Effect of dietary egg on human serum cholesterol and triglycerides[1-3]

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ABSTRACT One hundred sixteen male volunteers between the ages of 32 and 62 years (mean age 46) consumed two whole fresh eggs daily in their customary diets for 3 months and also eliminated eggs for 3 months before or after eating eggs. The men had had normal-range serum cholesterol and triglycerides for the past 7 years. Four-day food records kept by them in each experimental period were assessed for nutrient intake. A Latin square design allowed analyses for season and sequential effects on serum lipids. The serum cholesterol and triglyceride levels at the end of 6 months were compared with their initial levels on customary free choice diets as well as their levels after the first 3 months of study. No significant increase in mean serum cholesterol was found nor was there a significant association of dietary cholesterol intake with either serum cholesterol or triglyceride. *Am. J. Clin. Nutr.* 32: 1051–1057, 1979.

Investigators have studied the changes in serum cholesterol when dietary cholesterol is fed at different levels. Since a single egg contains 250 to 300 mg of cholesterol, about the amount recommended as the total daily dietary intake by the American Heart Association (1) and by the Senate Select Committee on Nutrition and Human Needs in their "Dietary Goals for the United States" (2), egg cholesterol has been investigated. Keys et al. (3) reported minimal changes in serum cholesterol at different levels of dietary eggs. Some researchers used crystalline cholesterol and a cholesterol-free diet and found a significant increase in serum cholesterol (4–7). Slater et. al. (8) studying two age groups of men who added one or two eggs in their diets over a period of eight weeks and then discontinued use of eggs for two weeks found no statistically significant changes in plasma cholesterol. In a previous Latin square-designed 6-month study of 114 normal healthy, freely-living men, we reported that their consumption of one whole egg daily over a period of 3 months did not significantly change their average serum cholesterol from 3 months of eating no eggs within their customary diets (9).

Of the men 90% in the one-egg-a-day study participated as volunteer subjects in the study reported here. The same design and experimental periods were used (9); they added two whole eggs daily for 3 months to their normally ingested diets and eliminated use of eggs in their regular diets for 3 months. No statistically significant increase in serum cholesterol and serum triglyceride were found when a reasonable intake of dietary egg cholesterol was used by healthy males leading their normal life styles and ingesting mixed solid food diets.

Materials and methods

Subjects

One hundred sixteen healthy men from the faculty and staff of the University of Missouri-Columbia, 90% of whom had participated in our one-egg-a-day study 2 years previously, and who had had normal range serum cholesterol and triglyceride for age by annual examination during the previous 7 years, who had total body fat less than 35% as measured by ^{40}K, and who were between

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the ages of 32 and 62 years with no family history of heart disease or diabetes were our volunteer subjects through informed consent.

Experimental design

Every subject passed through two sequential dietary regimen each 12 weeks long. In one, no whole eggs were eaten and in the other, two whole eggs were included daily. Subjects ate their customary diets, with restrictions on baked goods containing eggs and complete elimination of foods such as custards, French toast, etc.

A cross-over design was used: 56 men (group I) ate two whole eggs while the other 60 (group II) were without eggs from December 1, 1976 through February 28, 1977. From March 1, 1977 through May 30, 1977, group I ate no eggs and group II ate two eggs. Groups I and II were matched in age distribution (mean 46 years). Subjects continued their customary activities and kept their weight within 1 kg of initial weight. A 4-day diet record was taken in the middle of each dietary regimen with the assistance of a dietitian (R.K.). Table 1 shows a summary of the diets consumed. Nutrient calculations were derived with use of computerized nutrient-food tables form Agriculture Handbook 8 (10) and Handbook 456 (11) plus research and industry nutrient tables. For the study we provided large size eggs from White Leghorn chickens fed a normal commercial ration (representative of high quality grocery store eggs).

Collection and analysis of blood samples

Fasting venous blood samples were drawn initially and at the end of each dietary regimen. Total serum cholesterol was determined by totally enzymatic procedures using cholesterol esterase, cholesterol oxidase, and peroxidase (12). A comparison was made of 20 sera analyzed with the method of Henly (13) used in our first study (9) and a relative, correlation coefficient of 0.95 was obtained. Serum triglyceride was determined by the enzymatic procedure, using a commercial kit (Worthington Biochemical Corporation, Freehold, N.J.). The Gilford 330-N spectrophotometer (350-L in the system 3500) was the measuring instrument. A comparison was made of 20 sera analyzed for serum triglyceride by the method of Phillips (14) used in our previous study and a relative correlation coefficient of 0.96 was found. Statistical analyses were according to Snedecor and Cochran (15) and Gill and Hafs (16).

