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Studies on human Bile

IV. Influence of ingestion of cholesterol in the form of eggs on the composition of bile in healthy subjects

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With 12 tables

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The fact that ingestion of cholesterol in an easily absorbable form such as egg yolk tends to increase serum cholesterol in man is well established (1), but the possible influence of dietary cholesterol on the composition of human bile does not seem to have been examined.

The experiments described in the present paper were carried out in order to ascertain whether daily intake of a substantial amount of cholesterol in the form of whole eggs markedly influences the composition of bile in healthy subjects especially with respect to the molar percentages of each of the main bile acids in the total bile acids and the molar ratios between total bile acids and cholesterol, between lipid-soluble phosphorus and cholesterol, and between total bile acids and lipid-soluble phosphorus.

Experimental

Nine healthy volunteers, 19 to 22 years of age, received diets furnishing from 5 to 10 eggs per day through two consecutive periods of 3 weeks. The constituents of the daily rations were adjusted so as to keep the total amounts of protein and fat constant at 20 and 42 calorie per cent respectively. Further, the daily intake of calories was adjusted with a view to enable each volunteer to maintain constant body weight during the experiment.

Preparation and administration of the diets were undertaken by the Diet Kitchen of the Medical Department A of the University Hospital.

Examples of the diets used are shown in table 1 A. The volunteers were allowed to drink water, tea and coffee without sugar and cream. Other food items than those shown in the table were not introduced in any of the diets.

It was not considered necessary to begin the experiments with a period in which the subjects received a fixed diet without eggs, because continuous intake of 5 to 10 eggs per day is quite unusual and furnishes a much larger amount of cholesterol than does any commonly used diet. Further, previous experiments (2) had failed to show any significant change of the data to be determined in the present study as a consequence of replacing butter with a high-linoleic acid margarine.

Immediately before, and 3 and 6 weeks after beginning of the egg regimen, samples of duodenal bile were collected fasting after intravenous injection of cholecystokinin. Those

fractions of the bile samples having a pH higher than 6,9 were analyzed for bile acids, cholesterol and lipid-soluble phosphorus.

The methods for collection and analysis of the bile samples were as described in our previous studies (2, 3).

Simultaneously with the collection of bile, blood samples were taken for determination of serum total cholesterol.

Collection of bile was performed in the Gastro-enterological Laboratory, determination of serum cholesterol in the Central Laboratory of the University Hospital. Analysis of bile was undertaken in the Department of Biochemistry and Nutrition of the Polytechnic Institute to which the bile samples were speedily transferred.

The eggs used were chicken eggs of the Danish type „C", weighing from 55 to 60 g with shell. Their content of cholesterol was found to be 206 mg per egg. Estimates of the volunteers' daily intake of egg-cholesterol were based on this figure.

The cholesterol content was determined by hard-boiling of 6 eggs, cooling and division of each yolk in quarters by weight. One quarter from each yolk were combined and treated with 100 ml 60% KOH (w/v) on boiling water bath for 3 hours. The non-saponifiable fraction was shaken out quantitatively with ether. After washing with alkaline water and distilled water to neutrality, the ethereal solution was dried with anhydrous sodium sulfate, evaporated to dryness and dissolved in chloroform. The LIEBERMANN-BURCHARD reaction was performed on an aliquot of the solution as described previously (4) whereafter the absorbancy at 625 nm was measured and compared with the absorbancy obtained with a standard of pure cholesterol. Earlier one of us (5) had found a content of 239,5 mg total cholesterol per egg for chicken eggs of the size used in the present experiment. The commonly used food tables by SOUCI, FACHMANN & KRAUT (6) indicate 238 mg cholesterol for eggs of the size in question.

Results and Discussion

The results are shown in tables 1–12.[1)]

Table 1 shows the amounts of egg cholesterol consumed and the variation of serum cholesterol during the egg regimen.

