Journal of Animal and Feed Sciences, 3, 1994, 317–324

Effect of feeding egg yolk on total plasma cholesterol and atherosclerosis in young rabbits

Tadeusz Jezierski and Anna M. Konecka

Institute of Genetics and Animal Breeding, Polish Academy of Sciences
Jastrzębiec, 05-551 Mroków, Poland

(Received 17 October 1994; accepted 19 December 1994)

ABSTRACT

The experiment was aimed primarily at inducing moderate atherosclerosis to investigate the role of behavioural and emotional factors in diet-induced atherosclerosis in rabbits. The experimental rabbits (group E, n = 10) were offered 130 g standard pelleted feed mixed with 1 egg yolk daily, whereas the control rabbits (group C, n = 9) were fed the same feed but without egg yolk. Altogether the E rabbits were offered 64 egg yolks within a period of 2 months. Total plasma cholesterol was estimated before and after the experimental period. The degree of atheroma was estimated quantitatively as the percentage of the aorta surface affected. Mixing the egg yolk with pelleted feed resulted in the E rabbits consuming on average 20.1 g cholesterol during the experimental period as compared to 6.9 g cholesterol consumed by C rabbits in normal feed. There was no significant effect of feeding egg yolk on total plasma cholesterol. A significant diet x sex interaction effect ($P = 0.013$) on total plasma cholesterol after the experimental period was found. Not one of the rabbits either group E or C developed visible atherosclerosis.

KEY WORDS: egg yolk, feeding, plasma cholesterol, atherosclerosis, rabbits

INTRODUCTION

When using rabbits as models for studying the effect of behavioural and emotional factors on diet-induced atherosclerosis we found large individual differences in cholesterol consumed in feed and in atheroma size (Jezierski et al., 1993). The present experiment was aimed primarily at inducing moderate atherosclerosis, to make the effects of behavioural and emotional factors more discernible. This was attempted by using a simple method of inducing atherosclerosis in rabbits by feeding them one egg yolk daily mixed with normal, pelleted feed (Cornhill and Roach, 1974).

According to some recommendations for preventing coronary heart disease in humans, the daily intake of cholesterol should not exceed 300 mg (Brown, 1990; Cannon, 1990). Since egg yolk contains about 200-250 mg cholesterol, the general opinion is that eggs are a major source of dietary cholesterol, which may be responsible for inducing atherosclerosis. This opinion has led to a decline in egg consumption (Griffin, 1992). Many attempts have been made to reduce egg yolk cholesterol by feeding hens special diets, by pharmacological intervention and/or by genetic selection (Hargis, 1988). A question arises how far these manipulations of egg cholesterol are substantiated by the effects of egg consumption on changes in plasma cholesterol and, particularly, on inducing atherosclerosis. So far the majority of papers have discussed the influence of an egg diet on plasma cholesterol but this is rather a marker of risk for atherosclerosis and not its cause (Sieber, 1993).

In the present work, relationships between egg consumption, total plasma cholesterol and atheroma size were investigated in rabbits as a model.

MATERIAL AND METHODS

Nineteen White New Zeland rabbits (11 males and 8 females) stemming from the Institute's own outbred stock were used. The animals were three months old at the beginning of the experiment. The rabbits were kept in individual wire cages 60 cm x 60 cm x 32 cm at an average room temperature of 14-18°C. Water was available *ad libitum*. Between weaning and the beginning of the experiment the rabbits were fed *ad libitum* with standard pelleted rabbit feed containing 18.0% crude protein, 4.2% fat and 13.1% crude fibre.

During the experimental period lasting 2 months 10 rabbits (E = experimental group) were offered 130 g pelleted feed daily mixed with 1 egg yolk obtained from a commercial line of hens and dried for 12 hours, while the remaining 9 rabbits were fed the same feed without the egg yolk (C = controls). Since the rabbits sometimes did not consume their full daily dose, the unconsumed feed was weighed. Altogether the rabbits were offered 64 egg yolks. All the rabbits were weighed weekly.