Results

Table 1 gives the mean nutrient intake calculated from the diet records and shows that our subjects followed a diet that was similar whether they ate or did not eat eggs, except for lower absolute amounts of protein and total fat intake when they ate no eggs. The dietary cholesterol was increased about the amount of cholesterol within two whole eggs when the men ate eggs ($P < 0.0001$). When not eating eggs, the men derived about 17% of total calories from protein, 38% from

TABLE 1
Mean values and SEM for 4-day intakes WE[a] and NWE[b]

Diet	Calories	Protein	Total Fat	Saturated Fat	Linoleic acid	Cholesterol	Carbohydrates	Calcium	Iron	Vitamin A	Thiamin	Riboflavin	Ascorbic acid	Niacin
			g			*mg*	*g*	*mg*		*IV*		*mg*		
WE	2250 ±60	98[c] ±2.7	100[c] ±3	34 ±1.3	12 ±0.7	800[d] ±15	238 ±7	960 ±45	17 ±0.6	7300 ±600	1.2 ±0.1	2.1 ±0.1	115 ±7	20 ±0.8
NWE	2200 ±62	91[c] ±2.7	90[c] ±3	32 ±1.3	12 ±0.7	260[d] ±10	248 ±8	950 ±46	16 ±0.6	6100 ±530	1.4 ±0.1	2.0 ±0.1	122 ±7	22 ±0.7

[a] Two whole eggs daily for 3 months. [b] No whole eggs daily for 3 months. [c] Significant difference between mean values for WE versus NWE at $\alpha = 0.01$. [d] Significant difference between mean values for WE versus NWE at $\alpha = 0.0001$.

fat, and 45% from carbohydrate. While they were eating eggs, their calories were about 17% from protein, 39% from fat, and 43% from carbohydrate. This partition is not very different from the average American dietary.

The nutrient intake of the participants in our first study (1975) when the men ate one egg daily, are compared with the participants in our present study (1977) in Table 2. The dietary intakes are very similar except for cholesterol, which was designed as the variable.

Mean values, standard deviation and standard error of the mean of serum cholesterol and serum triglycerides of this study are compared with the values from our one-egg study in Table 3. Analysis of variance of mean values indicates that there were a variety of individual differences. Analyses of period and diet changes in mean values showed no differences. Changes among men and within men, comparing the true mean differences from period to period of initial serum cholesterol and triglycerides with the experimental serum sample values were found not to have statistically significant differences.

Data for cholesterol intake and change in serum cholesterol at the end of 3-month periods in the two-egg study are given in Table 4. Group I and II participants differed in their serum cholesterol response when they were not eating eggs: 82% of group I showed a decrease in serum cholesterol as compared to 55% of group II, while 18% increased serum cholesterol compared to 45% in group II. However, when the men were eating two eggs, the serum cholesterol of the two groups were not as dissimilar (45% group I and 55% group II decreased serum cholesterol). It was noted that 55% of group II participants decreased their serum cholesterol whether they ate two eggs or no eggs daily.

Discussion

In this study, as in our previous one (9) we elected to study normal "every day-living" humans and did not use exaggerated amounts or types of dietary cholesterol to produce an effect (two eggs are often eaten daily by persons who consume eggs). The design of this study is the same as the previous one. The daily diet ingested by each man was his

TABLE 2
Mean nutrient values and SEM for 4 day-no-egg dietary intake 1975 versus 1977 and 4 day-one-egg diet (1975) versus two egg diet (1977)

