Zusammenfassung

Die befragten rund 5500 Haushalte wurden nach der Größe der bewirtschafteten landwirtschaftlichen Nutzfläche (LN) geordnet. Dabei wurden folgende Gruppen gebildet: unter 5 ha LN, 5–10 ha LN, 10–15 ha LN, 15–20 ha LN, 20–25 ha LN, 25–30 ha LN, 30–40 ha LN, 40–50 ha LN, 50–100 ha LN, über 100 ha LN. Der vorliegende Teil der Gesamtuntersuchung bildet zugleich die Prämisse für die auf der Betriebsgrößenstruktur aufbauenden ernährungsphysiologischen Auswertungen des Lebensmittelverbrauchs dieser spezifischen Konsumentengruppe.

Während sich gegensätzlich zu den landsmannschaftlichen Verzehrsgewohnheiten beim Betriebsgrößenvergleich einige Antwortreihen nivellierend verhalten, ergeben sich bei anderen Befragungen um so differenziertere Resultate. Ein Einfluß seitens der Betriebsgröße auf die Fragestellung der Untersuchung ist unverkennbar. Die allgemeine Fiktion wird damit authentisch bestätigt. Art und Anzahl der täglichen Mahlzeiten, Art der verbrauchten Getränke zu den einzelnen Mahlzeiten, jahreszeitlicher Verbrauch wichtiger Lebensmittelgruppen, Abhängigkeit von Arbeitsspitzen, Art und Häufigkeit der verwendeten tisch- und kochfertigen Produkte nebst Bestimmungsgründen, Beachtung chemischer Zusätze beim Einkauf von Nahrungsmitteln sollen hier als Beispiel genannt werden, um einen deutlichen Überblick des Inhalts dieses Teiles der Abhandlung zu vermitteln.

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Anschrift des Verfassers:

Professor Dr. W. Wirths, Max-Planck-Institut für Ernährungsphysiologie
46 Dortmund, Rheinlanddamm 201

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

Alimentary production of gallstones in hamsters

22. The influence of a non-absorbable bile acid binding resin, cholestyramine, on formation of gallstones in hamsters

By E. Søndergaard, I. Prange and H. Dam

With 2 tables

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The question how continuous ingestion of a bile acid binding, non-absorbable substance such as cholestyramine would influence alimentary gallstone disease remained unsolved for several years.

In 1962 the substance, furnished by Merck, Sharp & Dohme Research Laboratories under the designation MK 135, was tested in our laboratory as addition at the level of 0.3% to the "fatfree glucose diet" (1) used for production of cholesterol gallstones in young hamsters. No effect on the very

high incidence of cholesterol gallstones was observed, and the results were not published. In 1963, an attempt was made to test the substance at the 3% level with the same basal diet, but at that time an infectious diarrhea was rampant among our hamsters causing early death of so many of the animals that decisive conclusions could not be reached. Since the substance had a very unpleasant smell and taste, and the diet with 3% cholestyramine, apparently, was only reluctantly eaten by the animals, further tests were not carried out.

Later, SCHOENFIELD and SJÖVALL (2) tested cholestyramine, now produced in a purer and less ill-tasting form under the trade name "Cuemid", as addition at the 1% level to an artificial, pelleted diet for guinea pigs. With this diet, gallstones containing about 50% cholesterol were formed. The animals lost weight, and when measures were taken for avoidance of weight loss (addition of hay to the diet or omission of the pelleting procedure) gallstone formation did not occur.

BERGMAN and VAN DER LINDEN (3) fed cholestyramine in the form of "Cuemid" to hamsters as addition at the 3% level to the "fat-free glucose diet" (1). As expected, the basal diet produced a high incidence of cholesterol gallstones after one month, but such gallstones did not occur among the animals receiving the diet with 3% cholestyramine. The cholestyramine-supplemented diet was also tested curatively on hamsters in which the presence of gallstones had been verified by biopsy. Considerable reduction of the incidence of cholesterol gallstones was found after 2 months of cholestyramine treatment, but a few animals still had cholesterol gallstones even after 3 months.

