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Effects of diet on the lipid composition of *Echinostoma caproni* (Trematoda) in ICR mice

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Abstract High-performance thin-layer chromatography (HPTLC) was used to determine neutral lipids and phospholipids in the intestinal trematode *Echinostoma caproni* from experimentally infected ICR mice fed a high-fat diet (hen's egg yolk) as compared with worms from mice fed a standard laboratory diet. Worms were removed from the hosts at 2, 3, and 4 weeks postinfection (p.i.). Analysis by TLC-densitometry showed significantly greater amounts of triacylglycerols and free sterols at 2, 3, and 4 weeks p.i. in worms from mice on the high-fat diet as compared with worms from mice on the standard laboratory diet. Significantly greater amounts of phosphatidylcholine and phosphatidylethanolamine were found in worms from mice on the high-fat diet as compared with worms from those on the standard diet at 2 weeks p.i. but not at 3 and 4 weeks p.i. The results of this study suggest that the host diet influences the lipid content of *E. caproni* adults.

Introduction

The relationship between host nutrition and parasitic worms has received considerable attention, and most studies have concerned the effects of high-carbohydrate or high-protein diets on cestodes and acanthocephalans (Crompton and Nesheim 1982; Nesheim 1984). Less information is available on the effects of host nutrition on intestinal trematodes, and the influence of host dietary lipids on the chemical constituents of digeneans has not been studied.

A particular intestinal trematode model, that of *Echinostoma caproni* (see Fried and Huffman 1996), has proved useful for studies on host-parasite interactions of echinostomes in murine hosts. Echinostomes are ubiquitous intestinal trematodes that affect humans, domestic animals, and wildlife and are valuable from an experimental standpoint because they can easily be cycled in the laboratory using pulmonate snails and mice (Fried and Huffman 1996). Using this model, Sudati et al. (1996) found that *E. caproni* adults obtained from ICR mice fed a high-fat diet (hen's egg yolk) showed reduced body growth, altered distribution in the host intestine, decline in worm recovery from hosts, and reduced fecundity, i.e., they produced fewer eggs than worms from mice maintained on a standard diet.

Chemical analysis of the lipid constituents of the worms from mice on either diet were not performed by Sudati et al. (1996). Intestinal trematodes such as echinostomes feed mainly on host mucosal contents (see Fried and Vonroth 1968), but the influence of host ingesta in the lumen of the gut has not been assessed for these parasites. The purpose of our study was to determine if a hen's-egg-yolk diet would influence the lipid contents of the worms. To accomplish this goal, worms maintained for 2–4 weeks postinfection (p.i.) in mice fed the high-fat diet were analyzed using high-performance thin-layer chromatographic (HPTLC) analysis, and the results were compared with those obtained in worms maintained similarly on mice fed a standard laboratory diet.

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Materials and methods

To obtain adult worms for this study, metacercarial cysts of *Echinostoma caproni* were removed from the kidney-pericardial cavity of experimentally infected *Biomphalaria glabrata* snails and were fed (approximately 25 cysts/host) by stomach tube to 36 outbred, female ICR mice aged 6–8 weeks (Sudati et al. 1996).

To obtain worms from hosts on a high-fat regimen, one-half of the mice infected with cysts were fed a dried hen's-egg-yolk diet (EYD) containing 16% protein, 31% lipid, 49% water, 2% carbohydrate, and 2% ash (Sigma Chemical Co., St. Louis, Mo.). The remaining mice (control mice) were fed a rat-mouse-hamster 3000 diet (RMH) containing 5% lipid, 23% protein, 61% carbohydrate, 5% salt, 4% fiber, and 2% vitamins (U.S. Biomedicals Co., Cleveland, Ohio). Food and water were provided ad libitum.

Mice were necropsied at 2, 3, and 4 weeks p.i. At necropsy the small intestines were removed from the pyloric sphincter to the ileocecal valve, and worms were removed from the intestine as described by Hosier and Fried (1986). Approximately 15 ± 5 worms were recovered per host at necropsy, and worms were washed in several changes of Locke's solution prior to use. Samples were prepared using 20, 10, and 5 worms per sample at weeks 2, 3, and 4, respectively. The average wet weight for each batch at 2, 3, and 4 weeks was 66, 58, and 26 mg, respectively.