Diet	Calories	Protein	Total fat	Satuated fat	Linoleic acid	Cholesterol	Carbo-hydrates	Calcium	Iron	Vitamin A	Thiamin	Ribo-flavin	Ascorbic acid	Niacin
			g			*mg*	*g*	*mg*		*IU*			*mg*	
NEW[a]														
1975	2400	100	96	34	12	300	264	1100	16	6300	1.4	2.3	110	23
	±55	±2.4	±3	±1	±1	±9	±6	±58	±0.5	±393	±0.1	±0.1	±5	±0.7
1977	2200	91	90	32	11	260	248	950	16	6100	1.4	2.0	122	22
	±62	±3	±3	±1	±1	±10	±8	±46	±0.6	±530	±0.1	±0.1	±7	±0.7
WE[b]														
One egg	2300	102	100	32	12	535[c]	246	1000	16	7000	1.3	2.1	103	23
(1975)	±56	±3	±3	±1	±1	±9	±6	±58	±0.5	±398	±0.1	±0.1	±5	±0.7
Two egg	2250	98	100	33	12	800[c]	234	960	17	7300	1.2	2.1	115	20
(1977)	±60	±3	±3	±1	±1	±15	±7	±45	±0.6	±600	±0.1	±0.1	±7	±0.8

[a] No whole eggs. [b] Whole eggs. [c] Significant difference between mean values one egg versus two egg $\alpha = 0.0001$.

TABLE 3
Comparison of one and two egg studies: mean ± SD (SEM) of serum cholesterol, serum triglycerides, and body weight

Group	Season	Diet	Body	Serum cholesterol	Serum triglycerides
			kg	*mg/dl*	
One egg (1975)[a,b]					
Group I n = 55	Initial		75 ± 11.(1.4)	223 ± 15 (1.9)	157 ± 45 (5.8)
	1	WE		226 ± 20 (2.6)	146 ± 38 (4.9)
	2	NWE		220 ± 21 (2.7)	159 ± 44 (5.8)
Group II n = 59	Initial		74 ± 9 (1.1)	226 ± 15 (1.9)	150 ± 43 (5.5)
	1	NWE		225 ± 15 (20)	135 ± 39 (4.9)
	2	WE		226 ± 15 (2.0)	140 ± 73 (9.4
Two egg (1977)[c,d]					
Group I n = 56	Initial		75 ± 10 (2)	214 ± 35 (5)	115 ± 58 (9)
	1	WE		213 ± 30 (4)	108 ± 65 (6)
	2	NWE		194 ± 31 (4)	102 ± 48 (6)
Group II n = 60	Initial		74 ± 10 (2)	203 ± 39 (6)	117 ± 59 (8)
	1	NWE		198 ± 36 (4)	103 ± 39 (5)
	2	WE		198 ± 40 (5)	100 ± 54 (7)

[a] Season 1 = September 17, 1974 to December 10, 1974; season 2 = December 11, 1974 to March 3, 1975. [b] Diet: include one whole egg (WE) exclude one whole egg (NWE). [c] Season 1 = December 1, 1976 to February 28, 1977; season 2 = March 1, 1977 to May 30, 1977. [d] Diet: include two whole eggs (WE) exclude two whole eggs (NWE).

usually eaten, mixed solid food diet with the addition of two whole eggs for 3 months, preceded or followed by the omission of eggs for 3 months. Their exercise habits were those which each subject preferred as a life style. In both of our studies the subjects were not initially adopted to a cholesterol-free diet. They were required to maintain their weight within 1 kg of their initial weight.

The participants were extremely cooperative and careful about food records which they kept for a 4-day sequence during each experimental period. Probably because most of the men are involved in their own research, they are conscious of the importance of reliable information and contributed recipes and labels from unusual foods and liquors ingested. Also, they wished to know whether their life style and diet affected their serum lipids, and therefore they kept good records.

Table 1 shows that the men in this study averaged 7 more g of protein and about 10 more g of total fat ($P < 0.01$) when they were eating two eggs than when they were not. Interestingly, two large whole eggs contain about 11 g of fat and 15 g of protein. The mean saturated fat and linoleic acid intake did not change in the diets (total saturated fat in two eggs is about 3.4 g, total unsaturated 6.0 g, and linoleic acid 1.3 g).

When the data of the dietary intake of the one-egg-a-day study are compared with those of the present two-egg-a-day study, the constancy of nutrient intake is noticeable (Table 2). The significant difference noted is in dietary cholesterol, the planned nutrient variable ($P < 0.0001$), which is explained by the addition of 250 or 500 mg of cholesterol present in one or two whole eggs respectively when the men were eating eggs. The average American diet contains 300 to 800 mg/day cholesterol; when the men ate eggs in our studies they averaged an intake of 530 to 800 mg/day cholesterol. The lower levels of dietary cholesterol intake (200 to 300 mg/day) can easily be attained by conscientious persons. When nutrient values were further analyzed so that only the data of the men who participated in both studies were assessed, the statistical data did not change.

