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Studies of human bile

VI. Influence of diets low in fat on the composition of bile in healthy subjects

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With 1 figure and 17 tables

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Gallstones occur less frequently in the population of Japan than in the populations of western countries [*Miyake*, sr. (1913), *Yagi* (1960), *Maki* et al. (1964)]. Early investigators sought an explanation of this phenomenon in circumstances possibly contributing to biliary stasis. Thus, *Miyake*, sr. (1913) suggested that the higher prevalence of gallstones among German women than among Japanese women could be due to the fact that the German women wore a corset phereas the Japanese women did not. Later, attention turned to the role played by differences in diet [*Yagi* (1960), *Maki* et al. (1964)]. A relation between the lesser frequency of gallstones in Japan and the fact that the consumption of fat and animal protein is much less in Japan than in western countries was emphasized by *Maki* et al. (1964). Further, a moderate increase of the autopsy incidence of cholelithiasis in Japan in the post war years was demonstrated by *Maki* et al. (1964), and linked to an increase of the intake of fat and animal protein. A change of the type of gallstones in Japan from the calcium-pigment type predominant in earlier years to a cholesterol containing type has been ascribed partly to a lessened frequency of infection of the gall bladder with bacteria and parasitic worms capable of inducing enzymatic deconjugation of bilirubin glucuronide [*Maki* (1961)], and partly to dietary changes [*Yagi* (1960)], *Nakayama* and *Miyake*, jr. (1970).

Some of the main features of the Japanese diet and of the diets from two western countries, Sweden and Denmark, are shown in table 1, from which it appears that not only the consumption of fat and animal protein but also the consumption of sugar is much lower in Japan than in western countries.

Table 1. Daily per caput intake in Japan and two western countries in periods with an interval of 26 years*)

		Japan		Sweden		Denmark	
		1934–38	1964–66	1934–38	1964–66	1934–38	1964–66
Calories		2180	2416	3110	2907	3420	3220
Fat	g	20.2	41.3	120.4	131.0	150.4	157.0
Animal protein	g	10.4	25.5	58.5	53.6	56.8	60.0
Veget. protein	g	53.4	46.9	35.6	26.8	34.2	29.8
Refined sugar	g	39.5	50.0	119.6	112.8	138.3	130.1
Non-centrifuged sugar, molasses or honey	g		1.5	6.0	5.0		3.4

*) From "Food Balance Sheets" issued by FAO, Rome, 1955 and 1971.

In the Japanese diet the main source of carbohydrate is rice. The fat is mainly vegetable and rich in linoleic acid [*Insull* et al. (1968)].

From data published by *Nakayama* and *Van der Linden* (1971) it seems that the ratios of bile salts to cholesterol and of phospholipid to cholesterol are higher in bladder bile from Japan than in bladder bile from Sweden.

Of further interest in connection with the less frequent occurrence of gallstones in Japan is the fact that serum cholersterol levels are lower in the population of Japan than in the U.S.A. [Vital and Health Statistics (1967)].

It would be of great interest to examine the question whether it is possible to reduce the occurrence of gallstones in western populations by restriction of the intake of fat and sugar (or the intake of the one or the other of these two nutrients).

A direct prophylactic attack on the problem will require comprehensive long term studies, difficult to carry out, but in the absence of such studies it may be of some interest to examine which changes, if any, the composition of the bile undergoes when the intake of fat is drastically lowered and maintained at a low level during a period of e.g. 6 weeks or more.

An attempt to carry out such a study is described in the following. The study also includes the influence of the experimental diets on the participants' serum cholosterol.

Experimental

Ten healthy female volunteers, 20–25 years of age, participated in the study. One of them participated twice with a time interval of 168 days. The low fat diets were given through a period of about 6 weeks, and in three cases the the dietary period was prolonged for an additional period of 4 weeks.

Duodenal bile was collected fasting after injection of cholecystokinin, twice before, and about 6 weeks after beginning of the dietary period. In the three cases in which the dietary period was prolonged, bile was also collected after 10 weeks.

Simultaneously with collection of bile, a blood sample was taken for determination of serum cholesterol.

Table 2. Constituents of diets I and II; grams per 2000 kcal.

	Diet I	Diet II
Chicken (breast, visible fat removed)	155.2	121.2+
Fish (plaice)	37.2	36.4
Cheese (10% fat in dry matter)	62.1	30.3
Skim milk	186.2	181.8
Butter milk	186.2	181.8
White bread	155.2	151.5
Rye bread, whole meal	93.1	90.9
Rice semi-polished	43.5	42.4
Potatoes	186.2	181.8
Carrots	186.2	181.8
Apple	310.4	303.0
Lettuce	24.8	24.2
Onion	37.2	36.3
Cucumber	62.1	60.6
Tomatoes	124.2	121.2
Banana (edible portion)	–	242.4
Limon juice	24.8	24.2
Marmalade	43.5	42.4
"Butter compound" (40% fat)[1])	24.8	12.1
Animal protein	83.2	58.1
Vegetable protein	33.3	36.3
Total protein	116.5	94.4
Total fat	19.7	15.2
Cholesterol	0.302	0.238
Linoleic acid[2])	5.16	3.00
Linolenic acid[2])	0.61	0.39
Sugars, as monosaccharides[3])	78.0	115.0

[1]) A special low fat margarine produced by Nordisk Margarinefabrik, Vanløse, Denmark.