Table 2 shows pH, per cent Dry Matter, and the millimolarities of Cholesterol (C), Lipid-soluble Phosphorus (P), and Total Bile Acids (TBA) in the bile samples before and after 3 and 6 weeks of egg diet. TBA is taken as the sum of Glycocholic Acid (GC), Glycochenodeoxycholic Acid (GCD), Glycodeoxycholic Acid (GD), Taurocholic Acid (TC), and Taurochenodeoxycholic plus Taurodeoxycholic Acids (TCD + TD). (If it had been possible to determine other bile acids that are present in small amounts, the value for TBA would have been a little higher).

Tables 3–7 show the molar percentages of GC, GCD, GD, TC and TCD + TD in TBA.

Tables 8 and 9 show the molar ratios between Glycine-conjugation and Taurine-conjugation (G/T), and between Dihydroxycholanoic Acids and Trihydroxycholanoic Acids (Di/Tri), respectively.

Tables 10–12 show the other ratios to be considered.

From table 1 it is obvious that the egg diet produced a more or less pronounced increase of serum total cholesterol, apparently without marked relation to the quantity of cholesterol consumed per kg body weight. The mean value of serum cholesterol was higher after 6 weeks than after 3 weeks. For one of the volunteers (LVM), whose

[1)] In tables 1 and 3–12, figures in parenthesis indicate per cent of the values before the egg diet.

body weight declined during the experiment, serum cholesterol remained practically constant.

The mean value of the molar precentage of GC in TBA is almost exactly the same after 3 and 6 weeks of egg diet as before the diet (table 3).

The mean values of the molar percentage of GCD and TCD + TD in TBA are also nearly the same after 3 and 6 weeks of diet as before the diet (tables 4 and 7).

The mean value of the molar percentage of GD in TBA seems to increase somewhat during the dietary reginem (table 5), and the mean value of the molar percentage of TC in TBA seems to decrease from the beginning to 3 weeks of diet and to remain at the same level after 6 weeks as after 3 weeks (table 6). Increase of GD and decrease of TC would mean a change in the direction of increased solubility of cholesterol in the bile, since among the six bile acids in question, GD is the most efficient, and TC the least efficient solubilizer for cholesterol in aqueous solution. (studies to be published later). It is, however, difficult to determine, whether the moderate increase of GD and the moderate decrease of TC represent significant changes, because, in the present experiment there is only one bile sample for each volunteer before the diet.

As far as the molar ratios G/T (table 8) and Di/Tri (table 9) are concerned, there is no obvious variation of the mean values during the dietary reginem.

The molar ratio TBA/C (table 10) was increased in 6 out of the 9 cases after 3 weeks and in 7 cases after 6 weeks on egg diet. The mean value of this ratio was slightly increased after 3 weeks and more after 6 weeks.

The molar ratio P/C (table 11) was increased in 7 out of the nine cases after 3 weeks and in 8 cases after 6 weeks. The mean value of P/C was slightly increased after 3 weeks and slightly higher after 6 weeks than after 3 weeks.

The mean value of the molar ratio TBA/P (table 12) did not show any consistent change (decrease after 3 weeks, increase after 6 weeks).

The mean value after 6 weeks was completely normal (compare reference 3).

All in all, the results of the present study do not give support to the assumption that liberal supply of cholesterol in the form of eggs changes the composition of the bile in a direction which is unfavorable to maintaining cholesterol in solution. The predominant, although moderate changes found, rather point in the opposite direction.

In the absence of direct observation on the effect of dietary cholesterol on gallstone formation in man it may be of some interest to mention the results of certain studies on the influence of ingested cholesterol on gallstone formation in experimental animals.

In young hamsters, addition of 1% cholesterol to a gallstone inducing diet has been found to decrease the incidence of cholesterol gallstones in both sexes and to increase the incidence of amorphous pigmented gallstones among the females (7).

In mice, cholesterol feeding has been found to produce cholesterol gallstones, only when cholic acid was also ingested (8).

According to an earlier less detailed report (9) addition of 1% cholesterol to a diet for guinea pigs caused production of gallstones „rich in calcium phosphate although they contained some cholesterol".

Thus, in three mammalian species, ingestion of cholesterol not accompanied by cholic acid did not produce formation of gallstones in which cholesterol is the major component, but in hamsters and guinea pigs it favored formation of gallstones of other types.