Both of our studies have the same results: the daily ingestion by normal healthy males of whole egg cholesterol at levels of one or two fresh eggs for three months does not increase serum cholesterol significantly. When we analyzed only those values of serum

cholesterol and triglycerides of the men who had participated in both studies, the statistical data did not change. Our results are similar to those of Keys et al. (3) who found long-term differences in dietary cholesterol were not associated with significant differences in serum cholesterol in healthy, unconfined men eating solid foods. They are also similar to those of Slater et al. (8) who reported that healthy male subjects who superimposed either one egg or two eggs on their usual diet for 8 weeks, showed no significant differences in average serum cholesterol between eating or abstaining from eating eggs. Kummerow et al. (17) found similar results when two fresh whole eggs were fed to hospitalized patients in Sofia, Prague and Urbana in studies up to 54 days in length.

We noted in our study of one-egg-a-day that the participants in group I may have more labile serum cholesterol (9). In that study we found 76% of the men decreased their serum cholesterol when they were not eating eggs. In the two-egg study 82% of group I had decreased serum cholesterol when they were not eating eggs (Table 4). Another explanation besides serum lability would be that the sequence of eating two eggs to eating no eggs has a greater effect on serum cholesterol than the sequence of not eating eggs to eating two eggs, although the statistics do not show this to be significant and the

TABLE 4
Cholesterol (CHOL) intake and change in serum cholesterol (SCHOL) at end of 3 months in two-egg study

Daily CHOL intake	Δ SCHOL (February–initial)					
	−10	−10 to 0	0 to 10	11 to 20	20	% Total
mg						
Group I (WE)[a] n = 56						
>800	3	4	6	2	2	30
601–800	10	6	11	2	7	65
401–600	2	0	1	0	0	5
201–400	0	0	0	0	0	0
0–200						
% Total	27	18	32	7	16	
	Δ SCHOL (May–March)					
Group I (NWE)[b] n = 56						
>800						
601–800						
401–600	6	0	0	0	0	11
201–400	29	6	4	1	0	71
0–200	5	0	3	1	1	18
% Total	71	11	12	4	2	
	Δ SCHOL (February–initial)					
Group II (NWE)[b] n = 60						
>800						
601–800						
401–600	2	1	1	0	1	8
201–400	8	14	13	5	1	68
0–200	5	3	3	1	2	24
% Total	25	30	28	10	7	
	Δ SCHOL (May–March)					
Group II (WE)[a] n = 60						
>800	7	10	11	2	1	52
601–800	9	7	5	1	7	68
401–600						
201–400						
0–200						
% Total	27	28	27	5	13	

[a] Include two whole eggs daily for 3 months. [b] Exclude eggs daily for 3 months.

cross-over design of our study was set up to test the possibility that factors other than diet influenced the results. The Latin square design of our study permitted statistical analyses to assess trends in serum lipid changes independent of dietary regimen. No significant influence of diet on serum cholesterol within each subject was noted and no interaction of season was found.

Mean values for serum triglycerides decreased with season in both groups in this study, with greater variability in serum triglycerides than in serum cholesterol, which we also found in previous study (Table 3). Large individual variability has been noted in serum triglycerides in response to dietary intake by other investigators (3, 18). The sensitive relationship of serum triglycerides to dietary fat makes small changes in blood lipids difficult to interpret. It should be noted in the two egg study that there was an average difference of 10 g total dietary fat between the diet with two eggs and the diet without eggs, yet the serum triglycerides decreased regardless of period. Also, caloric intake was about the same in both groups (Table 1), so its influence can be negated.

The initial serum cholesterol and serum triglyceride values shown in Table 3 for the subjects were significantly lower in the two egg study than in the one egg study done two years earlier ($P < 0.001$). Perhaps the reason was the fact that we used an enzymatic technique in the two-egg study. However, since each man served as his own control, each participating in 3 months' intake of two fresh whole eggs daily and three months of no eggs daily, the initial mean values of the serum lipids need not agree in order to demonstrate no significant increase in serum cholesterol when one or two eggs are ingested.