[2]) Assuming total fatty acids equal to 95% of total fat.

[3]) Lactose not counted. Data from *McCance* and *Widdowson* (1969).

Besides the ten volunteers mentioned above, one more volunteer (female, age 28 years) received the diet, but in her case collection of bile was unsuccessful, and only the blood samples were taken.

It was difficult to persuade volunteers to take diets corresponding closely to the specifications for Japanese diets found in Food Balance Sheets (1955). Therefore, six low fat diets were tried, but each volunteer received only one diet through the entire experimental period. The composition of the diets is shown in tables 2 and 3. Besides being low in fat, diets III, IV, V and VI, but not diets I and II, were also low in rapidly digestible sugars. The cholesterol content was fairly low, especially in diets III, IV, V and VI. The volunteers were allowed to drink water and tea and coffee without sugar and cream. The daily intake of calories was adjusted from time to time in order to enable each volunteer to maintain constant body weight during the experiment, but this measure was not successful in all cases.

Preparation and administration of the diets were undertaken by the Diet Kitchen of the Medical Department A of the University Hospital. It was not considered necessary to begin the experiments with a period in which the

Table 3. Constituents of diets III, IV, V and VI; grams per 2000 kcal.

	Diet III	Diet IV	Diet V	Diet VI
Beef, roast and salt, equal parts visible fat removed	10.6	10.0	10.0	10.0
Shrimps, frozen and canned, equal parts	64.5	60.8	60.8	60.8
Egg white	11.0	10.4	10.4	10.4
Skim milk	41.2	38.8	38.8	38.8
White bread	71.6	67.5	67.5	67.5
Graham bread	–	159.3	154.4	161.1
Rye bread, whole-meal	72.5	68.3	68.3	68.3
Textured vegetable protein	–	34.1	17.1	17.1
Rice, semi-polished	343.4	209.7	209.7	209.7
Potatoes	120.8	113.8	113.8	113.8
Carrots	37.0	34.8	34.8	34.8
Apple	52.4	49.4	49.4	49.4
Cabbage	36.2	34.1	34.1	34.1
Lettuce	48.3	45.5	45.5	45.5
Radish	36.2	34.1	34.1	34.1
Onion	15.3	14.5	14.5	14.5
Leek	36.2	34.1	34.1	34.1
Bamboo shoots	24.2	–	–	–
Bean sprouts	24.2	–	–	–
Seaweed	0.7	–	–	–
Soy sauce	47.3	44.6	44.6	44.6
Wine (sake)	50.6	47.7	47.7	47.7
"Butter-compound" (40% fat)[1])	8.6	16.2	–	–
Dietetic margarine (83% fat)[2])	–	–	17.1	12.0
Animal protein	16.2	15.2	15.2	15.2
Vegetable protein	46.7	64.5	53.3	52.7
Total protein	62.9	79.8	68.5	68.5
Total fat	11.7	13.9	23.7	19.8
Cholesterol	0.121	0.114	0.114	0.114
Linoleic acid[3])	3.85	4.61	9.70	7.84
Linolenic acid[3])	0.44	0.61	0.42	0.42
Sugars, as monosaccharides[4])	12.0	12.0	12.0	12.0

[1]) See table 3.
[2]) Brand "Prior" from Nordisk Margarinefabrik, Vanløse, Denmark.
[3]) Assuming total fatty acid equal to 95% of total fat.
[4]) Lactose not counted. Data from *McCance* and *Widdowson* (1969).

volunteers received a diet of fixed composition because their ordinary diets always contain a much higher amount of fat than that present in the experimental diets.

Collection of bile was carried out in the Gastro-enterological Laboratory of the University Hospital. The methods for collection, handling and analysis of the bile were as described in our previous publication [*Dam* et al. (1971)]. Serum cholesterol was determined in the Central Laboratory of the University Hospital by methods routinely used, utilizing the *Liebermann-Burchard* reaction.

The fat content of the diets was determined as follows:

Two per cent by weight of the diet, without "butter-compound" or margarine, was extracted in a blendor once with methanol and five times with chloroform-methanol 2:1 (v:v). The combined solutions were evaporated to dryness *in vacuo* and boiled with ether under reflux on water bath for 2 hours. The ethereal solution was quantitatively (with a liberal amount of ether) filtered into a round-bottomed flask, evaporated to dryness *in vacuo* and transferred quantitatively (with approximately 11 ml chloroform) into a 25 ml measuring flask. The substance which did not pass into solution by boiling with ether was boiled with chloroform under reflux on water bath for 2 hours. The chloroform solution was quantitatively (with a liberal amount of chloroform) filtered into a found-bottomed flask, evaporated to dryness *in vacuo*, transferred quantitatively (with approximately 10 ml chloroform) to the 25 ml measuring flask containing the first part of the chloroform extract, and filled up to 25 ml with chloroform. An aliquot (usually 1.5 or 3.0 ml) was used for determination of the dry matter by evaporation and weighing.