Table 1 A. Examples of diets with 6 eggs per day and different amounts of total calories.

	g	Cal.	Protein g	Fat g	Carbo-hydrate g	g	Cal.	Protein g	Fat g	Carbo-hydrate g
Butter	47,9	359,2	0,15	39,77	0,21	54,8	411,6	0,18	45,57	0,23
Rye bread, dark	78,0	186,7	4,99	0,78	40,00	85,8	205,4	5,50	0,86	43,93
Rice flour, white	30,0	105,3	2,16	0,20	23,70	33,0	115,8	2,38	0,21	26,10
Skimmed milk	150,0	50,8	5,25	0,11	7,20	165,0	55,8	5,78	0,12	7,92
Lean meat (protein 20%, fat 8%)	117,5	178,6	23,50	9,40	—	148,8	226,1	29,75	11,90	—
Lean fish (plaice)	37,7	28,5	6,45	0,30	—	41,5	31,4	7,09	0,33	—
Wheat bread, white	100,0	261,0	8,50	2,00	52,30	110,0	287,3	9,35	2,20	57,53
Potatoes	100,0	85,0	2,00	0,15	18,90	110,0	93,5	2,20	0,17	20,79
Apples	100,0	52,3	0,30	0,30	12,10	110,0	57,5	0,33	0,33	13,31
Tomatoes	50,0	9,4	0,48	0,11	1,64	55,0	10,4	0,52	0,12	1,80
Carrots	100,0	34,9	1,00	0,20	7,27	110,0	38,4	1,10	0,22	8,00
Bananas	50,0	45,1	0,55	0,10	10,50	55,0	49,6	0,61	0,11	11,55
6 eggs	—	460,6	39,00	33,84	—	—	460,6	39,00	33,84	—
Total g.			94,33	87,26	173,82			103,79	95,98	191,16
Total cal.		1857,4	377,32	785,34	695,28		2034,4	415,16	863,82	764,64

Each volunteer subject received per day the following vitamin supplement:

1 „B-combin"-tablet containing: thiamine chloride, 5 mg; riboflavin, 2,5 mg; nicotinamide, 24 mg; pyridoxin hydrocholride, 2,5 mg; calcium pantothenate, 5 mg. 1 „Adetamin"-pill containing 3000 IU vitamin A and 600 IU vitamin D. 1 Ascorbic acid tablet containing 50 mg ascorbic acid.

Table 1. Amount of Egg-Cholesterol consumed and variation of Serum Cholesterol

Initials	Volunteer's Sex	Age years	Body weight kg	Number of eggs per day	Egg-Cholesterol consumed per person per day mg	per kg body weight per day mg	Serum Cholesterol[1] Before egg diet mg%	3 weeks on egg diet mg%	6 weeks on egg diet mg%
LT	f	22	49,8	5	1030	20.7	161	219 (136)	262 (163)
TH	f	19	66,7	5	1030	15,5	159	193 (121)	227 (143)
IM	f	20	51.6	6	1235	23.9	187	209 (112)	256 (137)
HH	f	21	59.6	6	1235	20.7	308	303 (98)	343 (111)
NM	f	20	55.6	10	2060	37,0	257	349 (136)	334 (130)
LVM[2]	f	20	67.7	10	2060	30.4	244	246 (101)	252 (103)
RE	f	21	61.4	10	2060	33.6	163	244 (150)	256 (157)
HP	f	20	64.2	10	2060	32.0	219	269 (123)	232 (106)
LRH	f	20	65.1	10	2060	31.6	184	189 (103)	205 (111)
Mean values for the whole group:							209	247	263

[1]) mg% = mg per 100 ml; figures in parenthesis indicate per cent of value before egg diet.
[2]) The body weight of volunteer LVM declined 2 kg during the experiment.

Table 2. pH, per cent of Dry Matter, and millimolarities of Cholesterol (C), Lipid-soluble Phosphorus (P), and Total Bile Acids (TBA) in the bile samples before and during the egg regimen.

