EFFECT OF DIETARY FATS RICH IN 12:0, 14:0, 16:0, 18:1 and 18:2 ON PLASMA, HEPATIC AND BILIARY LIPIDS IN CHOLESTEROL-FED HAMSTERS.

Elke A. TRAUTWEIN, A. KUNATH-RAU, J. DIETRICH, S. DRUSCH and H.F. ERBERSDOBLER

Institute of Human Nutrition and Food Science, University of Kiel, Düsternbrooker Weg 17, 24105 Kiel, Germany

ABSTRACT

To test the effect of different dietary fats on plasma, hepatic and biliary lipids, male golden Syrian hamsters were randomly assigned to six groups and fed purified diets for seven weeks. The purified diets were made by blending different fats: olive oil (18:1) and sunflower oil (18:2). Dietary 18:2 (from safflower oil) was kept constant at 2% energy in these diets (except sunflower diet), in which fat supplied 12% of energy and cholesterol was added at 0.4%. Plasma lipids were analyzed at baseline and after 5 and 7 weeks on diet. Hepatic and biliary lipid concentration was assessed at the end after 5 and 7 weeks. All diets markedly increased plasma cholesterol and triglycerides compared to baseline. After 7 weeks, plasma cholesterol concentrations were highest with the palmstearin (343±70 mg/dL), coconut oil (343±47 mg/dL) and olive oil (357±25 mg/dL) diets and lowest with the rapeseed oil (259±10 mg/dL) and sunflower oil (290±45 mg/dL) diets while the butter diet was intermediate (327±25 mg/dL). Triglyceride concentration did not differ significantly between the six dietary fats. Hepatic cholesterol was highest in the olive oil diet (88±17 mg/g) and lowest in the palmstearin diet (56±10 mg/g). Biliary lipids, lithogenic index and bile acid profile did not differ significantly among the six diets.

INTRODUCTION

Syrian golden hamsters fed a low-fat diet with added cholesterol develop gallstones, but the incidence tends to be variable, in part, for genetic and dietary reasons. The type of fat, especially the fat saturation may also influence bile cholesterol concentration, bile acid composition and cholesterol cholelithiasis. Substitution of olive oil for butter prevented stone formation whereas palmitic acid added to the diet enhanced cholesterol gallstone formation (1,2).

In the present study, the impact of specific dietary fatty acids on cholesterol and bile acid metabolism was investigated using the cholesterol-fed hamster model. The fatty acid composition of the diets were modified in order to test whether substitution of monounsaturated (18:1) or polyunsaturated (18:2) fatty acids for specific saturated fatty acids (12:0, 14:0 and 16:0) would alter biliary lipids, bile acid profile and the incidence of cholesterol gallstones in the hamster.

MATERIALS AND METHODS

Animals and diets

Male golden Syrian hamsters (Charles River Lakeview strain, Sulzfeld, Germany) weighing 50 g were randomly assigned to six groups (n=10 per group) and fed purified cholesterol-rich diets for up to 7 weeks. The basal composition (g/kg dry weight) of the diet was as follows: Casein, 200; cornstarch, 385; glucose, 200; cellulose, 100; fat, 50; mineral mix, 46; vitamin mix, 12; cholin chloride, 3, and cholesterol, 4. Diets were fed as starch gels. The fatty acid composition of the diets are summarized in TABLE 1. Fat supplied 12% of energy and cholesterol was added at 0.4%. Dietary 18:2 (from safflower oil) was constant at 2% energy in all diets except the diet high in poly-

unsaturates. Hamsters were housed in groups of 3-4 animals per cage and kept in a controlled environment with a 12-hour light-dark cycle (lights on 18:00 h). Food and fresh water were provided daily *ad libitum*, and body weight were monitored on a weekly basis. All protocols were approved by the University Animal Care and Use Committee.

TABLE 1: Fatty acid composition of the fat blends

	Butter 14:0 +16:0	Palmstearin 16:0	Coconut oil 12:0 +14:0	Rapessed oil 18:1	Olive oil 18:1	Sunflower oil 18:2
			% of total fatty acids			
12:0	3.5 (0.4)	0.2 (0.0)	39.8 (4.8)	0.0 (0.0)	0.0 (0.0)	0.2 (0.0)
14:0	11.2 (1.3)	1.1 (0.1)	15.0 (1.8)	0.0 (0.0)	0.0 (0.0)	0.2 (0.0)
16:0	32.6 (3.9)	47.5 (5.7)	8.6 (1.0	5.6 (0.7)	12.8 (1.5)	7.6 (0.9)
16:1	1.8 (0.2)	0.3 (0.0)	0.0 (0.0)	0.0 (0.0)	1.1 (0.1)	0.0 (0.0)
18:0	7.8 (0.9)	3.8 (0.5)	2.3 (0.3)	1.5 (0.2)	2.6 (0.3)	3.6 (0.4)
18:1	19.3 (2.3)	28.9 (3.5)	7.5 (0.9)	58.2 (7.0)	63.7 (7.7)	22.6 (2.7)
18:2	16.2 (2.0)	16.8 (2.0)	17.2 (2.1)	20.8 (2.5)	19.0 (2.3)	63.0 (7.6)
18:3	0.7 (0.1)	0.2 (0.0)	0.1 (0.0)	11.4 (1.4)	0.1 (0.0)	1.1 (0.1)