Each batch of 20, 10, or 5 worms (for weeks 2, 3, and 4, respectively) was extracted in 3 ml of chloroform-methanol (2:1, v/v) in a glass homogenizer. The extract was filtered through glass wool and aqueous components were removed with 1.0 ml of Folch wash (0.88% aqueous KCl). The lipid extract was evaporated under nitrogen and reconstituted in 100–300 µl of choloroform-methanol (2:1, v/v).

HPTLC was performed as described by Masterson et al. (1993) using Whatman (Clifton, N.J.) LHP-KDF high-performance 10 × 20-cm channeled, preadsorbent silica-gel plates. The standard used was a neutral lipid standard 18-4A (Matreya, Pleasant Gap, Pa.), which contained cholesteryl oleate, methyl oleate, triolein, oleic acid, and cholesterol at 0.20 µg/µl each. The standard was used to identify the presence of cholesterol esters, methyl esters, triacylglycerols, free fatty acids, and free sterols, respectively, and for quantification of lipids in the samples. The standard and reconstituted sample solutions were spotted in 2.0-, 4.0-, 8.0-, and 16.0-µl aliquots on the same plate using a 25-µl Drummond (Broomall, Pa.) digital microdispenser. Plates were developed using the Mangold solvent system (petroleum ether-diethyl ether-acetic acid; 80:20:2, by vol.) to a distance of 7.5 cm past the preadsorbent-silica-gel interface in a glass, paper-lined, twin-trough HPTLC chamber. Development required 15 min.

The plates were dried using a hair dryer, and lipids were detected by spraying with 5% ethanolic phosphomolybdic acid (PMA) and heating for 15 min at 115 °C to produce blue lipid zones on a light yellow background. (See Masterson et al. 1993 for photographs of lipids separated in this HPTLC system.) Sample and standard zones were scanned using a Shimadzu CS-930 densitometer operated in the single-beam, single-wavelength mode at 700 nm. Calculation of lipid percentages based on the scan areas of the four standards and the single sample zone with an intensity closest to the two middle standards was performed as described by Higgs et al. (1990).

Phospholipid analysis was done as described by Gennaro et al. (1996). The standard used was Matreya polar lipid mix 1127, which contained cholesterol, phosphatidylethanolamine, phosphatidylcholine, and lysophosphatidylcholine at 0.25 mg/ml each. The silica-gel HPTLC plates were spotted and developed with chloroform-methanol-water (65:25:4, by vol.) as described above for neutral lipids. Phospholipids were detected as black spots on a white background by spraying of the dried plate with a 10% solution of cupric sulfate in 8% phosphoric acid and heating in an oven at 160 °C for 10 min. (See Gennaro et al. 1996 for a photograph of lipids separated in this HPTLC system.) Phospholipid zones were scanned at 370 nm.

Results

Worms from mice fed the egg-yolk diet or the standard laboratory chow contained free sterols (R_f = 0.23), triacylglycerols (R_f = 0.66), and cholesterol esters (R_f = 0.91). Visual analysis usually showed denser zones of free sterols, triacylglycerols, and cholesterol esters in worms from mice fed the high-fat diet as compared with those from mice on the laboratory chow diet. A free fatty acid fraction (R_f = 0.32) was often seen in worms from mice on the high-fat diet but not in those from control mice. Densitometric analysis confirmed that worms obtained from mice on high-fat diets had significantly greater amounts (Student's *t*-test, $P \leq 0.05$) of both free sterol and triacylglycerol at weeks 2, 3, and 4 than did those from the controls (see Table 1). Linearity correlation coefficients (*R* values) for lipid and phospholipid calibration curves were at least 0.95 during quantitative analysis.