The fat content of "butter compound" and margarine was determined separately and added to the fat content of the rest of the diets in the proper proportions.

Similarly, the fatty acid patterns of the diets minus "butter compound" and margarine, and of "butter compound" and margarine were determined separately by the method and equipment indicated previously [*Søndergaard, Prange, Dam* (1973)] and combined in the proper proportions.

The data for total fat, linolenic and linoleic acids indicated in tables 2 and 3 are based on these determinations.

The distribution of the diets among the volunteers appears from table 4.

Total cholesterol in the animal components of the diets was determined as follows: for shrimps and cheese by the method indicated by *Pihl* (1952); for chicken, fish, skim milk, butter milk and beef by the method indicated in the paper by *Dam, Prange* and *Søndergaard* (1952).

The cholesterol contents, mg per 100 g, were:

Chicken, breast, boiled	153.4
Fish, raw	81.9
Skim milk	2.5
Butter milk	3.9
Roast beef	65.8
Salt beef, boiled	41.3
Frozen shrimps, boiled	133.7
Canned shrimps, boiled	224.7
Boiled egg white	1.1

Results and discussion

The results are presented in tables 4–17 and in fig. 1.

Maintenance of body weight (table 4)

Accepting a difference of 0.2 kg as possibly due to errors in the weighings, the body weight may be considered as having remained constant within about 6 weeks in 4 cases, increased in 1 case and decreased in 6 cases, the deepest decrease being 2.8 kg (volunteer PC).

In the cases in which the dietary period was extended to 10 weeks, the body weight at 10 weeks was higher than at 6 weeks in 1 case, but lower in the 3 other cases.

One of the volunteers (DD) terminated participation in the team after 32 days. Nevertheless, her data at 32 days yield information about the changes under study, and therefore, are listed together with the data for the other volunteers at about 6 weeks.

Table 4. Volunteers' age, diet, and body weight at different times of the experiment

Volunteer	Age Years	Diet no.	Body-weight at start of experiment kg	Body-weight at about 6 weeks of experiment kg	Body-weight at about 10 weeks of experiment kg
MO	20	I	53.5	52.1	
BN	24	I	49.0	49.0	
IP	21	II	56.2	55.7	
GH	20	II	53.2	53.9	
SB	20	III	63.0	63.0	
LB	21	IV	66.0	65.9	
DD	23	IV	52.5	51.4*)	
LB	21	V	68.5	68.3	67.7
IN	20	VI	67.0	65.2	
PC	20	VI	69.0	66.2	66.7
LC	25	VI	54.1	52.2	51.8
AC	28	VI	55.6	54.4	53.1

*) 32 days only

Table 5. Serum cholesterol (total) millimol/l

Volunteer	Diet no.	Before diet 1st sample	2nd sample	Mean value	During low-fat diet after about 6 weeks	after about 10 weeks
MO	I	–	6.03	(6.03)	5.32	
BN	I	5.17	4.60	4.88	4.57	
IP	II	7.15	6.81	6.98	5.20	
GH	II	5.29	5.56	5.42	4.85	
SB	III	4.65	–	(4.65)	4.92	
LB	IV	5.92	5.24	5.53	4.82	
DD	IV	6.66	6.02	6.34	4.32*)	
LB	V	5.57	5.23	5.40	4.70	4.88
IN	VI	6.03	5.72	5.87	5.31	
PC	VI	6.14	6.43	6.28	4.52	4.12
LC	VI	5.77	5.16	5.46	4.38	5.45
AC	VI	5.74	5.27	5.51	4.30	4.88
Mean values**)		5.83^{11}	5.64^{11}	5.70^{10}	4.76^{12}	4.83^{4}
st. d.		±0.21	±0.19	±0.19	±0.11	±0.27

*) 32 days only
**) In the 10 cases where 2 determinations were available before beginning of the low-fat regimen, the mean value of the lower values before diet was 5.55 mM/l, st. d. 0.20; the mean value after about 6 weeks was 4.70 mM/l, st. d. 0.11.

Table 6. pH, per cent dry matter, and millimolarities of cholesterol (C) lipidsoluble phosphorus (P), and total bile acids (TBA)[1]) in the bile samples before and during the Low Fat Regimen

