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

Alimentary Production of Gallstones in Hamsters

21. The Content of Cholesterol in Livers of Hamsters reared on Diets with Different Influence on Gallstone Formation

By I. Prange, F. Christensen and H. Dam

With 2 tables

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Previous studies from this laboratory have shown that diets without fat give rise to abundant formation of cholesterol gallstones in young hamsters when the carbohydrate is entirely or mainly represented by glucose (1, 2), whereas there is little or no formation of gallstones when the carbohydrate is entirely represented by rice starch (1, 3). A diet of a more complicated composition ("the curative diet") was found capable of bringing already-formed cholesterol gallstones into solution (4).

Analyses of bladder bile from young hamsters reared on diets of the aforementioned three types (3) showed that the ratios between bile acids and cholesterol were highest with the "curative diet", somewhat lower with the "rice starch diet", and lowest with the "glucose diet". The ratios between lipid-soluble phosphorus and cholesterol varied similarly. The concentrations of cholesterol were lowest with the "curative diet" and highest with the "glucose diet".

Incorporation of a single dose of intraperitoneally injected 1-^{14}C-acetate into cholesterol *in vivo* was found to be greater with the "glucose diet" than with the "rice starch diet" and the "curative diet" (5).

If cholesterol in the bile originates, partly, by "elution" of cholesterol contained in the liver through the action of secreted bile acids (and lecithin), the conditions for production of cholesterol in young hamsters receiving the fat-free "glucose-diet" might be thought to be due to a particularly high content of unesterified cholesterol in the livers of hamsters reared on that diet.

We have, therefore, determined the content of cholesterol, esterified and unesterified, in the livers of hamsters reared on diets of the above-mentioned three types.

The results showed clearly that the animals on the "curative" diet had less esterified and unesterified cholesterol per g liver than the animals on the "glucose diet". But the animals on the "rice starch diet" had almost exactly the same content of unesterified cholesterol per g liver as the animals on the "glucose diet". The animals on the "rice starch diet" had somewhat more esterified cholesterol per g liver than the animals on the "glucose diet", but the difference was of low significance.

Experimental

The hamsters were young females from our stock colony, 36–41 days of age at the beginning of the experiment. They were housed in individual cages with wire screen bottom, and given the diets indicated in table 1. Diet and water were available *ad libitum*. After

having received the experimental diets for 56–58 days the animals were killed with chloroform, autopsied and examined for gallstones as previously described (6). The livers were taken out, weighed, wrapped in aluminum foil (paper coated on the inside with a watertight layer of aluminum) and stored at minus 20 °C until analysis.

Table 1. Diets

	Glucose diet g	Rice starch diet g	"Curative diet" g
Casein, "Vitamin Test"[1]	20.0	20.0	
Casein, crude[2]			20.0
Glucose	62.3		
Rice starch	12.0	74.3	
Ground polished rice			28.3
Dried yeast, Fleischmann 50 B[3]			36.0
Lard			10.0
Salt mixture[4]	5.0	5.0	5.0
Vitamin mixture[5]	0.5	0.5	0.5
Cholin chloride	0.2	0.2	0.2
	100.0	100.0	100.0

[1]) From Genatosan Ltd., Loughborough, England.
[2]) "Dairinex", from Dansk Mejeri Industri & Export Kompagni, Stege, Denmark.
[3]) From Standard Brands, Inc., New York, N. Y., U.S.A.
[4]) The salt mixture specified in (12).
[5]) The vitamin mixture specified in (12).

For analysis the whole liver was cut into small cubes and extracted in an electrically driven homogenizer (from Measuring and Scientific Equipment Ltd., London, England) with chloroform:methanol 2:1 (v/v), 20 times the weight of the liver, according to the procedure of FOLCH et al. (7).

The neutral lipid fraction was separated from the phosphatide fraction by column chromatography on silicic acid according to GLENN et al. (8). An aliquot part of the neutral lipid fraction in chloroform was subjected to thin layer chromatography on Silica gel G (Merck, Darmstadt), thickness of the layer 0.25 mm. The sample was applied to the plate as a long streak. Standards of pure cholesterol and cholesteryl palmitate were applied to the plate as spots. The solvent system used as mobile phase was a mixture of petroleum ether:diethyl ether:glacial acetic acid 70:30:1 (v:v:v). After development the spots were visualized with iodine vapor.