Volunteer	Sample	pH	Dry matter % (w:v)	C mM	P mM	TBA mM
LT	Before diet	6,9	4,2	1,9	5,9	28,2
LT	3 weeks on diet	7,3	9,4	7,7	21,5	78,1
LT	6 weeks on diet	7,8	9,5	5,3	17,7	69,0
TH	Before diet	7,1	5,6	4,1	10,7	42,8
TH	3 weeks on diet	7,5	5,3	3,1	8,8	38,0
TH	6 weeks on diet	7,1	6,5	3,3	16,3	41,4
IM	Before diet	7,9	3,9	2,2	5,0	24,8
IM	3 weeks on diet	7,6	3,7	2,7	7,3	27,0
IM	6 weeks on diet	7,5	3,1	1,6	4,3	26,6
HH	Before diet	7,1	2,3	2,2	4,5	11,9
HH	3 weeks on diet	7,3	5,1	4,2	10,0	27,9
HH	6 weeks on diet	7,7	3,1	2,2	8,5	24,8
NM	Before diet	7,5	4,7	3,0	9,2	38,2
NM	3 weeks on diet	7,8	7,3	2,6	12,1	35,0
NM	6 weeks on diet	7,5	5,8	2,2	8,5	37,0
LVM	Before diet	8,0	10,5	8,6	19,9	74,5
LVM	3 weeks on diet	7,4	5,1	3,3	9,7	40,1
LVM	6 weeks on diet	7,6	6,7	6,5	13,6	51,0
RE	Before diet	7,5	5,2	4,0	8,4	27,8
RE	3 weeks on diet	7,2	5,1	3,0	7,5	28,6
RE	6 weeks on diet	7,9	5,1	2,8	7,1	25,3
HP	Before diet	7,4	4,4	3,4	10,3	34,5
HP	3 weeks on diet	7,3	6,2	4,9	14,6	37,6
HP	6 weeks on diet	7,0	5,2	3,0	10,5	48,0
LRH	Before diet	7,6	5,4	4,7	11,6	20,1
LRH	3 weeks on diet	6,9	7,0	5,4	15,1	35,2
LRH	6 weeks on diet	7,3	4,8	4,4	11,5	29,3

Table 3. Mol per cent Glycocholic Acid in Total Bile Acids (100 GC/TBA)

Volunteer	Before diet	3 weeks on diet	6 weeks on diet
LT	13,2	16,8 (128)	15,5 (117)
TH	30,7	27,9 (91)	23,5 (77)
IM	12,5	15,5 (124)	20,8 (166)
HH	15,0	25,9 (173)	14,9 (99)
NM	34,6	31,6 (91)	19,7 (57)
LVM	35,1	29,8 (85)	43,7 (124)
RE	20,7	11,1 (54)	23,5 (113)
HP	28,2	21,8 (77)	12,7 (45)
LRH	12,5	25,7 (206)	26,9 (215)
Mean value	22,5	22,9	22,4

Table 4. Mol per cent Glycochenodeoxycholic Acid in Total Bile Acids (100 GCD/TBA)

Volunteer	Before diet	3 weeks on diet	6 weeks on diet
LT	17,6	16,5 (94)	22,5 (128)
TH	24,1	20,0 (83)	22,9 (95)
IM	13,7	14,3 (104)	15,0 (110)
HH	18,7	18,2 (97)	24,1 (129)
NM	20,0	14,4 (72)	13,7 (69)
LVM	18,4	30,9 (168)	6,3 (34)
RE	36,6	17,6 (48)	19,9 (54)
HP	4,3	23,2 (540)	18,3 (426)
LRH	20,6	13,2 (64)	23,7 (115)
Mean value	19,3	18,7	18,5

Table 5. Mol per cent Glycodeoxycholic Acid in Total Bile Acids (100 GD/TBA)

Volunteer	Before diet	3 weeks on diet	6 weeks on diet
LT	21,4	19,8 (92)	16,8 (79)
TH	15,1	18,2 (120)	28,9 (191)
IM	2,3	6,6 (287)	5,9 (256)
HH	12,6	11,2 (89)	21,1 (168)
NM	9,7	7,2 (74)	13,1 (135)
LVM	3,6	7,6 (211)	10,6 (294)
RE	18,2	36,7 (202)	27,9 (153)
HP	19,7	14,3 (73)	9,6 (49)
LRH	16,5	21,5 (130)	16,4 (100)
Mean value	13,2	15,9	16,7