Blood samples were taken into heparinized tubes from an ear vein before and after the experimental period. Prior to blood sampling the rabbits fasted for 24 hours. After collection, the blood samples were centrifuged at 3000 x g for 15 min. Total plasma cholesterol content in mg/100ml was measured in triplicate using Biochemtest No. 1344-690-718061 (POCh, Gliwice, Poland). The intra assay variability was 2%. Analysis of the food revealed that the diet for E rabbits contained 295 (S.D. = 39) mg cholesterol/100g dry feed and the diet for C rabbits contained 98 (S.D. = 7.2) mg cholesterol/100 g dry feed.

After killing each rabbit, the aorta ascendens, arcus aortae and aorta descendens up to the 6 th or 7 th arteria intercostalis were removed and dissected. These arteries were stained with oil-red O (Romeis, 1968), pinned out and photographed in colour. Two colour tints were distinguised: (a) deep red indicating severe atheroma and (b) pink or pale indicating weak or no atheroma. Atheroma size was estimated by placing a transparent grid over the photograph, counting the deep red squares and presenting them as a percentage of the total number of counted squares.

For the statistical analysis of feed consumption, weight gains, total plasma cholesterol and atheroma size the following mixed - model two-way analysis of variance was used:

$$Y(ijk) = u + F(i) + S(j) + FS(ij) + e(ijk)$$

where:

u – mean value
F(i) – effect of diet (i = 1,2) - fixed effect
S(j) – effect of sex (j = 1,2) - random effect
FS(ij) – effects of feeding x sex interaction
e(ijk) – error

Also, correlation coefficients between the investigated traits were calculated.

RESULTS AND DISCUSSION

Feed consumption, weight gains and feed efficiency (feed per 1 g weight gain) did not differ significantly between groups E and C (Table 1). The females of both groups grew faster ($P \leqslant 0.006$) and consumed less feed per 1 g weight gain than did the males ($P \leqslant 0.006$).

Mixing the egg yolk with pelleted feed resulted in the E rabbits consuming approximately three times more cholesterol than the controls (Table 1) but this was not accompanied by an increase in the concentration of total plasma cholesterol. After consuming 64 egg yolks the rabbits had an even lower mean total plasma cholesterol than the control ones (Table 2). There was a significant diet x sex interaction effect in respect to total plasma cholesterol after the period of feeding egg yolk, ($P \leqslant 0.013$). The females in the group fed egg yolk demonstrated a greater increase of total plasma cholesterol than males, but the opposite tendency was observed in the control group (Table 2). The correlation coefficients between the investigated traits, calculated separately within the E and C groups were mostly inconsistent (Table 3).

Not one of the rabbits fed egg yolk developed atherosclerosis. The lack of any effect of feeding egg yolk cholesterol on atherosclerosis in this experiment was

TABLE 1

Consumption of food with and without egg yolk and weight gain in male and female rabbits

Group of rabbits		Feed consumption, g		Weight gain, g		Feed per 1 g weight gain		Cholesterol consumed, g	
		mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
E. (egg yolk in food)									
males	n=6	6686	343	855	118	7.93	1.04	19.79	1.01
females	n=4	6957	198	1072	76	6.52	0.61	20.59	0.58
total group E		6795	313	942	149	7.37	1.12	20.11	0.93
C. (no egg yolk in food)									
males	n=5	7086	153	776	142	9.33	1.36	6.99	0.15
females	n=4	6818	281	960	85	7.12	0.33	6.73	0.28
total group C		7009	214	829	151	8.70	1.55	6.92	0.21

Results of ANOVA

	D.F.	Significance of F			
diet	1	0.361	0.143	0.093	0.000 +++
sex	1	0.992	0.006 +	0.006 +	0.480
diet x sex	1	0.072	0.789	0.481	0.172

TABLE 2

Total plasma cholesterol in male and female rabbits

Group of rabbis		Total plasma cholesterol, mg/100 ml				Increase in total plasma cholesterol, mg/100 ml during the experiment	
		before experiment		after experiment			
		mean	s.d.	mean	s.d.	mean	s.d.
E (egg yolk in food)							
males	n=6	108.6	30.5	116.1	19.3	7.4	36.6
females	n=4	136.9	40.9	187.5	38.3	50.0	58.2
total group E		121.5	33.2	144.4	45.1	24.5	48.6
C (no egg yolk in food)							
males	n=5	127.9	21.3	189.8	61.9	61.9	64.5
females	n=4	112.8	62.5	128.0	54.4	15.2	8.0
total group C		123.6	31.8	172.1	62.9	48.5	57.5