The zone representing unesterified cholesterol was scraped off and the cholesterol eluted by extraction 3 times with 5 ml of a mixture of equal volumes of chloroform and diethyl ether. The solvent was evaporated under nitrogen and the residue transferred to chloroform. Aliquots of the chloroform solution were used for the LIEBERMANN-BURCHARD reaction as described by DAM et al. (9). The absorbancy was read at 625 nm and compared with the absorbancy obtained with a standard of pure cholesterol.

The cholesterol ester zone was scraped off, eluted as described for unesterified cholesterol, evaporated to a small volume and subjected again to thin-layer chromatography as described. After elution, the amount of esterified cholesterol was determined by the LIEBERMANN-BURCHARD reaction. Pure cholesteryl palmitate was used as standard, but the results were expressed as the amount of free cholesterol equivalent with the palmitate.

Table 2. Data for young female hamsters on three different diets

Diet characteristics	Animal number	Gall-stones*)	Body weight after 56 days of feeding g	Liver weight g	Non-phosphatide lipid g/100 g liver	Unesterified cholesterol mg/100 g liver	Esterified cholesterol mg/100 g liver	Total cholesterol mg/100 g liver	Esterified cholesterol as per cent of total cholesterol
"Glucose 62.3% + rice starch 12.0%"	1258/53	0	80	3.67	2.54	209	96	305	31
„	1260/102	C	79	3.47	2.16	191	41	232	18
„	1260/19	C	51	2.06	2.38	174	38	212	18
„	1254/34	C	65	3.03	2.04	192	56	248	23
„	1258/11	C	61	2.34	2.39	205	50	255	20
„	1259/1	C	66	2.83	2.50	185	40	225	18
„	1258/74	C	59	2.45	2.58	210	103	313	33
„	1259/8	C	51	2.55	2.16	190	53	243	22
„	1260/61	C	64	2.33	2.32	202	43	245	18
„	1258/57	C	45	1.79	2.23	185	94	279	34
	Mean values:		62.1	2.65	2.33 ± 0.06	194 ± 4	75 ± 9	256 ± 10	24
"Rice starch 74.3%"	1261/44**)	0	65	2.44[a)]	2.25[a)]	187[a)]	45[a)]	232[a)]	19[a)]
„	1261/86**)	0	68						
„	1262/150	0	82	3.15	2.35	230	125	355	35
„	1262/54	0	84	3.11	2.38	215	99	314	32
„	1262/113	0	77	3.10	2.26	205	86	291	30
„	1261/140	0	84	2.95	2,82	216	180	396	46
„	1261/122	0	87	2.96	2.60	196	72	268	27
„	1262/23	0	61	2.48	2.46	179	56	235	24
„	1262/97	0	65	2.36	2.89	197	151	348	43
„	1262/41	0	62	2.25	2.27	171	42	213	20
	Mean values:		73.5	2.72	2.45 ± 0.07[b)]	198 ± 6[b)]	90 ± 7[b)]	288 ± 9[b)]	30

"Curative"	1263/3	0	82	3.53	1.56	143	13	156	8
"	1264/69	0	84	3.19	1.82	158	14	172	8
"	1264/128	0	83	3.25	1.92	160	17	177	10
"	1263/101	0	84	2.93	1.71	159	17	176	10
"	1264/145	0	76	2.84	1.97	158	21	179	12
"	1264/141	0	91	3.60	1.78	191	16	207	7
"	1263/60	0	70	2.75	1.78	152	20	172	12
"	1263/115	0	82	2.67	1.95	214	27	241	11
"	1263/83	0	82	2.98	1.92	109	17	126	13
"	1264/98	0	84	3.54	1.67	124	16	140	11
	Mean values:		81.8	3.13	1.81 ± 0.04	157 ± 10	18 ± 1	175 ± 10	10

*) C = cholesterol gallstones; 0 = no gallstones.

**) Through a mistake, the livers from the two animals 1261/44 and 1261/86 were analyzed together. The figures marked a) are, therefore, counted twice in the calculation of mean values, and the figures for standard deviations marked b) represent minimal values only.

Results

The results are presented in table 2.

It is seen that the mean value for unesterified cholesterol per g liver obtained with the "glucose diet" was almost identical with that obtained with the "rice starch diet". The mean value for non-phosphatide lipid per g liver was also almost the same whether the one or the other of these two diets was used. The mean value for esterified cholesterol per g liver was somewhat higher with the "rice starch diet" than with the "glucose diet", but the difference was of low significance.

