Department of Biochemistry and Nutrition
Polytechnic Institute, Copenhagen (Denmark)

Alimentary production of gallstones in hamsters

29. Influence of dietary cholesterol and squalene on the composition of bladder bile

I. Prange, E. Søndergaard and H. Dam

With 1 figure and 3 tables

(Received July 22, 1974)

In previous studies we have shown that dietary cholesterol (1, 2) and dietary squalene (2) counteract formation of cholesterol containing gallstones in young hamsters. In our study (2) it was found that almost complete protection against formation of cholesterol containing gallstones could be obtained when either cholesterol or squalene was added at the level of 1 percent to a gallstone producing diet containing 20 % casein, 67.3 % glucose, 7 % palmkernel oil plus vitamins and salts and a non-absorbable antioxidant.

The present study deals with the influence of dietary cholesterol and squalene on the composition of the bladder bile of hamsters with respect to cholesterol, phospholipid and bile salts (bile acids).

Experimental

The hamsters used in the present study were those belonging to Experimental Series G 160 in our previous study (2) where other details of the experiment are described. The series consisted of three groups, each comprising 20 male and 20 female hamsters. The hamsters in the first group (1414) received the gallstone producing diet without addition, those in the second group (1415) received the diet with addition of 1 % cholesterol, whereas those in the third group (1416) received the diet with addition of 1 % squalene. The animals were killed after having received the diet for a period of 42–44 days. Sampling of bile and methods for determination of cholesterol, phospholipid and bile acids in the bile were as described in our paper (3) with the exception that the separation of cholesterol was carried out by thin layer chromatography instead of paper chromatography (silicagel G, moving phase: petroleum ether/diethyl ether/glacial acetic acid (70/30/1).

In most cases it was necessary to pool bile from 2 or 3 animals in order to obtain enough for the analyses. It was not possible to obtain bile from all animals in each group.

Results and discussion

The results are presented in tables 1, 2 and 3, and in fig. 1.

From the tables it can be seen that both the diet with 1 % cholesterol and the diet with 1 % squalene have produced increase of the mean value of the ratio of Total Bile Acids to Cholesterol (TBA/C) and of the mean value of the ratio of Phospholipid to Cholesterol (P/C) with the exception that the mean value of the ratio P/C for the males in the cholesterol fed group changed from 3.9 to 3.7.

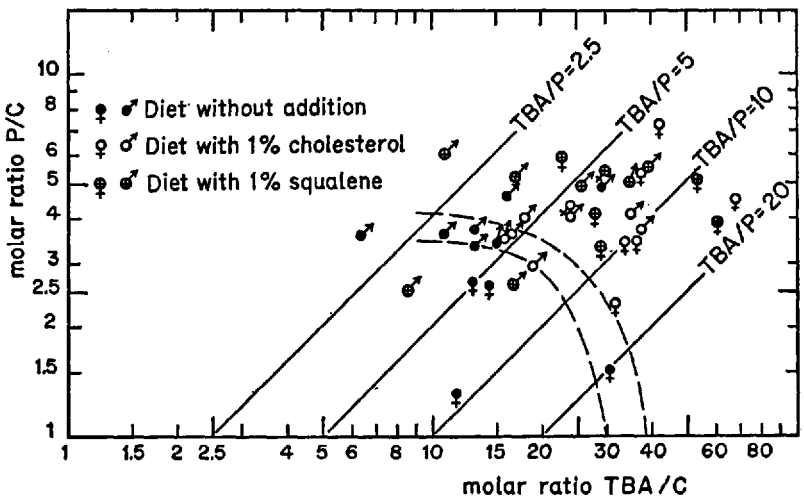

Fig. 1. Abscissa: molar ratios of Total Bile Acids to Cholesterol (TBA/C). Ordinate: molar ratios of Phospholipid to Cholesterol (P/C). The curved lines indicate limits for solubility of cholesterol in aqueous solutions of bile salts and lecithin. The inner curved line is based on the data from *Hegardt* and *Dam* (ref. 4), and *Holzbach* et al. (ref. 5). The outer curved line is based on data from *Mufson* et al. (ref. 6 and 7). The four oblique lines indicate values of the molar ratio of Total Bile Acids to Phospholipid = 2.5; 5; 10; and 20 resp.

