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# Alimentary production of gallstones in hamsters

## 23: Influence of hydrogenated palm oil and hydrogenated palm oil in mixture with sunflower seed oil, on the ratio between lipid-soluble phosphorus and cholesterol in the bladder bile

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With 4 tables

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In a previous study (1) it was found that the ratios between lipid-soluble phosphorus and cholesterol and between total bile acids and cholesterol in the bladder bile of young hamsters was higher when the diet contained 10% of the fat from a high linoleic acid margarine than when the diet contained 10% butter fat. The main source of carbohydrate was glucose; in consequence thereof the incidence of cholesterol gallstones was high among the hamsters receiving the butter-fat diet, but very low among the hamsters receiving the margarine-fat diet.

In the present study we are comparing the ratio between lipid-soluble phosphorus and cholesterol in the bladder bile of groups of hamsters reared on:

1. A basal diet having rice starch as the carbohydrate component and containing no added fat,
2. The basal diet modified by incorporation of 10% hydrogenated palm oil at the expense of the same weight of rice starch,
3. The basal diet modified by incorporation of 4% of hydrogenated palm oil and 6% of sunflower seed oil at the expense of same weight of rice starch.

The purpose being to examine the influence of a margarine fat component almost completely devoid of essentiel fatty acids, and the influence of a mixture of the same component with sunflower seed oil in a proportion corresponding to that in a dietetic margarine fat containing 39–40% linoleic acid.

The use of diets in which the carbohydrate component is rice starch almost completely excludes the appearance of gallstones even when the diet contains no added fat (2). This effect is associated with the observation that compared with the "fat-free glucose diet", the "fat-free rice starch diet" produces higher ratios between lipid-soluble phosphorus and cholesterol, an, especially, higher ratios between total bile acids and cholesterol, in the bladder bile of young hamsters (2). For practical reasons the present study had to be limited to determination of the ratio between lipid-soluble phosphorus and cholesterol.

### Experimental

The animals were young females from our stock colony of hamsters, 34–37 days old at the beginning of the experimental feeding which lasted from 56 to 64 days. They were housed in individual cages with wire screen bottom. Diet and water were available *ad libitum* and

were changed twice a day. The diet was removed from the cages about 20 hours before, and the water about 4 hours before the animals were killed with chloroform and autopsied. Collection of bladder bile and analyses of the bile with respect to lipid-soluble phosphorus and cholesterol were carried out as in our previous studies (1, 2). In some cases bile from two or more animals had to be pooled in order to obtain a quantity sufficient for the determinations.

The composition of the diets is shown in table 1.

*Table 1.* Composition of diets

| | Diet no. 1<br>g | Diet no. 2<br>g | Diet no. 3<br>g |
|---|---|---|---|
| Casein, crude[1] | 20.0 | 20.0 | 20.0 |
| Rice starch | 74.3 | 64.3 | 64.3 |
| Hydrogenated palm oil | 0.0 | 10.0 | 4.0 |
| Sunflower seed oil | 0.0 | 0.0 | 6.0 |
| Salt mixture[2] | 5.0 | 5.0 | 5.0 |
| Vitamin mixture[3] | 0.5 | 0.5 | 0.5 |
| Choline chloride | 0.2 | 0.2 | 0.2 |
| | 100.0 | 100.0 | 100.0 |

[1] „Dairinex", from A/S Dansk Mejeri Industri & Export Kompagni, Stege, Denmark.
[2] See footnote 2 to table 1 in reference (1).
[3] See footnote 3 to table 1 in reference (1).

The fatty acid composition of the two fats – shown in table 2 – was determined by gas-liquid chromatography of the methyl esters using the same methods and equipment as in our previous studies (1, 3).

*Table 2.* Fatty acid composition of the two fats used. Per cent of the methyl esters of the individual fatty acids in the methyl esters of the total fatty acids from each fat.

| Fatty acid<br>(Number of carbon atoms<br>and double bonds) | Hydrogenated<br>palm oil,<br>mp. 44–46 °C[1] | Sunflower<br>seed oil[2] |
|---|---|---|
| 8:0 | 0.25 | — |
| 10:0 | 0.39 | — |
| 12:0 | 4.64 | — |
| 14:0 | 2.29 | 0.19 |
| 16:0 | 47.72 | 5.47 |
| 16:1 | — | 1.10 |
| 18:0 | 9.79 | 4.49 |
| 18:1 (oleic) | — | 30.42 |
| 18:1 (partly isomers of oleic) | 34.15 | — |
| 18:2 (linoleic) | — | 65.76 |
| 18:2 (isomers of linoleic) | 0.74 | — |
| 18:3 | — | 1.18 |
| 20:1 | — | 0.69 |
| 22:1 | — | 0.69 |

[1] Obtained from A/S Grindstedværket, Brabrand 8220, Denmark.
[2] Obtained from Margarine-Compagniet A/S, Copenhagen 1563, Denmark.