Volunteer	Bile sample	pH	Dry Matter %(w:v)	C mM/l	P mM/l	TBA mM/l
MO	7 days before diet	7.2	9.92	8.43	21.62	59.16
MO	immediately before diet	7.1	5.18	5.20	11.75	40.46
MO	42 days on diet	7.3	7.41	5.13	14.04	50.64
BN	8 days before diet	7.6	6.08	4.09	12.51	31.40
BN	immediately before diet	7.5	8.70	7.68	19.84	71.97
BN	46 days on diet	7.6	7.59	5.36	13.68	71.31
IP	15 days before diet	7.2	6.73	6.68	17.30	51.04
IP	immediately before diet	7.2	3.38	1.63	3.30	21.38
IP	41 days on diet	7.5	8.51	3.26	12.39	55.62
GH	13 days before diet	7.2	3.22	1.67	5.31	29.02
GH	immediately before diet	5.81	6.90	7.75	15.88	39.53
GH	43 days on diet	7.8	6.14	3.66	11.98	48.80
SB	7 days before diet	7.6	1.78	0.94	2.89	12.07
SB	immediately before diet	7.5	4.32	2.73	9.68	30.03
SB	42 days on diet	7.3	5.76	3.24	9.46	45.52
LB	7 days before diet	7.4	7.5	6.85	14.27	34.21
LB	immediately before diet	7.1	10.52	8.14	25.23	78.25
LB	42 days on diet	7.5	5.12	5.29	11.75	35.33
DD	8 days before diet	7.0	2.92	1.93	5.25	22.22
DD	immediately before diet	7.8	1.87	0.53	1.63	13.36
DD	32 days on diet	8.2	4.03	1.53	5.47	41.75
LB	7 days before diet	7.3	8.05	6.44	18.50	44.65
LB	immediately before diet	7.2	6.24	8.96	16.34	44.05
LB	42 days on diet	7.7	7.44	5.67	14.47	42.67
LB	70 days on diet	7.7	3.24	3.86	5.84	24.76
IN	7 days before diet	7.4	9.28	10.13	20.37	82.69
IN	immediately before diet	7.2	10.54	9.64	21.28	69.53
IN	42 days on diet	7.5	6.94	5.71	13.84	57.35
PC	6 days before diet	6.3	9.65	6.57	20.93	42.08
PC	immediately before diet	6.6	8.72	6.40	22.11	54.02
PC	41 days on diet	6.8	13.40	10.00	35.10	89.30
PC	69 days on diet	6.7	10.50	9.00	22.10	66.50
LC	7 days before diet	7.0	4.44	2.00	8.60	19.20
LC	immediately before diet	7.0	5.18	2.90	9.70	19.10
LC	49 days on diet	7.1	2.44	1.00	3.10	9.61
LC	70 days on diet	7.8	3.70	1.61	5.38	23.79

[1]) TBA is taken as the sum of GD, GCD, GC, TD + TCD and TC, cf. text.

Serum cholesterol (table 5)

In each of the 10 cases where two determinations of serum cholesterol were available before the experiment, the value of serum cholesterol at about 6 weeks was lower than the lower of the two values before the experiment. The mean value of the 10 determinations at about 6 weeks (4.70 mM/L, st. d. 0.11) was significantly lower than the mean value of

Table 7. Mol per cent glycocholic acid in total bile acids (100 GC/TBA)

Volunteer	Before diet 1st sample	2nd sample	Mean value	During low-fat diet after about 6 weeks	after about 10 weeks
MO	15.4	24.1	19.8	29.0	
BN	8.1	20.8	14.4	22.5	
IP	21.2	26.2	23.7	24.2	
GH	13.8	16.9	15.3	20.8	
SB	22.5	26.8	24.7	22.7	
LB	17.8	24.8	21.3	21.0	
DD	21.2	19.2	20.2	34.1*)	
LB	31.2	29.4	30.3	17.3	11.0
IN	20.1	21.9	21.0	25.7	
PC	17.7	23.9	20.8	18.2	32.8
LC	39.6	7.3	23.5	20.8	30.0
Mean values	20.78	21.94	21.34	23.30	24.6
st. d.	±2.54	±1.82	±1.32	±1.47	±6.85

*) 32 days only

Table 8. Mol per cent glycochenodeoxycholic acid in total bile acids (100 GCD/TBA)

Volunteer	Before diet 1st sample	2nd sample	Mean value	During low-fat diet after about 6 weeks	after about 10 weeks
MO	15.1	12.7	13.9	16.1	
BN	19.6	28.7	24.2	27.0	
IP	11.2	18.2	14.7	9.9	
GH	18.0	17.3	17.7	11.1	
SB	25.9	17.5	21.7	7.3	
LB	20.2	20.1	20.1	20.3	
DD	15.7	26.5	21.1	27.4*)	
LB	7.7	13.2	10.5	15.7	19.9
IN	13.1	15.3	14.4	21.3	
PC	30.3	44.0	37.1	27.3	17.2
LC	26.0	12.0	19.0	11.3	17.1
Mean values	18.44	20.5	19.49	17.70	18.07
st. d.	±2.07	±2.85	±2.14	±2.24	±0.92

*) 32 days only

the corresponding 10 lower determinations before the experiment (5.55 mM/L, st. d. 0.20). In two cases only one determination of serum cholesterol was available before the experiment, and in one of these cases the value at about 6 weeks was lower, in the other higher than the value before the experiment. Among the 4 cases in which the dietary period

Table 9. Mol per cent glycodeoxycholic acid in total bile acids (100 GD/TBA)