Results of ANOVA	D.F.	Significance of f		
diet	1	0.894	0.754	0.720
sex	1	0.723	0.846	0.940
diet x sex	1	0.253	0.013 +	0.121

TABLE 3

Correlation coefficients between traits investigated

Traits	Total plasma cholesterol				Increase of total plasma cholesterol	
	before experiment		after experiment			
	group E	C	E	C	E	C
Cholesterol consumption	.11	−.21	.40	.03	.29	.15
Weight gains	.39	−.22	.69	−.91*	.35	−.88*
Feed per 1 g gain	−.41	.10	−.64	.87*	−.29	.90*
Total plasma cholesterol before experiment			.30	.42	−.46	−.10

* significant at $P < 0.05$

rather surprising, since feeding one egg yolk daily for a period of approximately 60 days was successfully used as a method for inducing atherosclerosis in rabbits by Cornhill and Roach (1974). However, the results of experiments on the effect of egg consumption or cessation of egg consumption on serum cholesterol are contradictory. While in some works (Slater et al., 1976; Kummerow et al., 1977; Porter et al., 1977; Flynn et al., 1979; Voster et al., 1992) egg consumption was demonstrated to have no effect on total serum cholesterol in humans, Bronsgeest-Schoute et al. (1979) ascertained a small but significant decrease in serum cholesterol after removal of eggs from the diet of habitually egg-eating people. According to Beynen and Katan (1985), after 3 weeks during which no eggs or egg-containing products were consumed, the serum cholesterol decreased by 6-16 mg/dl in one and by 12-14 mg/dl in another experiment and individual responses varied from −39 to +19 mg/dl. On the other hand, Vizioli et al. (1988) found a decrease of total serum cholesterol when beef and pork in the diet of elderly people were completely replaced by eight eggs per week. Beynen et al. (1986) suggest that in animals individual differences in response to dietary cholesterol are greater than in man, due to the existence of hypo- and hyper-responders. In a majority of experiments on humans, the investigations concentrated on the effects of eliminating eggs from a diet rather than on the effect of including eggs into a diet that had previously not contained eggs at all. In the experiment by Richard et al. (1990), young goats receiving cholesterol as egg yolk had a significantly higher cholesterol concentration in blood plasma than did goats supplemented with crystaline cholesterol.

As one of the possible explanations for egg intake having no effect on serum cholesterol Vizioli et al. (1988) and Voster et al. (1992) suggest that the phospholipid in eggs may be hypocholesterolemic and may counteract any possible hypercholesterolemic effect of the cholesterol in eggs.

In conclusion, the present experiment demonstrated that a three-fold increase of cholesterol consumption resulting from mixing egg yolk with normal feed had no significant effect on total plasma cholesterol and no visible atherosclerosis – promoting effect in rabbits.

ACKNOWLEDGEMENT

We are grateful to Mrs. Anna Stawicka for her care of the experimental animals

REFERENCES

Beynen A.C., Katan M.B., 1985. Reproductibility of the variation between humans in the response of serum cholesterol to cessation of egg consumption. Atherosclerosis, 57, 19-31

Beynen A.C., Katan M.B., van Zutphen L.F.M., 1986. Hypo- and hyperresponders to dietary cholesterol. In: A.C.Beynen (Editor), Nutritional Effects on Cholesterol Metabolism. Transmondial, Voorthuizen, 99-109

Bronsgeest-Schoute D.C., Hermus R.J.J., Dallinga-Thie G.M., Hautvast J.G.A., 1979. Dependence of the effects of dietary cholesterol and experimental conditions on serum lipids in man. III. The effect on serum cholesterol of removal of eggs from the diet of free-leving habitually egg-eating people. Amer.J. Clin. Nutr. 32, 2193-2197

Brown W.V.,1990. Dietary recommendations to prevent coronary heart disease. Ann. New York Acad. Sci. 598, 376-388