*Table* 3. Millimolar concentrations of cholesterol (C), lipid-soluble phosphorus (P) and ratio P/C in bladder bile of hamsters reared on Diets 1, 2 and 3

| Diet no. 1 | | | | | Diet no. 2 | | | | | Dit no. 3 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Group no. / animal no. | Days on diet | C | P | P/C | Group no./ animal no. | Days on diet | C | P | P/C | Group no./ animal no. | Days on diet | C | P | P/C |
| 1287/118/187 | 56 | 1.6 | 8.1 | 5.1 | 1292/86/28 | 56 | 2.8 | 11.8 | 4.2 | 1297/92 | 56 | 1.7 | 13.9 | 8.2 |
| 1288/53 | 56 | 0.7 | 4.7 | 6.7 | 1293/11 | 56 | 1.4 | 4.7 | 3.4 | 1297/127 | 56 | 1.4 | 10.9 | 7.8 |
| 1289/5 | 58 | 1.7 | 7.2 | 4.2 | 1292/97 | 56 | 1.1 | 6.5 | 5.9 | 1297/37/1298/81/40/67/73 | 56 | 1.4 | 10.8 | 7.7 |
| 1289/90 | 58 | 1.4 | 8.5 | 6.1 | 1293/51 | 56 | 1.9 | 16.8 | 8.8 | 1298/101 | 58 | 2.5 | 14.4 | 5.8 |
| 1289/1290/122/71 | 58 | 2.8 | 15.2 | 5.4 | 1293/144 | 58 | 3.2 | 12.7 | 4.0 | 1299/13/15 | 58 | 1.2 | 6.8 | 5.7 |
| 1290/24 | 58 | 1.6 | 7.6 | 4.8 | 1294/27/64 | 58 | 2.0 | 11.4 | 5.7 | 1299/57 | 58 | 3.9 | 17.7 | 4.5 |
| 1291/43/6 | 64 | 1.6 | 5.2 | 3.3 | 1294/46 | 58 | 1.3 | 9.5 | 7.3 | 1300/56 | 58 | 1.2 | 11.0 | 9.2 |
| 1291/2 | 64 | 2.3 | 5.7 | 2.5 | 1295/9 | 58 | 2.7 | 10.8 | 4.0 | 1300/47 | 58 | 3.0 | 23.9 | 8.0 |
| 1291/62 | 64 | 1.4 | 5.7 | 4.1 | 1295/34 | 58 | 2.4 | 14.1 | 5.9 | 1301/79 | 64 | 0.9 | 5.4 | 6.0 |
| 1290/75 | 64 | 1.3 | 4.4 | 3.4 | 1295/65 | 64 | 3.1 | 18.2 | 5.9 | 1300/32/1301/49 | 64 | 2.5 | 16.0 | 6.4 |
| 1290/148 | 64 | 2.6 | 10.3 | 4.0 | 1295/1296/91/131 | 64 | 1.8 | 6.9 | 3.8 | 1301/48 | 64 | 1.1 | 8.0 | 7.2 |
| | | | | | 1296/95 | 64 | 2.2 | 4.6 | 2.1 | | | | | |
| Mean value[1] of P/C | | | | | | | | | | | | | | |
| all samples | | | 4.5 ± 0.4 | | all samples | | | 5.1 ± 0.5 | | all samples | | | 7.0 ± 0.5 | |
| 8 non-pooled samples | | | 4.5 ± 0.5 | | 9 non-pooled samples | | | 5.3 ± 0.7 | | 8 non-pooled samples | | | 7.1 ± 0.5 | |

[1] With standard deviation.

## Results

The data determined in the bladder biles are shown in table 3; the weights of the animals appear from table 4.

*Table 4.* Mean body weight at beginning of the experiment and weight increase during 6 and 7 weeks, with standard deviation of the mean.

| Diet no. | Number of hamsters | Mean body weight at beginning of experiment g | Weigh increase during 6 weeks g | Weight increase during 7 weeks g |
|---|---|---|---|---|
| 1 | 14 | 50.6 ± 2.5 | 22.8 ± 3.1 | 26.5 ± 3.2 |
| 2 | 16 | 50.5 ± 2.0 | 31.6 ± 3.4 | 37.2 ± 3.5 |
| 3 | 17 | 48.7 ± 1.5 | 33.6 ± 3.7 | 37.7 ± 3.9 |

None of the hamsters had gallstones.