In the following a reexamination of the preventative effect of cholestyramine in the form of "Cuemid" given at the level of 3% is presented. The basal diet used was the more convenient version of the "fat-free glucose diet" used in our laboratory during the last few years, in which trace elements are included in the salt mixture, and the vitamin mixture is slightly modified (table 1). Since the bile acid binding substance might interfere with the

Table 1

	Unsupplemented diet g	Cholestyramine-supplemented diet g
Casein, crude[1])	20.0	20.0
Glucose	74.3	71.3
Salt mixture[2])	5.0	5.0
Vitamin mixture[3])	0.5	0.5
Choline chloride	0.2	0.2
Cholestyramine[4])		3.0
	100.0	100.0

[1]) "Dairinex", from Dansk Mejeri Industri & Export Kompagni, Stege, Denmark.

[2]) The salt mixture indicated in Reference (11).

[3]) The vitamin mixture indicated in Reference (11), but with doubled amounts of vitamins A, D_3 and E.

[4]) "Cuemid", generously furnished by the representative of Merck, Sharp & Dohme, Inc., in Copenhagen.

absorption of fat-soluble vitamins, the amounts of vitamins A, D_3 and E were doubled. As a check on the absorption of vitamin A from the cholestyramine-supplemented diet, vitamin A was determined in the livers of all the animals autopsied.

Experimental

The hamsters were young males and females from our stock colony, 30 to 32 days of age at beginning of the experiment. They were housed in individual cages with wire screen bottom. The diets were as indicated in table 1. Diet an water were available ad libitum. After having received the diets for 42–43 days the animals were killed with chloroform, autopsied and inspected for gallstones as previously described, the type of stones being determined by the aid of dissecting and polarizing microscopes (4). The results were based only on animals living through the entire experimental period and not having had diarrhea.

The vitamin A content of the livers was determined by a modified version of the simplified method of AMES et al. (5): To about 3 grams of liver tissue were added 10 g anhydrous sodium sulfate and 60 ml chloroform, whereafter the mixture was homogenized for 4 minutes in an electrically driven homogenizer (from Measuring and Scientific Equipment Ltd., London, England). The homogenate was filtered on a fritted glass filter funnel and the filtrate made up to a known volume with chloroform (usually 100 ml). To a small aliquote of the chloroform solution made up to 1 ml were added one drop of acetic anhydride and 5 ml of a saturated antimony trichloride solution (25 g antimony trichloride in 100 ml chloroform); the intensity of the resulting blue color was measured at 620 nm using a Beckman Colorimeter Model C. The amount of vitamin A present was determined by reference to a standard curve prepared using vitamin A acetate.

Results and discussion

The results are presented in table 2.

It is seen that addition of 3% cholestyramine has brought about a considerable decrease of the frequency of cases with cholesterol gallstones, although not to the same degree as found by BERGMAN and VAN DER LINDEN (3). It was noted that the number of cholesterol gallstones in the gallbladder of the hamsters having such stones was considerable less in the group of animals receiving the cholestyramine supplemented diet than in the group fed the unsupplement diet.

However, the degree of protection against formation of cholesterol gallstones obtained by addition of 3% cholestyramine to the basal diet was much less than that obtained in numerous previous experiments simply by replacing the total amount of glucose contained in the basal diet by rice starch (6, 7).

The addition of cholestyramine has increased the frequency of cases with amorphous pigmented gallstones, and this increase was not predominantly found among the females, such as was the case when amorphous pigmented gallstones were produced by certain other dietary measures, e. g., by addition of 1% cholesterol to a diet containing 10% butter-fat (8). It is possible that the composition of this type of gallstones is variable and to some extent dependent upon the diet.