The single values of these ratios are plotted in fig. 1 which also shows the limits for solubility of cholesterol in aqueous solutions of bile salts and lecithin based on data from *Hegardt* and *Dam* (4) and *Holzbach* et al. (5) (the inner curve), and on data from *Mufson* et al. (6, 7) (the outer curve). The sources of error in the determination of the solubility pointed out by *Mufson* et al. make it seem likely that the curve based on their data is to be preferred. Points to the right and above the curve represent unsaturation; points to the left and below the curve represent supersaturation

Comparison with the outer curve shows that for the diet given without addition, 9 out of the 11 points represent saturation or more or less pronounced supersaturation, and only 2 of the points represent unsaturation, whereas for the diet given with addition of 1 % cholesterol, 11 out of the 14 points represent unsaturation, and for the diet given with addition of 1 % squalene, 11 out of the 13 points represent unsaturation.

These observations are in accordance with the fact that occurrence of cholesterol containing gallstones is frequent in the group of hamsters receiving the diet without addition, and rare in the groups receiving the diet with either 1 % cholesterol or 1 % squalene.

The values of the ratio of Total Bile Acids to Phospholipid (TBA/P) show a much greater scattering than did the values of this ratio in our earlier experiments (3, 8) which were carried out with hamsters from our own breeding colony. This circumstance may be due to the origin and preexperimental feeding of the hamsters used in the present study, and, possibly, also to the particular basal diet used.

Table 1. Data for bladder bile from hamsters receiving the diet without addition (Group No. 1414)

Hamster No.	Type of gall-stones	Days on diet	Millimolar concentrations of			Mol per cent in TBA of						Molar ratios of			
			C	P	TBA	GC	GCD	GD	TC	TCD +TD	G / T	Di / Tri	TBA / C	P / C	TBA / P
Males															
1	0														
2	0	42	3.1	14.8	89.5	10.9	40.1	25.8	16.5	6.6	3.3	2.6	28.9	4.8	6.0
4	C														
5	C	42	6.7	23.8	72.6	6.5	11.4	39.7	4.0	38.4	1.4	8.6	10.8	3.6	3.1
8	0														
9	C	42	5.3	17.4	70.1	11.4	22.7	20.2	35.5	10.1	1.2	1.1	13.2	3.3	4.0
10	0														
11	0														
12	0	42	4.9	16.7	73.8	19.9	26.7	2.4	12.6	38.3	1.0	2.1	15.1	3.4	4.4
14	0														
15	0	43	5.5	20.2	73.2	15.2	39.5	22.0	3.4	19.1	3.3	4.4	13.3	3.7	3.6
17	C														
18	C	43	4.6	16.4	29.4	41.2	45.2	4.8	3.7	5.1	10.3	1.2	6.4	3.6	1.8
19	C														
20	C	43	3.9	18.0	62.0	17.3	40.8	1.0	14.2	26.8	1.4	2.7	15.9	4.6	3.4
38	0														
Mean values for all 7 samples:			4.9	18.2	67.2	17.5	32.3	16.6	12.8	20.6	3.1	3.2	14.8	3.9	3.8
with st. d.			0.4	1.1	7.7	4.3	4.7	5.4	4.3	5.4	1.3	1.0	2.6	0.2	0.5