Table 6. Mol per cent Taurocholic Acid in Total Bile Acids (100 TC/TBA)

Volunteer	Before diet	3 weeks on diet	6 weeks on diet
LT	14,1	15,3 (108)	12,1 (86)
TH	10,5	12,8 (122)	9,9 (94)
IM	37,6	26,8 (71)	30,9 (82)
HH	20,9	17,2 (82)	9,1 (43)
NM	14,4	11,5 (80)	13,8 (96)
LVM	21,7	11,8 (54)	14,2 (65)
RE	11,0	7,1 (64)	6,0 (55)
HP	21,7	15,0 (69)	22,0 (101)
LRH	19,4	12,3 (63)	11,8 (61)
Mean value	19,0	14,4	14,4

Table 7. Mol per cent Taurochenodeoxycholic Acid + Taurodeoxycholic Acid in Total Bile Acids (100 (TCD + TD/TBA))

Volunteer	Before diet	3 weeks on diet	6 weeks on diet
LT	33,7	31,6 (94)	33,1 (98)
TH	19,7	21,1 (107)	14,9 (76)
IM	33,9	36,8 (108)	27,3 (80)
HH	32,9	27,5 (84)	30,8 (93)
NM	21,3	35,3 (166)	39,8 (186)
LVM	21,2	19,9 (93)	25,3 (119)
RE	13,4	27,6 (206)	22,7 (170)
HP	26,2	25,7 (98)	37,4 (143)
LRH	31,2	27,3 (87)	21,2 (68)
Mean value	25,9	28,1	28,1

Table 8. Molar ratio Glycine-conjugation / Taurine-conjugation (Ratio G/T)

Volunteer	Before diet	3 weeks on diet	6 weeks on diet
LT	1,10	1,13 (103)	1,22 (111)
TH	2,31	1,95 (85)	3,04 (132)
IM	0,40	0,57 (142)	0,72 (180)
HH	0,86	1,24 (144)	1,51 (176)
NM	1,82	1,14 (63)	0,87 (48)
LVM	1,33	2,16 (163)	1,53 (115)
RE	3,09	1,88 (61)	2,49 (81)
HP	1,09	1,46 (134)	0,68 (62)
LRH	0,98	1,52 (155)	2,03 (207)
Mean value	1,44	1,45	1,57

Table 9. Molar ratio Dihydroxycholanoic acids / Trihydroxycholanoic acids (Ratio Di/Tri)

Volunteer	Before diet	3 weeks on diet	6 weeks on diet
LT	2,67	2,12 (80)	2,63 (99)
TH	1,43	1,46 (102)	2,00 (140)
IM	1,00	1,36 (136)	0,93 (93)
HH	1,79	1,20 (67)	1,19 (67)
NM	1,04	1,51 (145)	1,99 (191)
LVM	0,76	1,40 (184)	0,73 (96)
RE	2,15	4,52 (210)	2,40 (112)
HP	1,73	1,72 (170)	1,88 (109)
LRH	2,13	1,63 (77)	1,59 (75)
Mean value	1,63	1,88	1,70

Table 10. Molar ratio Total Bile Acids / Cholesterol (Ratio TBA/C)

Volunteer	Before diet	3 weeks on diet	6 weeks on diet
LT	14,83	10,15 (69)	13,06 (88)
TH	10,46	12,12 (116)	12,41 (119)
IM	11,51	10,13 (88)	16,33 (142)
HH	5,52	6,62 (120)	11,25 (204)
NM	12,62	13,69 (108)	16,81 (133)
LVM	8,68	12,13 (140)	7,87 (91)
RE	6,96	9,63 (138)	9,08 (130)
HP	10,20	7,68 (75)	15,82 (155)
LRH	4,29	6,49 (151)	6,66 (155)
Mean value	9,45	9,85	12,14

Table 11. Molar ratio Lipid-soluble Phosphorus / Cholesterol (Ratio P/C)