Table 2. Experimental data for hamsters on gallstone-producing diets with and without 3% cholestyramine

Diet	Animal no.	Sex	Number of days on diet	Weight at start of Exp. g	Weight after 6 weeks g	Weight increase during 6 weeks g	Weight of liver, g	Vitamin A microg/g liver	Type of gallstones C[1])	M[2])	A[3])	O[4])
Without cholestyramine	4	m	42	40	55	15	3.33	131	C			
	6	m	42	58	81	23	4.59	127	C			
	11	m	42	38	53	15	3.09	127	C			
	12	m	42	58	78	20	3.49	124				O
	14	m	42	62	84	22	5.07	122	C			
	16	m	42	69	90	21	5.23	144	C			
	17	m	42	62	89	27	4.99	125	C			
	43	m	42	59	85	26	4.41	136	C			
	46	m	42	62	92	30	5.08	136	C			
	49	m	42	63	80	17	4.57	150	C			
				57.1	78.7	21.6	4.39	132±3				
Without cholestyramine	7	f	42	49	76	27	5.50	124	C		A	
	8	f	42	51	74	23	4.02	129	C			
	10	f	42	41	52	11	2.14	153	C			
	45	f	42	47	82	35	4.54	111	C			
	47	f	42	61	90	29	6.72	100	C			
				49.8	74.8	25.0	4.58	123±9				
With 3% cholestyramine	5	m	42	50	67	17	3.46	132	C	M	A	
	13	m	42	50	75	25	3.76	124	C			
	28	m	42	58	71	13	4.46	93				O
	29	m	42	56	75	19	4.85	124	C			
	31	m	42	49	76	27	4.67	110	C	M	A	
	33	m	42	50	74	24	3.79	111			A	
	34	m	43	52	75	23	4.04	113				O
	35	m	43	52	82	30	4.74	83				O
	37	m	43	58	68	10	3.63	118	C			
	40	m	43	58	68	10	3.16	135			A	
	48	m	43	70	80	10	3.87	112	C			
	50	m	43	59	78	19	4.44	111			A	
	51	m	43	58	86	28	3.85	128			A	
				55.4	75.0	19.6	4.06	115±4				
With 3% cholestyramine	1	f	43	41	55	14	3.59	105				O
	2	f	43	48	62	14	3.66	101				O
	3	f	43	53	74	21	4.23	115	C			
	9	f	43	45	62	17	4.28	111	C	M	A	
	15	f	43	64	78	14	4.88	93			A	
	18	f	43	62	72	10	4.37	87				O
	25	f	43	55	82	27	5.51	90			A	
	30	f	43	48	65	17	4.19	87				O
	35	f	43	49	80	31	5.29	91				O
	41	f	43	52	78	26	4.71	88			A	
				51.7	70.8	19.1	4.47	97±4				

[1]) C = Cholesterol gallstones. [2]) M = Mixed gallstones.
[3]) A = Amorphous pigmented gallstones. [4]) O = No gallstones.

The occurrence of a few cases with mixed gallstones among the hamsters reared on the cholestyramine-supplemented died further emphasizes the ability of the cholestyramine supplement to produce gallstones containing amorphous pigmented material.

Our previous experiments show that elimination of formation of cholesterol gallstones in the hamster by giving the carbohydrate in the form of rice starch is not accompanied by formation of amorphous pigmented gallstones.

In the present experiment, the average weight increase during 6 weeks was slightly less for the hamsters receiving cholestyramine than for those receiving the unsupplemented diet, but this difference was of low significance.

None of the animals in either group showed gross signs of deficiency of fat-soluble vitamins, and the amount of vitamin A in the liver was of the order of magnitude of 100 micrograms per g in both groups, although significantly lower in the group receiving cholestyramine than in the group of animals fed the unsupplemented diet. Since vitamin K was given in water-soluble form, an influence of cholestyramine on the degree of absorption of this vitamin was not to be expected and was therefore not examined.

Regarding the mode of action of cholestyramine in counteracting the formation of cholesterol gallstones the following tentative considerations may apply.