Females														
66 67	0 C	42	2.9	3.7	33.5	21.5	24.2	16.1	0	38.2	1.6	3.7	11.6	1.3	9.7
68 70	C 0	43	2.5	6.7	32.4	24.7	41.4	11.7	0	22.2	3.5	3.1	13.0	2.7	4.8
69 73	0 C	43	2.1	5.4	30.1	5.6	30.6	33.6	3.7	26.6	2.3	9.8	14.3	2.6	5.6
74 76	0 C	43	1.7	2.6	51.6	20.3	47.3	16.1	10.5	5.8	5.1	2.3	30.4	1.5	19.8
Mean values for all 4 samples:			2.3	4.6	36.9	18.0	35.9	19.4	3.6	23.2	3.1	4.7	17.3	2.0	10.0
with st. d.			0.3	0.9	5.0	4.2	5.2	4.9	2.5	6.7	0.8	1.7	4.4	0.4	3.4

Abbreviations used:

For type of gallstones: C = Cholesterol gallstones; A = Amorphous gallstones; 0 = No gallstones.

For components of bladder bile: C = Cholesterol; P = Phospholipid; TBA = Total Bile Acids, defined as the molar sum of Glycocholic Acid (GC), Glycochenodeoxycholic Acid (GCD), Glycodeoxycholic Acid (GD), Taurocholic Acid (TC), and Taurochenodeoxycholic + Taurodeoxycholic Acid (TCD + TD); $\frac{G}{T}$ = Glycine-conjugation/Taurine-conjugation; $\frac{Di}{Tri}$ = Dihydroxycholanoic Acids/Trihydroxycholanoic Acids; $\frac{TBA}{C}$ = Total Bile Acids/Cholesterol; $\frac{P}{C}$ = Phospholipid/Cholesterol; $\frac{TBA}{P}$ = Total Bile Acids/Phospholipid.

Table 2. Data for bladder bile from hamsters receiving the diet with 1% cholesterol (Group No. 1415)

Hamster No.	Type of gall-stones	Days on diet	Millimolar concentrations of			Mol per cent in TBA of					Molar ratios of				
			C	P	TBA	GC	GCD	GD	TC	TCD +TD	$\frac{G}{T}$	$\frac{Di}{Tri}$	$\frac{TBA}{C}$	$\frac{P}{C}$	$\frac{TBA}{P}$
Males															
16	0														
21	A	42	2.3	9.5	81.9	22.1	28.3	10.9	13.4	25.3	10.2	1.8	35.6	4.1	8.6
23	0														
24	A														
25	0	42	2.4	8.7	39.4	24.9	27.2	11.9	13.2	22.8	1.8	1.6	16.4	3.6	4.5
26	0														
27	0														
28	0	42	3.3	12.1	123.4	22.3	22.9	17.7	10.6	26.4	1.7	2.0	37.4	3.7	10.2
29	0														
30	0														
31	0	42	2.4	9.7	43.5	9.0	33.6	17.0	20.0	20.5	1.5	2.5	18.1	4.0	4.5
34	0														
35	A														
36	0	43	3.4	13.7	82.2	17.1	52.8	3.2	9.0	17.9	2.7	2.8	24.2	4.0	6.0
37	0														
40	0	43	5.5	16.0	103.6	22.3	19.2	6.3	10.9	41.3	0.9	2.0	18.8	2.9	6.5
54	A	43	4.6	16.2	72.3	12.4	42.2	11.9	12.6	20.9	2.0	3.0	15.7	3.5	4.5
Mean values for all 7 samples:			3.4	12.3	78.0	18.6	32.3	11.3	12.8	25.0	3.0	2.2	23.7	3.7	6.4
with st. d.			0.5	1.2	7.5	2.2	4.4	2.0	1.3	2.9	1.2	0.2	3.5	0.2	0.8