Cannon G.,1990. Healthy eating. The Experts Agree HMSO, London

Cornhill J.F., Roach M.R., 1974. Quantitative method for the evaluation of atherosclerotic lesions. Atherosclerosis 20, 131-136

Flynn M.A., Nolph G.B., Flynn T.C., Kahrs R., Krause G., 1979. Effect of dietary egg on human serum cholesterol and trigycerides. Amer. J. Clin. Nutr. 32, 1051-1057

Griffin H.D., 1992. Manipulation of egg yolk cholesterol: a physiologist's view. World Poultry Sci. J. 48, 101-112

Hargis P.S., 1988. Modifying egg yolk cholesterol - a review. World Poultry Sci. J. 44, 17-29

Jezierski T., Mckking P., Wiepkema P.R., 1993. Handling and diet induced atherosclerosis in rabbits. Lab. Anim. 27, 235-239

Kummerow F.A., Kim Y., Pollard J., Hull J., Illnow P., Dorossiev D.L., Valek J., 1977. The influence of egg consumption on the serum cholesterol level in human subjects. Amer. J. Clin. Nutr. 30, 664-673

Porter M.W., Yamaneka W., Carlson S.D., Flynn M.A., 1977. Effect of dietary egg on serum cholesterol and triglyceride of humanmales. Amer. J. Clin. Nutr. 30, 490-495

Richard M.J., Lynn D.D., Jacobson N.L., 1990. The domestic goat: a useful model to determine effects of diet and excercise on cholesterol accumulation in the body. Comp. Biochem. Physiol. 95 A, 2, 275-280

Romeis B., 1968. Mikroskopische Technik. Munchen-Wien R.Oldenburg Verlag, 259

Sieber R., 1993. Cholesterol removal from animal food - can it be jusified?. Lebensmittel-Wiss. Technol. 26, 375-387

Slater G.J., Mead J., Dhopeshwarker G., Alfi-Slater A.B., 1976. Effects of eating eggs on plasma cholesterol levels in young and middle-aged men. Nutr. Rept. Int. 14, 249-253.
Vizioli A., Schipani A., Capriotti L., Biagiarelli C., Bevilacqua L., Menteleone A., 1988. Changes in the lipid spectrum after an egg diet. Clin. Diet. 15, 205-212
Voster H., Benade A.J.S., Barnard H.C., Locke M.M., Silvis N., Venter C.S., Smuts C.M., Engelbrecht G.P., Marais M.P., 1992. Egg intake does not change plasma lipoprotein and coagulation profiles, Amer. J. Clin. Nutr. 55, 400-410

STRESZCZENIE

Wpływ żywienia żółtkiem jaj na poziom cholesterolu całkowitego i arteriosklerozę u młodych królików

Pierwotnym celem doświadczenia było badanie roli czynników behawioralnych i emocjonalnych w powstawaniu arteriosklerozy wywoływanej podawaniem cholesterolu w paszy u królikow. Królikom doświadczalnym (grupa E, n = 10) podawano dziennie 130 g standardowej paszy granulowanej z dodatkiem 1 żółtka jaja kurzego. Króliki kontrolne (grupa K, n = 9) otrzymywały tą samą dawkę paszy bez żółtka. Ogółem kroliki z grupy E spożyły 64 żółtka w ciągu 2 miesięcy. Przed i po okresie doświadczalnym w osoczu krwi królików oznaczono poziom cholesterolu całkowitego. Stopień arteriosklerozy oznaczano ilościowo jako procent powierzchni aorty ze zmianami miażdżycowymi. Dodawanie żółtka do paszy spowodowało, że króliki z grupy E pobrały w okresie doświadczalnym 20,1 g cholesterolu, podczas gdy króliki kontrolne po 6,9 g cholesterolu zawartego w paszy standardowej. Nie stwierdzono istotnego wpływu podawania żółtka na poziom cholesterolu całkowitego w osoczu. Stwierdzono istotny wpływ interakcji dieta x płeć na poziom cholesterolu po okresie doświadczenia. U żadnego z królików doświadczalnych i kontrolnych nie stwierdzono widocznych objawów arteriosklerozy w aortach.