ERIKSSON (9) has shown that in rats, conversion of cholesterol into bile acids is greatly accelerated when the bile is continuously drained through a fistula for several days. It seems possible, therefore, that reduction of the production of cholesterol gallstones in hamsters by continuous removal of bile acids bound to the non-absorbable substance, cholestyramine, is due to a similar mechanism leading to increase of the ratio between bile acids and cholesterol in the bile. The validity of this explanation remains to be established, however. It might be pertinent to remember that interruption of the enterohepatic circulation in hamsters for a relatively short time (17–20 hours) has the opposite effect, i. e., it decreases the ratio bile acids/cholesterol (10).

Summary

The influence of the bile acid binding, non-absorbable substance cholestyramine on alimentary production of gallstones in young hamsters was examined by rearing one group of the animals on a diet without added fat containing 74.3% glucose as the carbohydrate component (and twice the usual amount of vitamins A, D_3 and E), and another group on the same diet supplement with 3% cholestyramine at the expense of glucose. The animals were killed and autopsied after having received the experimental diets for 42–43 days from the age of 30–32 days.

The cholestyramine supplement markedly reduced the production of cholesterol gallstones, but not to the same degree as that obtained in numerous previous experiments by replacing the content of glucose in the basal diet by rice starch.

Further, the cholestyramine supplement markedly favored production of amorphous pigmented gallstones, an effect not encountered when cholesterol gallstones are eliminated by replacing the content of glucose in the basal diet by rice starch.

The growth of the animals was not significantly retarded by the cholestyramine supplement, and none of the animals showed gross signs of deficiency of fat-soluble vitamins.

The amount of vitamin A in the livers was of the order of magnitude of 100 microgram per gram in both groups, but significantly lower in the group receiving cholestyramine than in the group receiving the unsupplemented diet.

Zusammenfassung

Der Einfluß der gallensäure-bindenden, nicht resorbierbaren Substanz, Cholestyramin, auf die alimentäre Entwicklung von Gallensteinen in jungen Hamstern wurde untersucht.

Eine Gruppe von Hamstern wurde mit einer Cholesterin-Gallensteine hervorrufenden, 74,3% Glucose enthaltenden Nahrung ohne Fettzulage gefüttert. Eine zweite Gruppe erhielt dieselbe Nahrung mit Zusatz von 3% Cholestyramin an Stelle von 3% Glucose. Das Alter der Tiere beim Versuchsbeginn war 30–32 Tage. Die Fütterung dauerte 42–43 Tage.

Der Cholestyramin-Zusatz bewirkte eine beträchtliche Minderung der Bildung von Cholesterin-Gallensteinen. Die Schutzwirkung diesem Gallenstein-Typus gegenüber war aber geringer als diejenige, welche man durch Ersatz der Glucose durch Reisstärke erreichen kann.

Der Cholestyramin-Zusatz begünstigte die Bildung von amorphen, pigmentierten Gallensteinen beträchtlich, eine Komplikation, welche bei der Elimination der Cholesterin-gallenstein-Bildung durch Ersatz der Glucose durch Reisstärke nicht auftritt.

Die Gewichtszunahme der Tiere wurde durch den Cholestyramin-Zusatz nicht signifikant gehemmt, und Symptome eines Mangels an fettlöslichen Vitaminen wurden nicht beobachtet. (Um eine Hemmung der Resorption der fettlöslichen Vitamine durch die Bindung von Gallensäuren an Cholestyramin entgegenzuwirken, war die Nahrung beider Gruppen von Tieren mit der doppelten der gewöhnlich benutzten Menge der Vitamine A, D_3 und E versehen). Unter diesen Umständen war der mittlere Gehalt der Leber an Vitamin A von der Größenordnung 100 Mikrogramm per g in beiden Gruppen, aber signifikant geringer in der mit Cholestyramin-Zusatz als in der ohne Cholestyramin-Zusatz gefütterten Gruppe.

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Authors' address:

Dr. E. Søndergaard, Dr. I. Prange, and Prof. Dr. H. Dam
Polytechnic Institute, Øster Voldgade 10 L[4]
DK-1350 Copenhagen