Volunteer	Before diet 1st sample	2nd sample	Mean value	During low-fat diet after about 6 weeks	after about 10 weeks
MO	7.2	11.3	9.3	8.8	
BN	16.3	18.8	17.6	11.2	
IP	9.7	10.1	9.9	9.9	
GH	21.6	24.2	22.9	18.9	
SB	21.2	22.0	21.6	7.1	
LB	4.2	12.6	8.4	9.8	
DD	5.5	3.4	4.5	6.7*)	
LB	9.1	6.5	7.8	1.9	4.3
IN	18.3	19.3	18.8	7.3	
PC	17.0	2.2	9.6	23.4	11.6
LC	4.2	32.4	18.3	22.7	12.5
Mean values st. d.	12.21 ±2.05	14.80 ±2.83	13.52 ±1.92	11.61 ±2.10	9.47 ±2.60

*) 32 days only

was prolonged to 10 weeks, the individual serum cholesterol value declined further in 1 case and rose in the 3 other cases, but only in one of these did the value at 10 weeks aproach the same volunteer's mean value before the experiment. Thus it may be concluded that change of the valunteers' diet from the customary diet before the experiment to any of the low-fat diets used, generally lowered serum cholesterol within the duration of the experiment.

The decreases of serum cholesterol could be thought to be due not only to the diet, but in some cases, also to decline of body weight. However, decrease of serum cholesterol occurred in two cases in which the body weight increased (volunteer GH from beginning until 6 weeks, volunteer PC from 6 weeks until 10 weeks) and in 3 of the 4 cases in which the body weight was considered constant within 6 weeks. In the fourth of these cases (volunteer SB) a decrease in serum cholesterol was not observed but here, only 1 determination was available before the experiment. Thus the diet seems to have been more important than weight decline as a factor contributing to low serum cholesterol.

How far the relatively low cholesterol content of the experimental diets has contributed to the decrease of serum cholesterol cannot be decided on the basis of the present experiments, but other investigators, *Walker* et al. (1953), have found that on a low fat diet the cholesterol intake had no influence on the serum lipids including serum cholesterol.

The decrease of serum cholesterol was not due to increased supply of linoleic acid, and gas-chromatographic analysis from 9 of the volunteers on various stages of the experiment did not show an increase, but in some cases a decrease of linoleic acid in the total fatty acids of the bile lecithin as the experiment progressed.

Table 10. Mol per cent taurocholic acid in total bile acids (100 TC/TBA)

Volunteer	Before diet 1st sample	2nd sample	Mean value	During low-fat diet after about 6 weeks	after about 10 weeks
MO	24.1	22.5	22.3	11.5	
BN	18.2	12.7	15.5	20.2	
IP	18.0	11.4	14.7	23.2	
GH	13.2	18.1	15.7	22.0	
SB	18.2	14.4	16.3	16.6	
LB	24.0	18.2	21.1	16.7	
DD	27.2	32.6	29.9	19.1*)	
LB	21.0	22.7	21.8	27.2	30.7
IN	14.2	17.4	15.8	9.9	
PC	7.8	14.4	11.1	7.6	16.1
LC	7.8	18.8	13.3	13.9	14.9
Mean values	17.61	18.47	17.95	17.08	20.57
st. d.	±1.93	±1.79	±1.61	±1.81	±5.08

*) 32 days only

Table 11. Mol per cent taurochenodeoxycholic acid + taurodeoxycholic acid in total bile acids (100 [TCD + TD]/TBA)

Volunteer	Before diet 1st sample	2nd sample	Mean value	During low-fat diet after about 6 weeks	after about 10 weeks
MO	38.1	29.3	33.7	34.7	
BN	37.7	19.0	28.4	19.1	
IP	39.9	34.1	37.0	33.0	
GH	33.5	23.5	28.5	27.2	
SB	12.3	19.2	15.8	46.2	
LB	33.7	24.3	29.0	32.2	
DD	30.5	18.4	24.4	12.7*)	
LB	30.9	28.1	29.5	37.7	35.4
IN	34.2	26.4	30.3	36.0	
PC	27.2	16.5	21.9	23.4	22.4
LC	22.4	29.3	25.6	31.2	25.6
Mean values	30.95	24.37	27.65	30.31	27.80
st. d.	±2.41	±1.69	±1.72	±2.80	±3.91

*) 32 days only

The distribution of the different bile acids in the total bile acids

Tables 6–11 swoh the molar percentages of the bile acids which can be separated by the methods used, viz. Glycocholic Acid (GC), Glycochenodeoxycholic Acid (GCD), Glycodeoxycholic Acid (GC), Taurocholic Acid (TC) and Taurochenodeoxycholic plus Taurodeoxycholic Acid (TCD + TD)

Table 12. Molar ratio glycine-conjugation/taurine-conjugation (ratio G/T)

Volunteer	Before diet 1st sample	2nd sample	Mean value	During low-fat diet after about 6 weeks	after about 10 weeks
MO	0.61	0.93	0.77	1.04	
BN	0.79	2.16	1.48	1.55	
IP	0.73	1.20	0.97	0.78	
GH	1.14	1.40	1.27	1.03	
SB	2.28	1.98	2.13	0.59	
LB	0.73	1.35	1.04	1.05	
DD	0.73	0.96	0.84	2.14*)	
LB	0.92	0.96	0.94	0.54	0.54
IN	1.06	1.29	1.18	1.19	
PC	1.85	2.23	2.04	2.22	1.60
LC	2.40	1.08	1.74	1.21	1.47
Mean values	1.20	1.41	1.31	1.21	1.20
st. d.	±0.20	±0.15	±0.14	±0.17	±0.33