Therefore, the marked difference between the "glucose diet" and the "rice starch diet" with respect to formation of gallstones cannot be ascribed to differences in the contents of cholesterol or non-phosphatide lipid in the liver.

The mean values for unesterified and esterified cholesterol and for non-phosphatide lipid per g liver were lower with the "curative diet" than with the two other diets. Whether this circumstance is of importance to previously established superiority of the "curative diet" over the "rice starch diet" with respect to avoidance of gallstone formation is an unsettled question.

The fact that the content of cholesterol in the liver was lower for hamsters on the "curative diet" than for hamsters on the two diets without added fat is probably due to the presence of fat in the "curative diet". In previous experiments with chicks reared on diets without added cholesterol and with and without added fat (10% peanut oil), the livers contained less total cholesterol when the diet contained fat than when the diet was fat-free (10). Similarly, ALFIN-SLATER et al. (11) found higher contents of total and unesterified cholesterol per g liver of rats, when the diet was fat-free than when the diet contained 12.5% cottonseed oil.

Summary

Esterified and unesterified cholesterol were determined in the livers from groups of young female hamsters reared for 56–58 days on three different diets, viz.: 1. a cholesterol-gallstone producing diet containing no added fat and having glucose as the major carbohydrate; 2. a diet likewise containing no added fat but having rice starch as the only carbohydrate, a type of diet with which the tendency to production of gallstones is very low; and 3. a diet of a more complicated composition containing 10% lard and no sugar, a type of diet previously found capable of bringing already formed cholesterol gallstones into solution.

Compared with the animals on diets 1 and 2, the animals on diet 3 had lower contents of esterified and unesterified cholesterol per g liver.

No significant difference could be found between the animals on diet 1 and the animals on diet 2 with respect to the content of unesterified cholesterol per g liver. The amount of esterified cholesterol per g liver was somewhat higher for the animals on diet 2 than for the animals on diet 1, but the difference was of low significance. Thus, the marked difference between diet 1 and diet 2 with respect to production of gallstones is not dependent upon differences in the content of cholesterol in the liver.

Zusammenfassung

Gruppen von jungen weiblichen Hamstern wurden während 56–58 Tage mit drei verschiedenen künstlichen Nahrungen gefüttert. Die Nahrungen waren: 1. eine Cholesterin-Gallensteine in reichlicher Menge hervorrufende Nahrung ohne Fettzusatz und mit Glucose als hauptsächlichste Kohlenhydratkomponente; 2. eine Nahrung, gleichfalls ohne Fettzusatz, aber mit Reisstärke als einzigstes Kohlenhydrat, ein Nahrungstypus, bei welchem die Neigung zu Gallensteinbildung nur ganz gering ist; und 3. eine etwas komplizierter zusammengesetzte Nahrung, welche 10% Schweineschmalz und keinen Zucker enthielt, ein Nahrungstypus, welcher früher zur Auflösung schon gebildeter Cholesterin-Gallensteine benutzt worden ist.

Nach Ablauf der Fütterungsperiode wurde der Gehalt der Leber an verestertem und unverestertem Cholesterin bestimmt.

Die Tiere, welche mit der Nahrung 3 gefüttert worden waren, hatten weniger esterifiziertes und unesterifiziertes Cholesterin pro Gramm Leber als die Tiere, welche die Nahrungen 1, bzw. 2 erhalten hatten.

Zwischen den mit Nahrung 1 gefütterten Tieren auf der einen Seite und den mit Nahrung 2 gefütterten Tieren auf der anderen Seite, konnte kein signifikanter Unterschied in bezug auf unesterifiziertes Cholesterin pro Gramm Leber festgestellt werden. Die mit Nahrung 2 gefütterten Tiere hatten einen etwas höheren Gehalt an esterifiziertem Cholesterin pro Gramm Leber als die mit Nahrung 1 gefütterten Tiere. Dieser Unterschied war aber von niedriger Signifikanz. Der markierte Unterschied zwischen den Nahrungen 1 und 2 in bezug auf Gallensteinbildung kann somit nicht auf Verschiedenheiten in bezug auf den Cholesteringehalt der Leber zurückgeführt werden.

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Authors' address:
Professor, Dr. Henrik Dam, Polytechnic Institute, Østervoldgade 10‴,
DK – 1350 Copenhagen, Denmark