Females														
71	0	42	1.1	2.5	35.4	27.1	7.9	31.3	23.2	10.4	2.0	1.0	32.1	2.3	14.1
75	0														
80	0	42	1.4	4.8	48.5	4.3	28.6	26.6	6.0	34.4	1.5	8.7	34.6	3.4	10.1
81	A														
82	A	42	0.6	4.3	25.5	21.6	22.0	6.7	10.6	39.2	1.0	2.1	42.5	7.2	5.9
83	0														
84	A	43	2.3	9.9	55.6	15.6	50.9	4.0	6.3	22.7	2.4	3.5	24.2	4.3	5.6
86	0														
87	A	44	2.3	7.9	83.7	21.0	36.3	8.6	14.3	19.7	1.9	1.8	36.4	3.4	10.5
88	0														
89	0	44	1.1	5.0	74.0	13.8	41.4	25.4	10.3	9.2	4.1	3.2	68.0	4.5	14.8
91	A														
95	A														
96	A	44	1.1	5.8	41.3	15.3	63.2	2.4	0	19.1	4.2	5.6	37.5	5.3	7.1
Mean values for all 7 samples:			1.4	5.7	52.0	17.0	35.8	15.0	10.1	22.1	2.4	3.7	39.3	4.3	9.7
with st. d.			±0.2	±0.9	±7.9	±2.7	±7.0	±4.6	±2.8	±4.3	±0.5	±1.0	±5.2	±0.6	±1.4

Abbreviations as in Table 1.

Table 3. Data for bladder bile from hamsters receiving the diet with 1% squalene (Group No. 1416)

Hamster No.	Type of gall-stones	Days on diet	Millimolar concentrations of			Mol per cent in TBA of					Molar ratios of				
			C	P	TBA	GC	GCD	GD	TC	TCD +TD	$\frac{G}{T}$	$\frac{Di}{Tri}$	$\frac{TBA}{C}$	$\frac{P}{C}$	$\frac{TBA}{P}$
Males															
32	0	42	2.5	12.3	64.6	5.0	22.1	11.0	2.5	59.4	0.6	12.5	25.8	4.9	5.3
33	0														
41	0	42	5.2	13.7	86.6	17.1	31.3	6.7	9.9	35.0	1.2	2.7	16.7	2.6	6.3
42	0														
43	0	42	8.5	21.4	73.8	15.1	26.4	5.4	12.7	40.5	0.9	2.6	8.6	2.5	3.4
44	0														
47	0														
49	0	43	1.7	9.3	65.3	23.0	34.0	12.9	10.7	19.4	2.3	2.0	38.4	5.5	7.0
51	0														
53	0	43	3.5	21.0	38.1	17.8	3.4	44.3	3.1	31.2	1.9	3.8	10.9	6.0	1.8
55	0														
56	0	43	3.2	16.4	54.0	23.7	41.5	5.2	12.6	17.0	2.4	1.8	16.9	5.1	3.3
57	0														
58	0	43	2.5	12.6	87.3	28.4	14.7	16.5	7.7	33.2	1.5	1.8	34.9	5.0	6.9
59	0														
Mean values for all 7 samples:			3.9	15.2	67.1	18.6	24.8	14.6	8.5	33.7	1.5	3.9	21.7	4.5	4.9
with st. d.			0.9	1.7	6.5	2.9	4.8	5.2	1.6	5.3	0.3	1.5	4.4	0.5	0.8

Females														
103 105	0 0	42	1.1	6.5	25.5	12.5	58.0	6.7	6.7	16.1	3.4	4.2	23.2	5.9	3.9
106 107	0 0	42	0.9	3.5	55.3	17.4	50.3	17.2	11.8	3.4	2.1	2.4	61.4	3.9	15.8
108 109	0 0	42	3.6	11.8	104.7	20.2	41.5	8.5	10.9	18.8	2.4	2.2	29.1	3.3	8.9
110 112	0 0	42	1.9	7.7	53.3	17.8	58.9	3.4	16.1	3.7	4.0	1.9	28.1	4.1	6.9
113 114	0 0	42	1.9	9.7	102.1	24.7	19.7	28.6	6.8	20.3	2.7	2.2	53.7	5.1	10.5
115 116	0 0	42	1.7	9.1	50.8	34.6	36.4	1.4	17.1	10.4	2.6	0.9	29.8	5.4	5.6
Mean values for all 6 samples:			1.9	8.1	65.3	21.2	44.1	11.0	11.6	12.1	2.9	2.3	37.6	4.6	8.6
with st. d.			0.4	1.2	8.0	3.1	6.1	4.2	1.8	3.0	0.3	0.4	6.5	0.4	1.7

Abbreviations as in Table 1.