Values in parentheses () represent percent energy contributed by individual fatty acid.
To all diets except the sunflower oil diet safflower oil was added to equalize 18:2

Plasma lipid analysis

Blood samples were drawn from animals fasted overnight at baseline and after 5 and 7 weeks. Blood samples were collected under light anaesthesia into a EDTA-wetted syringe by cardiac puncture and plasma was separated immediately by centrifugation at 12,000 g for 5 min. Total plasma cholesterol, HDL-cholesterol and triglycerides were determined by enzymatic assays as previously described (3,4)

Necropsy and gallstone evaluation after 5 and 7 weeks

Hamsters were exsanguinated under anesthesia and liver and cecum excised, blotted and weighted. A portion of the liver was frozen for hepatic cholesterol analysis. Gallbladder bile was aspirated and analyzed for biliary lipids and bile acids. The gallbladder was dissected from the liver, opened under a dissecting microscope, and examined along with the remaining gallbladder bile for cholesterol crystals, cholesterol and pigment gallstones under regular and polarized light by light microscopy as previously described (3,4).

Biliary and hepatic lipids analysis

Biliary lipids, bile acid profile and hepatic cholesterol concentration were analyzed as previously described (3,4).

RESULTS AND DISCUSSION

During the 5 and 7 week period, hamsters from all diet groups were healthy and demonstrated normal weight gain. No significant differences were noted in the initial and final body weight, the daily weight gain or the cecal and epididymal fat weight. Livers of hamsters fed the butter and palmstearin diet were significantly heavier after 5 weeks but after 7 weeks no differences in liver weight existed between the 6 diets.

Plasma cholesterol concentrations markedly increased in all diets compared to baseline (TABLE 2). After 7 weeks, plasma cholesterol concentrations were highest with the olive oil, palmstearin and coconut oil diets and lowest with the rapeseed oil and sunflower oil diets while the butter diet was intermediate . Plasma triglyceride concentrations also increased compared to the baseline value, but no significant differences were observed between diets, even though the rapeseed oil and sunflower oil

diets tended to decrease triglycerides after 7 weeks (TABLE 2).

Hepatic cholesterol was highest in the olive oil diet (88±17 mg/g) and lowest in the palmstearin diet (56±10 mg/g)). Biliary lipids, lithogenic index and bile acid profile did not differ significantly among the six diets. The lithogenic index did not differ significantly between the six diets and exceeded 1.0 in all diets.

Thus, rapeseed and sunflower oil diets revealed the lowest plasma cholesterol and triglyceride concentration whereas olive oil failed to demonstrate a cholesterol-lowering effect when compared with the diets rich in saturated fatty acids. Since 18:2 was kept constant in these diets, the different response of rapeseed and olive oil could possibly be attributed to either their different amount of 16:0 or the amount of 18:3 in rapeseed oil.

TABLE 2: Plasma lipid concentration in hamsters fed diets with different fatty acids

	Butter 14:0+16:0	Palmstearin 16:0	Coconut 12:0+14:0	Rapeseed 18:1	Olive oil 18:1	Sunflower oil 18:2
				mg/dL		
Baseline						
TC: 100±11						
TG: 164±32						
after 5 weeks						
TC	287±71	366±102[a]	248±41	223±48[a]	271±29	247±44
TG	794±895	501±466	174±47	246±70	191±37	228±98
HDL-C	149±72	202±56	190±31	130±62	179±32	142±14
	(50±14)	(56±13)	(69±9)	(56±17)	(66±12)	(54±11)
after 7 weeks						
TC	327±25	343±39	343±47[a]	259±10[ab]	357±70[b]	290±45
TG	250±74	202±134	269±111	138±22	375±188	190±122
HDL-C	195±43	230±26	217±61	169±46	215±26	164±45
	(59±9)	(65±4)	(62±13)	(65±17)	(61±8)	(56±9)

Mean±SD

* values in parentheses () are HDL-C as % TC

Means in a row sharing a common superscript are significantly different ($p<0.05$) using ANOVA and Tukey Compromise Test.

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REFERENCES

1) Cohen, B.I., Mosbach, E.H., and McSherry C.K. (1990) Dietary fat modulates cholesterol cholelithiasis in the hamster. *Hepatology* 12, 898.
2) Cohen, B.I., Mosbach, E.H., Ayyad, N., Miki, S., and Mc-Sherry, C.K. (1992). Dietary fat and fatty acids modulate cholesterol cholelithiasis in the hamster. *Lipids* 27, 526-532.
3) Trautwein, E.A., Siddiqui, A., and Hayes, K.C.(1993).Modelling plasma lipoprotein-bile lipid relationships: Differential impact of psyllium and cholestyramine in hamsters fed a lithogenic diet. *Metabolism* 42, 1531-1540.
4) Trautwein, E.A., Liang J., and Hayes, K.C. (1993). Cholesterol gallstone induction in hamsters reflects strain differences in plasma lipoproteins and bile acid profiles. *Lipids* 28, 305-312.