*) 32 days only

Table 13. Molar ratio dihydroxycholanoic acids/trihydroxycholanoic acids (ratio Di/Tri)

Volunteer	Before diet 1st sample	2nd sample	Mean value	During low-fat diet after about 6 weeks	after about 10 weeks
MO	1.53	1.14	1.34	1.48	
BN	2.80	2.38	2.59	1.34	
IP	1.55	1.66	1.61	1.11	
GH	2.70	1.85	2.28	1.41	
SB	1.46	1.42	1.44	1.54	
LB	1.39	1.32	1.36	1.65	
DD	1.07	0.93	1.00	0.88*)	
LB	0.91	0.95	0.93	1.25	1.39
IN	1.92	1.54	1.73	1.82	
PC	2.91	1.67	2.29	2.81	1.04
LC	1.11	2.82	1.96	1.88	1.23
Mean values	1.76	1.61	1.68	1.56	1.22
st. d.	±0.22	±0.17	±0.16	±0.16	±0.10

*) 32 days only

in the Totale Bile Acids (TBA), the latter taken as the molar sum of the bile acids mentioned. Tables 12 and 13 show the molar ratios of Glycine conjugation to Taurine conjugation (G/T), and the molar ratios of Dihydroxycholanoic Acids to Trihydroxycholanoic acids (Di/Tri). These parameters did not exhibit changes of importance to the problem under

Table 14. Molar ratios total bile acids/cholesterol (ratio TBA/C)

Volunteer	Before diet 1st sample	2nd sample	Mean Value	During low-fat diet after about 6 weeks	after about 10 weeks
MO	7.02	7.71	7.37	9.87	
BN	7.68	9.37	8.52	13.30	
IP	7.54	13.12	10.38	17.06	
GH	17.38	5.10	11.24	13.33	
SB	12.84	11.00	11.92	14.05	
LB	4.99	9.61	7.30	6.68	
DD	11.51	25.21	18.36	27.29*)	
LB	6.93	4.92	5.92	7.53	6.41
IN	8.16	7.21	7.69	10.04	
PC	6.40	8.44	7.42	8.93	7.40
LC	9.60	6.60	8.10	9.61	14.78
Mean values	9.10	9.84	9.47	12.52	9.53
st. d.	±1.08	±1.70	±1.04	±1.75	±2.64

*) 32 days only

Table 15. Molar ratios phospholipid/cholesterol (ratio P/C)

Volunteer	Before diet 1st sample	2nd sample	Mean value	During low-fat diet after about 6 weeks	after about 10 weeks
MO	2.56	2.26	2.41	2.84	
BN	3.06	2.58	2.82	2.55	
IP	2.59	2.02	2.31	3.80	
GH	3.17	2.05	2.61	3.27	
SB	3.07	3.55	3.31	2.92	
LB	2.08	3.10	2.59	2.22	
DD	2.72	3.08	2.90	3.58*)	
LB	2.87	1.82	2.35	2.55	1.51
IN	2.01	2.21	2.11	2.44	
PC	3.18	3.45	3.32	3.51	2.46
LC	4.30	3.34	3.82	3.10	3.34
Mean values	2.87	2.68	2.78	2.98	2.44
st. d.	±0.19	±0.19	±0.16	±0.16	

*) 32 days only

study, since small or moderate variations in the distribution of the in-individual bile acids in the total bile acids do not materially influence the solubility of cholesterol when the ratio of phospholipid to bile acids is as high as is the case in human bile (*Hegardt* and *Dam*, 1971).

Table 16. Molar ratios total bile acids/phospholipid (ratio TBA/P)

Volunteer	Before diet 1st sample	2nd sample	Mean value	During low-fat diet after about 6 weeks	after about 10 weeks
MO	2.74	3.44	3.09	3.61	
BN	2.51	3.62	3.07	5.21	
IP	2.95	6.68	4.71	4.49	
GH	5.48	2.49	3.99	4.07	
SB	4.18	3.10	3.64	4.81	
LB	2.40	3.10	2.75	3.00	
DD	4.23	8.20	6.22	7.63*)	
LB	2.41	2.70	2.55	2.95	4.24
IN	4.06	3.27	2.56	4.14	
PC	2.01	2.44	2.22	2.54	3.00
LC	2.30	1.92	2.11	3.10	4.42
Mean values	3.21	3.72	3.36	4.14	3.89
st. d.	±0.33	±0.58	±0.37	±0.43	±0.45

*) 32 days only

The Molar ratios of Total Bile Acids to Cholesterol (TBA/C), of Phospholipid to Cholesterol (P/C), (tables 14, 15 and fig. 1)

The change of the ratio TBA/C from the mean of the two values before the experiment to the value obtained at about 6 weeks was a moderate or small increase in 10 of the 11 cases, and a small decrease in one case.