Volunteer	Before diet	3 weeks on diet	6 weeks on diet
LT	3,09	2,80 (91)	3,35 (108)
TH	2,61	2,79 (107)	4,88 (187)
IM	2,33	2,75 (118)	2,66 (114)
HH	2,10	2,37 (113)	2,68 (128)
NM	3,03	4,74 (156)	3,87 (128)
LVM	2,31	2,93 (127)	2,11 (91)
RE	2,10	2,54 (121)	2,55 (121)
HP	3,04	2,98 (98)	3,45 (113)
LRH	2,48	2,79 (113)	2,60 (105)
Mean value	2,57	2,97	3,12

Table 12. Molar ratio Total Bile Acids / Lipid-soluble Phosphorus (Ratio TBA/P)

Volunteer	Before diet	3 weeks on diet	6 weeks on diet
LT	4,79	3,63 (76)	3,90 (82)
TH	4,01	4,33 (108)	2,54 (63)
IM	4,95	3,69 (75)	6,13 (124)
HH	2,63	2,79 (106)	4,20 (160)
NM	4,15	2,89 (70)	4,34 (105)
LVM	3,76	4,14 (110)	3,74 (100)
RE	3,31	3,79 (115)	3,56 (108)
HP	3,36	2,58 (77)	4,59 (136)
LRH	1,73	2,33 (136)	2,56 (148)
Mean value	3,64	3,35	3,95

Summary

Nine healthy young volunteers received diets furnishing from 5 to 10 eggs (1.03–2.06g egg cholesterol) per day through two consecutive periods of 3 weeks. The amounts of proteins and fat per 100 calories were the same in all the diets.

Immediately before, and 3 and 6 weeks after beginning of the egg regimen, samples of duodenal bile were collected, after injection of cholecystokinin, and analyzed with respect to pH, dry matter, cholesterol, lipid-soluble phosphorus, glycocholic acid, glycochenodeoxycholic acid, glycodeoxycholid acid, taurocholic acid and taurochenodeoxycholic plus taurodeoxycholic acids. Simultaneously with the collection of bile, blood samples were taken for determination of serum total cholesterol. The main results were as follows:

Serum total cholesterol increased during the egg regimen.

In the bile no great changes occurred in the distribution of the different bile acids in the total bile acids. The predominant change of the ratios between total bile acids and cholesterol and between lipid-soluble phosphorus and cholesterol was a slight to moderate increase.

Thus, the results did not provide evidence for a lessening of the solubility of cholesterol in the bile in spite of the fact that serum cholesterol increased.

Zusammenfassung

Neun junge gesunde Versuchspersonen erhielten während zwei aufeinander folgenden Perioden von je 3 Wochen Diäten, welche 5 bis 10 Eier (1.03–2.06 g Ei-Cholesterin) pro Tag zuführten. Die Mengen von Protein und Fett pro 100 Calorien waren in allen Diäten gleichbleibend.

Unmittelbar vor und 3 und 6 Wochen nach dem Beginn der Ei-Diät wurde Duodenal-Galle, nach Injektion von Cholecystokinin, gesammelt. Die Gallen wurden auf pH, Trockensubstanz, Lipid-Phosphor, Glycocholsäure, Glycochenodesoxycholsäure, Glycodesoxycholsäure, Taurocholsäure und Taurochenodesoxycholsäure plus Taurodesoxycholsäure analysiert. Gleichzeitig mit der Aufnahme von Duodenal-Galle wurden Blutproben für Bestimmung von Gesamt-Cholesterin im Serum entnommen.

Die Hauptergebnisse waren:

Mehr oder weniger ausgeprägte Erhöhung des Gesamt-Cholesterins im Serum.

In der Galle wurde keine erhebliche Veränderung in der Verteilung der einzelnen Gallensäuren in den Gesamt-Gallensäuren festgestellt. Die vorherrschende Veränderung der Verhältnisse zwischen Gesamt-Gallensäuren und Cholesterin, und zwischen Lipid-Phosphor und Cholesterin war eine geringe bis mässige Erhöhung.

Die Versuche deuten somit nicht darauf, daß die Einnahme von Eiern eine Minderung der Löslichkeit des Cholesterins in der Galle herbeigeführt hat.

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