The results of our two studies are contrary to the data which the American Heart Association and the Senate Select Committee on Nutrition and Human Needs have used in their deliberation and recommendations to change the American diet by limiting dietary cholesterol intake to 300 mg/day (1, 2). Neither one nor two whole fresh eggs eaten daily for three months have been shown to increase serum cholesterol significantly in our investigations with normal, healthy, freely-living males who ate their habitual diets and who have documented serum lipids within normal range.

Summary

The results of this study show high individual variability of serum cholesterol and serum triglyceride response to the daily ingestion of two whole fresh eggs, within a habitual mixed American diet. This is similar to the response of eating only one egg daily shown in our previous study. We found no significant increase in serum cholesterol with dietary intake of two eggs daily for 3 months in men who ate a mixed solid food diet. Our data further support the suggestion that indiscriminate exclusion of eggs may be a useless preventive measure to maintain low serum cholesterol in all normal healthy men.

References

1. Atherosclerosis Study Group. primary prevention of the atherosclerotic diseases. Circulation 42: (Suppl. 1) I, 1970.
2. Dietary Goals for the United States. Washington, D.C.: Superintendent of Documents, 1977.
3. KEYS, A., J. T. ANDERSON, O. MICKELSEN, S. F. ADELSON AND F. FIDANZA. Diet and serum cholesterol in man: lack of effect of dietary cholesterol J. Nutr. 59: 39, 1956.
4. CONNER, W. E., R. E. HODGES, R. F. HODGES AND R. E. BLEILER. The serum lipids in men receiving high cholesterol and cholesterol-free diets. J. Clin. Invest. 40: 894, 1961.
5. ERICKSON, B. A., R. H. COOTS, F. H. MATTSON AND A. M. KLINGMAN. The effect of partial hydrogenation of dietary fats, of the ratio of polyunsaturate to saturate fatty acid, and of dietary cholesterol on plasma lipids in man. J. Clin. Invest. 43: 2017, 1964.
6. MATTSON, F. H., B. A. ERICKSON AND A. M. KLIGMAN. Effect of dietary cholesterol on serum cholesterol in man. Am. J. Clin. Nutr. 25: 589, 1972.
7. ANDERSON, J. T., F. GRANDE AND A. KEYS. Independence of the effects of cholesterol and degree of saturation of the fat in the diet on serum cholesterol in man. Am. J. Clin. Nutr. 29: 1184, 1976.
8. SLATER, G., J. MEAD, G. DHOPESHWARKER AND A. B. ALFIN-SLATER. Effects of eating eggs on plasma cholesterol levels in young and middle-aged men. Nutr. Rept. Internat 14: 249, 1976.
9. PORTER, M. W., W. YAMANAKA, S. CARLSON AND M. A. FLYNN. Effect of dietary egg on serum cholesterol and triglyceride of human males. Am J. Clin. Nutr. 30: 490, 1977.
10. WATT, B. K., AND A. MERRILL. Composition of foods. Agriculture Handbook no. 8, U.S.D.A. Washington, D.C.: United States Government Printing Office, 1963.
11. ADAMS, D. F. Nutritive value of American foods in

common units. Agriculture Handbook no. 456, U.S.D.A. Washington, D.C.: United States Government Printing Office, 1975.
12. ALLAIN, C. C., L. S. POON, C. S. G. CHAN, W. RICHMOND AND P. C. FU. Enzymatic Determination of total serum cholesterol. Clin. Chem. 20: 470, 1974.
13. HENRY, R. Method of Henly. In: Chemistry. New York: Harper and Row, 1974.
14. PHILLIPS, R. E. The fluorometric determination of serum triglycerides (neutral fat) In: A Clinical Procedure. G. K. Turner Associates, 1969.
15. SNEDECOR, G. W., AND W. G. COCHRAN. Statistical Methods (6th ed). Ames: Iowa State University Press, 1967, p. 369.
16. GILL, J. L., AND H. D. HAFS. Analysis of repeated measurements of animals. J. Animal Sci. 33: 331, 1971.
17. KUMMEROW, F. A., Y. KIM, M. D. HULL, J. POLLARD, P. ILINOV, D. DOROSSIEV AND J. VALEK. The influence of egg consumption on the serum cholesterol level in human subjects. Am. J. Clin. Nutr. 30: 664, 1977.
18. KEYS, A., J. T. ANDERSON AND F. GRANDE. Serum cholesterol response to changes in the diet. II. The effect of cholesterol in the diet. Metabol. Clin. Exptl. 14: 759, 1965.