The change of the ratio P/C from the mean of the two determinations before the experiment until about 6 weeks were: in 7 cases a slight to moderate increase, and in 4 cases a small or moderate decrease.

Among the 3 cases in which values were available at 10 weeks, the changes from 6 to 10 weeks were as follows: TBA/C increased further in 1 case and declined slightly in the other 2 cases, whereas P/C was almost constant in 1 case and declined in the 2 other cases.

It is scarcely possible to point out any relationship of the observed changes of the ratios to change or constancy of body weight or to differences in composition between the several diets used.

Only for one or perhaps two of the volunteers (DD and IP) did the values of TBA and P/C obtained at about 6 weeks correspond to unsaturation of the bile with cholesterol when compared with the solubility limit for cholesterol in aqueous solutions of bile salts and lecithin established by *Mufson* et al., i.e. the outer curved line in fig. 1, which is, probably, the most reliable of all the solubility limits hitherto proposed. (Conclusions regarding over- or under-saturation presuppose, of course, that an influence of bile components other than those determined can be left out of consideration.)

The mean values for all volunteers at about 6 weeks:

TBA/C = 12.52; P/C = 2.98; TBA/P = 4.14

represent only moderate or small increases from the mean values at the beginning of the experiment:

TBA/C = 9.47; P/C = 2.78; TBA/P = 3.36

The smallest mean increase is that of P/C.

Further, the mean values obtained at about 6 weeks are not much different from the mean values previously found for 42 volunteers not having undergone dietary or other treatment:

TBA/C = 11.84; P/C = 3.17; TBA/P = 3.75

[*Dam* et al. (1971)],

or from the mean values found for bladder biles from 27 surgical patients not having gallstones:

TBA/C = 11.59; P/C = 2.99; TBA/P = 3.98

[*Dam* et al. (1971)].

The results from the 3 experiments in which the dietary period was prolonged to 10 weeks do not make it seem likely that continuation of the treatment with the diets used will lead to further increases, especially regarding the ratio P/C.

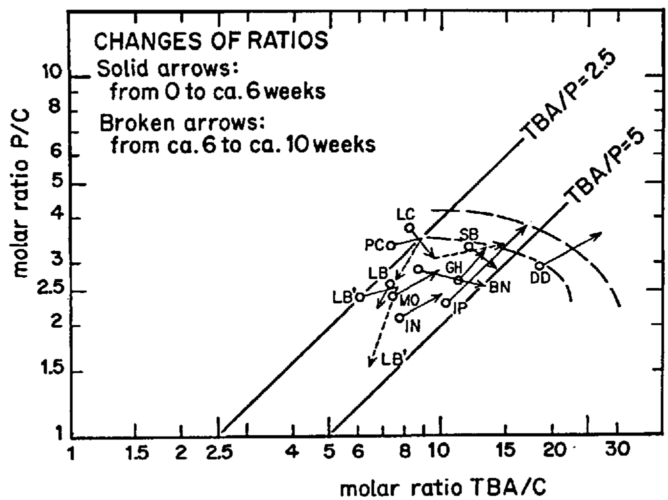

Fig. 1. Abscissa: molar ratio of total bile acids to cholesterol (TBA/C). Ordinate: molar ratio of phospholipids to cholesterol (P/C). Logarithmic coordinates. Arrows indicate direction and magnitude of the changes for each volunteer. Arrows in solid line refer to changes from the mean value before the experiment to the value obtained after about 6 weeks. Arrows in broken line refer to changes from 6 weeks to 10 weeks. The two curved lines indicate limits for solubility of cholesterol in aqueous solutions of lecithin and bile salts. The inner curved line is based on the data of *Hegardt* and *Dam* (1971) and *Holzbach* et al. (1973). The outer curved line is based on data from *Mufson* et al. (1973, 1974).

Table 17. Linoleic, arachidonic and linolenic acids as percent of the total bile acids in bile lecithin

Volunteer		Before diet 1st sample	2nd sample	During low-fat diet after about 6 weeks	after about 10 weeks
	18:2		32.6	27.6	
JP	20:4		5.8	6.6	
	18:3				
	18:2		27.2	26.3	
GH	20:4		6.7	6.9	
	18:3		4.3	1.5	
	18:2		34.6	32.3	
SB	20:4		4.4	2.5	
	18:3		0.6	0.9	
LB	18:2	35.3	33.7	34.5	
(1. exper.)	20:4	3.3	4.2	3.0	
	18:3	0.8	0.9	0.9	
	18:2	34.1		21.8	
DD	20:4	4.9		4.3	
	18:3	0.5		0.8	
	18:2	39.5	33.0	32.1	30.8
LB	20:4	5.9	6.5	7.8	6.3
(2. exper.)	18:3	1.35	1.1	0.8	0.3
	18:2	28.9	32.3	19.3	
IN	20:4	9.8	7.8	9.2	
	18:3	0.7	0.8	0.8	
	18:2	31.0	31.0	31.6	29.6
PC	20:4	10.3	7.8	10.3	8.6
	18:3	1.0	1.2	1.0	1.0
	18:2	31.2	30.3	28.6	
LC	20:4	7.3	8.6	8.5	
	18:3	0.7	0.8	0.8	

It is possible that in a population used to a high intake of fat, subsistence on a low fat intake over a long period of time will tend to lessen the ratio P/C.

