolated just before (A) and 10 days after (B) egg yolk consumption. On the top the subject no. is indicated. The location of apoprotein E and apoprotein A-I are indicated. Apoprotein B does not enter the gel. The location of apo E was verified using the purified protein. The gels were only run in the experiment of 1981.

the cholesterol concentrations in the HDL₁ and HDL₂ density fractions were most pronounced (Table 2). The increases in these fractions were highest for those subjects who also showed the highest response in LDL cholesterol. As a result the HDL₂/LDL cholesterol ratio actually became higher after egg yolk feeding. In essence, the effects of egg yolk feeding on the group mean levels of cholesterol in the various lipoprotein fractions were reproducible from one year to another (Table 2).

Table 3 documents the effect of egg yolk consumption on the composition of the LDL fraction in the experiment of 1981. The ultracentrifugal measurements were done twice in each dietary period. (In retrospect it is unfortunate that these analyses were not carried out on the samples of the experiment of 1982). Within the LDL core elements egg yolk feeding caused a marked increase in the ratio of esterified cholesterol to triglycerides. Furthermore, as shown in Table 3, there was a significant elevation of the mean ratio of total LDL core components (cholesterol esters and triglycerides) to surface components (free cholesterol, phospholipids and protein).

Fig. 1 shows the apoprotein pattern observed on SDS-polyacrylamide gel electrophoresis of the HDL₁ and HDL₂ fractions. The fractions mainly contain apo A-I, but apo B is also present in the HDL₁ fractions of certain subjects. In 2 subjects (no. 3 and 4) there tended to be an increase in the relative content of apo E in the HDL fractions.

Discussion

In man a moderate rise in the concentration of group mean serum total cholesterol has been observed frequently [2-5] when cholesterol intake is increased. Many workers, but not all, have observed that steady state levels of serum cholesterol are reached within 10 to 14 days [5,6,8,26,27]. Thus it is reasonable to anticipate that in the present study no sizeable further changes in serum lipid levels would have occurred after feeding the egg yolks for longer periods.

Within 1 experiment large differences in response between persons were seen, but we also found that from one year to another the response of a given subject was not reproducible. These observations can be explained by 'spontaneous' intra-individual fluctuations in the level of serum cholesterol [25] as discussed under Results. Despite the lack of reproducibility per subject, the reproducibility of the group mean effects of egg yolk feeding was rather good (Table 2). Thus this study allows valid conclusions about the mean effects of egg yolk feeding on the concentration and composition of serum lipoproteins in groups of subjects.

Egg yolk is a convenient source of cholesterol for dietary trials. Its effects, however, may be confounded by the relatively large amount of fat that comes with it. In our experiments the addition of 6 egg yolks to the diet caused a replacement of 7% of energy as carbohydrates by an equivalent amount of fat. As this fat was mainly monounsaturated, such an exchange by itself would probably not affect the total cholesterol concentration [28] nor presumably the LDL cholesterol concentration. Monounsaturated fat might even have a hypocholesterolemic action [29]. This suggests that the increase in serum total cholesterol, which was located largely in LDL, was due to the cholesterol component of the egg yolk. Whether the increase in

the mean size of the core of the LDL particles, and the enrichment of the core with cholesterol ester at the expense of triglycerides were due to the cholesterol in the diet or to the change in carbohydrate intake cannot be determined. Whatever the cause, the possible atherogenicity, as based on in vitro studies, of such cholesterol-rich, large LDL particles is of considerable interest [30]. This is substantiated by a recent report that elevated levels of 'light', large LDL particles are more closely related to angiographically defined atherosclerosis than the smaller 'heavy' component of the LDL fraction [31].

Schonfeld et al. [9] concluded that dietary cholesterol does not affect LDL composition. However, they based their conclusion on the cholesterol/protein and cholesterol/phospholipid ratios, which in our experiment also remained virtually constant.

Packard et al. [10] have demonstrated that egg feeding (6 eggs/day) for 4 weeks does not alter the composition of LDL but increases the number of LDL particles. However, these workers had balanced the low- and high-cholesterol diets for the amounts of fat and protein in the eggs so that the amount of cholesterol was the only dietary variable. This suggests that the replacement of dietary carbohydrates by fat in our study caused the observed change in LDL composition. It is also possible that this change is transient and is not observed after 4 weeks of egg yolk feeding [10]. Many experiments [32] have shown that replacement of dietary carbohydrates by fat will reduce fasting triglyceride and VLDL concentrations and increase the concentration of cholesterol in HDL, especially in the lighter HDL₂ fraction. This is also what we observed in this experiment. Thus the decrease in VLDL and IDL cholesterol concentrations and much of the increase in HDL cholesterol might have been caused by the fat in the egg yolks rather than the cholesterol.

Mahley et al. [33] have shown that feeding of cholesterol to animals causes the appearance of HDL_c, a high density lipoprotein that is rich in cholesterol esters and apolipoprotein E and that is precipitated by heparin-manganese reagent. Mahley et al. [34] presented indirect evidence that such lipoproteins are also induced in humans after excessive egg yolk consumption. If HDL_c were present in our subjects it would be expected to appear in the density range of the HDL₁ and HDL₂ particles [33]. After analysis of these fractions by polyacrylamide gel electrophoresis (Fig. 1) there were some signs in certain subjects (no. 3 and 4) of an increase in apolipoprotein E concentration in these fractions. If HDL_c is formed it should show up in the HDL density fractions after ultracentrifugation but not in the HDL supernatant after heparin-manganese treatment, because HDL_c contains apo E and is insoluble in heparin-manganese. There was a slight but persistent and significant difference between the increase after egg yolk feeding in heparin-manganese soluble HDL cholesterol (+0.08 mmol/l) and the total increase in cholesterol in the ultracentrifugal density range $d > 1.075$ g/ml (+0.14 mmol/l). The difference amounted to 0.06 ± 0.09 mmol/l (mean \pm SD; $n = 12$, $P < 0.05$). This is a conservative estimate, because the HDL₁ fraction ($1.055 < d < 1.075$) was omitted from these calculations so as to exclude any possible contamination with LDL. Indeed, in almost all subjects the HDL₁ fraction was found to contain apo B (Fig. 1). Inclusion of HDL₁ would have increased the difference to 0.11 ± 0.10 mmol/l (mean \pm SD, $n = 12$, $P < 0.01$).

Thus we have some evidence that egg yolk feeding causes an increase in apo E-containing lipoproteins in the density range of HDL₁ and HDL₂ particles.

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