It is seen that the mean values of the molar ratio P/C is lowest with diet no. 1 containing no added fat (P/C = 4.5), slightly higher with diet no. 2 containing 10% hydrogenated palm oil (P/C = 5.1), and considerably higher with diet no. 3 containing 4% hydrogenated palm oil and 6% sunflower seed oil (P/C = 7.0).

Calculations of significance based on the values from non-pooled samples show that the difference with respect to the ratio P/C between the hamsters on diet no. 1 and the hamsters on diet no. 2 is not significant ($0.4 > P > 0.3$), whereas the difference with respect to the same ratio between the hamsters on diets no. 1 and 3 is highly significant ($0.01 > P > 0.001$). The corresponding difference between the hamsters on diets no. 2 and 3 is lower, however ($0.1 > P > 0.05$).

The different efficiency of the fats used in diets no. 2 and 3 in causing an increase of the ratio P/C over the value obtained with the "fat-free" diet no. 1 is assumed to be due to the high content of linoleic acid in the fat of diet no. 3 and the practical absence of linoleic acid in the fat used in diet no. 2.

The gains in weight during 6 weeks and during 7 weeks are higher for the hamsters on the diets containing fat than for the hamsters on the diet without added fat. The weight gain is almost the same with both types of fat, i. e., apparently independent of the content of linoleic acid in the fats.

The mean value 5.1 of the ratio P/C obtained with 12 samples from female hamsters receiving diet no. 2 may be compared with the mean value 4.3 of the same ratio obtained with 10 samples from female hamsters receiving a diet with 10% butter fat and glucose as the main carbohydrate in our previous study (1); similarly, the mean value 7.0 of the ratio P/C obtained with 11 samples from female hamsters receiving diet no. 3 may be compared with the mean value 6.3 of the same ratio obtained with 9 samples from female hamsters receiving a diet with 10% high linoleic acid margarine fat, and glucose as the main carbohydrate in our previous study (1).

The fact that the growth rates for hamsters on diets 2 and 3 were nearly equal is reflected in our study (1) where the growth rates were nearly equal for hamsters on butter fat diet and for hamsters on a diet containing the fat of a high linoleic acid margarine.

Apparently, the content of linoleic acid in the diet plays no major role as growth limiting factor within a feeding period of 6 or 7 weeks under the conditions of the present and the former experiments (1).

*Summary*

Groups of young female hamsters were reared for 56 to 64 days on the following diets:

1. A basal diet having rice starch as the carbohydrate component and containing no added fat.

2. The basal diet modified by incorporation of 10% hydrogenated palm oil at the expense of the same weight of rice starch.

3. The basal diet modified by incorporation of 10% of a mixture of 4 parts of hydrogenated palm oil and 6 parts of sunflower seed oil at the expense of the same weight of rice starch.

At the end of the feeding period the molar ratio between lipid-soluble phosphorus and cholesterol in the bladder bile was lowest with diet no. 1, slightly but not significantly higher with diet no. 2, and considerably and significantly higher with diet no. 3.

The gain in weight of the animals was almost the same with diet no. 2 as with diet no. 3, and significantly greater than with diet no. 1.

*Zusammenfassung*

Gruppen von jungen weiblichen Hamstern wurden während 56–64 Tagen mit den folgenden Nahrungen gefüttert:

1. Eine Basal-Nahrung mit Reisstärke als Kohlenhydrat- Komponente, ohne Zulage von Fett.

2. Nahrung Nr. 1 durch Austausch von 10% Reisstärke gegen 10% gehärtetes Palmöl modifiziert.

3. Nahrung Nr. 1 durch Austausch von 10% Reisstärke gegen 4% gehärtetes Palmöl und 6% Sonnenblumenöl modifiziert.

Am Ende der Fütterungsperiode wurde das molare Verhältnis zwischen lipid-löslichem Phosphor und Cholesterin in der Blasengalle bestimmt.

Dieses Verhältnis (P/C) war am niedrigsten für die mit Nahrung Nr. 1 gefütterten Hamster, etwas, aber nicht signifikant höher für die mit Nahrung Nr. 2 gefütterten Hamster, und beträchtlich und signifikant höher für die mit Nahrung Nr. 3 gefütterten Hamster.

Die Gewichtszunahme während der Fütterung war fast genau dieselbe für die mit Nahrung Nr. 2 gefütterten wie für die mit Nahrung Nr. 3 gefütterten Hamster, und signifikant größer als die Gewichtszunahme der mit Nahrung Nr. 1 gefütterten Hamster.

## References

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