Further investigation on this subject will require diets that are more acceptable than those used in the present study, so that constancy of body weight can be secured in all cases.

Questions worthwhile to consider in future experiments are whether the results would be different if the dietary fat was supplied partly as phospholipids in stead of entirely in the form of triglycerides or if the volunteers had belonged to age groups in which the occurrence of gallstones is frequent.

It is of some interest to compare the mean values of the ratios obtained in our study after about 6 weeks with the mean values of the corresponding ratios for Japanese bladder bile that can be calculated from the results of *Nakayama* and *Van der Linden* (1971). In their study the ratios TBA/C and P/C (and implicitly TBA/P) were presented by weight. Assuming a mean molar weight of 471 for the mixed bile acids, and a molar weight of 775 for the phospholipid, the weight ratios can be transformed to molar ratios and will be:

TBA/C = 12.0; P/C = 3.0; TBA/P = 4.05.

These figures are very close to the mean values found for our volunteers at about 6 weeks and close to the mean values found for Danish bladder biles from surgical patients not having gallstones [*Dam* et al. (1971)]. They represent moderate supersaturation with cholesterol when compared with the solubility limits shown in fig. 1.

Comparison of the data from the study of *Nakayama* and *Van der Linden* (1971) with our data may not be entirely relevant, however, because the methods used in the two studies were different, and the mean molar ratios for Swedish bladder biles than can be calculated from the study of *Nakayama* and *Van der Linden:*

TBA/C = 5.2; P/C = 1.85; TBA/P = 2.8

are different from those found for Danish bladder biles by *Dam* et al. (1971).

Summary

Healthy youg females volunteers received diets with low fat through periods of maximally 10 weeks.

Total dietary fat varied from 11.7 to 19.8 g, linoleic acid from 3.00 to 9.7 g, cholesterol from 114 to 302 mg per 2000 kcal. of the experimental diets.

The main results were:

Serum cholesterol. Mean values in mM/l declined from 5.70 before the dietary regimen to 4.76 at about 6 weeks of low-fat regimen (12 experiments with 11 volunteers) and to 4.83 at 10 weeks of low-fat regimen (4 experiments with 4 volunteers).

Duodenal bile. Mean values of the molar ratios of total bile acids to cholesterol (TBA/C) and of phospholipid to cholesterol (P/C) increased from TBA/C = 9.47, P/C = 2.78 before the dietary regimen to TBA/C = 12.52, P/C = 2.98 at about 6 weeks of dietary regimen (11 experiments with 10 volunteers). In 3 experiments with 3 volunteers the mean values were: TBA/C = 7.15, P/C = 3.16 before the dietary regimen; TBA/C = 8.69, P/C = 3.05 at 6 weeks, and TBA/C = 9.53, P/C = 2.44 at 10 weeks of low-fat diet.

All mean the ratios correspond to moderate supersaturation with cholesterol when compared with the limits for solubility of cholesterol in aqueous solutions of bile salts and lecithin established in recent years.

Zusammenfassung

Gesunde junge weibliche Versuchspersonen erhielten Nahrungen mit niedrigem Fettgehalt während Versuchsperioden von maximal 10 Wochen.

Pro 2000 kcal enthielten die Nahrungen: 11,7–19,8 g Gesamt-Fett, 3,00–9,7 g Linolsäure und 114–302 mg Cholesterin.

Die Hauptergebnisse waren:

Serumcholesterin: Die Mittelwerte in mM/l nahmen ab von 5,70 vor dem Versuchsbeginn bis 4,76 nach ungefähr 6 Wochen fettarmer Ernährung (12 Versuche mit 11 Versuchspersonen) und 4,83 nach 10 Wochen fettarmer Ernährung (4 Versuche mit 4 Versuchspersonen).

Duodenalgalle: Die Mittelwerte der molären Verhältnisse von Gesamt-Gallensäuren und Cholesterin (TBA/C) und von Phospholipid zu Cholesterin (P/C) stiegen an von TBA/C = 9,47, P/C = 2,78 vor Versuchsbeginn zu TBA/C = 12,52, P/C = 2,98 nach 6 Wochen fettarmer Ernährung (11 Versuche mit 10 Versuchspersonen). In 3 Versuchen mit 3 Versuchspersonen waren die Mittelwerte: TBA/C = 7,15, P/C = 3,16 vor Versuchsbeginn; TBA/C = 8,69, P/C = 3,05 nach 6 Wochen und TBA/C = 9,53, P/C = 2,44 nach 10 Wochen fettarmer Ernährung. Sämtliche Mittelwerte der Verhältnisse entsprechen mäßiger Übersättigung mit Cholesterin, wenn man sie vergleicht mit den in den späteren Jahren ermittelten Grenzen der Löslichkeit des Cholesterins in wässerigen Lösungen von Gallensalzen und Lecithin.

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