Summary

Samples of bladder bile from hamsters on three different diets were analyzed for cholesterol, phospholipid and bile acids.

The diets were:

Diet 1: a gallstone-producing diet containing 20 % casein, 67.3 % glucose, 7 % palmkernel oil, plus vitamins, salts and 0.05 % of a non-absorbable antioxidant.

Diet 2: diet 1 with addition of 1 % cholesterol.

Diet 3: diet 1 with addition of 1 % squalene.

Graphical plotting of the ratios TBA/C and P/C for each of the samples analyzed, together with the most recently established limit for solubility of cholesterol in aqueous solutions of bile salts and lecithin shows that for diet 1, only two out of eleven samples represent unsaturation, whereas for diet 2, eleven out of fourteen samples, and for diet 3, eleven out of thirteen samples represent unsaturation.

These findings are in accordance with the fact that occurrence of cholesterol containing gallstones is frequent with diet 1, and rare with diets 2 and 3.

Zusammenfassung

Proben von Blasengalle von Hamstern, die mit drei verschiedenen künstlichen Nahrungen gefüttert waren, wurden in bezug auf Cholesterin, Phospholipid und Gallensäuren analysiert.

Die Nahrungen waren:

Nahrung 1: Eine Gallensteine hervorrufende Nahrung, die aus 20 % Casein, 67,3 % Glucose, 7 % Palmenkernöl, Vitaminen, Salzen und 0,05 % eines nichtresorbierbaren Antioxydans bestand.

Nahrung 2: Nahrung 1 mit Zugabe von 1 % Cholesterin.

Nahrung 3: Nahrung 1 mit Zugabe von 1 % Squalen.

Graphische Abbildung der Verhältnisse TBA/C und P/C für die einzelnen analysierten Proben zusammen mit der spätestens ermittelten Löslichkeitsgrenze für Cholesterin in wässerigen Lösungen von Gallensalzen und Lezithin zeigte, daß für Nahrung 1 nur zwei von insgesamt elf Proben ungesättigt in bezug auf Cholesterin waren, während für Nahrung 2 elf von insgesamt vierzehn Proben und für Nahrung 3 elf von insgesamt dreizehn Proben ungesättigt waren.

Diese Befunde entsprechen der Tatsache, daß das Vorkommen von cholesterinhaltigen Gallensteinen unter den mit Nahrung 1 gefütterten Hamstern häufig und unter den mit den Nahrungen 2 oder 3 gefütterten Hamstern selten ist.

References

1. *Dam, H., I. Prange* and *E. Søndergaard,* Z. Ernährungswiss. **9,** 43 (1968). – 2. *Dam, H., I. Prange* and *E. Søndergard,* Z. Ernährungswiss. **13,** 208, (1974). – 3. *Prange, I., F. Christensen* and *H. Dam,* Z. Ernährungswiss. **3,** 59 (1962). – 4. *Hegardt, F.* and *H. Dam,* Z. Ernährungswiss. **10,** 223 (1971). – 5. *Holzbach, R. T., M. Marsh, M. Olszewski* and *K. Holan,* J. Clin. Invest. **52,** 1467 (1973). – 6. *Mufson, D.,* personal communication (1973). – 7. *Mufson, D., K. Trianond, J. E. Zarembo* and *L. J. Ravin,* J. Pharmaceut. Sci. **63,** 327 (1974). – 8. *Dam, H., I. Prange* and *F. Christensen,* Z. Ernährungswiss. **6,** 97 (1965).

Authors' address:

Prof. Dr. *H. Dam,* Danmerks Tekniske Højskole,
Øster Voldgade 10 L, DK-1350 København K (Danmark)