Worms from mice fed the egg-yolk diet or the standard laboratory chow diet contained phosphatidylcholine (R_f = 0.41) and phosphatidylethanolamine (R_f = 0.61). Qualitative TLC analysis showed relatively equal zones of each constituent in worms from mice fed either diet. A faint lysophosphatidylcholine band (R_f = 0.26) was often seen in worms from mice on the high-fat diet but not in worms from mice fed laboratory chow.

Densitometric analysis showed that at 2 weeks p.i., worms from mice on the high-fat diet had significantly greater amounts of both phosphatidylcholine and phosphatidylethanolamine (Student's *t*-test, $P < 0.05$) than did worms on the control diet (Table 2). There was no significant difference in the amount of

Table 1 Levels of neutral lipids detected in worms from mice on the control diet or the high-fat diet

Group	Week p.i.	Number of trials	Mean percentage of wet weight ± SE	
			Free sterols	Triacylglycerols
Control diet:				
*A	2	3	0.38 ± 0.04	0.39 ± 0.05
*B	3	4	0.39 ± 0.06	0.3 ± 0.1
*C	4	4	0.5 ± 0.2	3.3 ± 0.3
High-fat diet:				
*A′	2	3	0.78 ± 0.02	0.67 ± 0.03
*B′	3	4	0.86 ± 0.02	1.7 ± 0.4
*C′	4	4	3.3 ± 0.3	7.9 ± 0.3

*$P < 0.05$ for values recorded for worms from groups of mice on high-fat diets (A′, B′, C′) as opposed to those on control diets (A, B, C) for both free sterols and triacylglycerols at all weeks p.i. (Student's *t*-test)

Table 2 Levels of phospholipids detected in worms from mice on the control diet or the high-fat diet

Group	Week p.i.	Number of trials	Mean percentage of wet weight ± SE	
			Phosphatidylcholine	Phosphatidylethanolamine
Control diet:				
*A	2	3	0.050 ± 0.005	0.054 ± 0.002
B	3	3	0.27 ± 0.05	0.07 ± 0.01
C	4	3	0.8 ± 0.4	1.0 ± 0.3
High-fat diet:				
*A′	2	3	0.18 ± 0.02	0.58 ± 0.05
B′	3	3	0.29 ± 0.09	0.11 ± 0.03
C′	4	3	1.4 ± 0.4	1.5 ± 0.4

$P < 0.05$ for values recorded for group A′ as compared with group A for both phosphatidylcholine and phosphatidylethanolamine (Student's *t*-test)

phosphatidylcholine or phosphatidylethanolamine detected between worms from control mice versus worms from mice on high-fat diets at 3 or 4 weeks p.i. (Table 2).

Discussion

Previous silica-gel TLC studies have provided qualitative information on the presence of lipids in echinostomes. Thus, the major neutral lipid found in *Echinostoma trivolvis* (referred to as *E. revolutum* in that study) and *E. caproni* adults was free sterol (Fried and Boddorf 1978; Horutz and Fried 1995), but the major neutral lipid detected in *E. malayanum* was triacylglycerol (Yusufi and Siddiqi 1976). The major phospholipids found in *E. trivolvis* adults (also referred to as *E. revolutum* in that study) were phosphatidylcholine and phosphatidylethanolamine (Fried and Shapiro 1979). The present study provides for the first time quantitative information on the major neutral lipids and phospholipids of *E. caproni* adults.

The results of our study suggest that the host diet influences the lipid content of *E. caproni* adults. Thus, mice fed hen's egg yolk (which contains abundant amounts of triacylglycerol and free sterol) have worms with significantly greater amounts of free sterol and triacylglycerol than do control mice. Our study shows that host ingesta can influence the lipid content of an intestinal trematode. However, it should also be noted that murine hosts on an abnormal diet such as hen's egg yolk may have an altered mucosal environment with possible changes in their normal lipid constituents. Indeed, Horutz and Fried (1995) used qualitative TLC and showed that the intestinal mucosa of mice infected with *E. caproni* adults had more free fatty acids than did that of uninfected hosts. The increased levels of free fatty acids observed in the aforementioned study may have resulted from an altered intestinal mucosa caused by worms grazing on that site or by the release of worm excretory-secretory products. Therefore, the increase in the lipid content of worms from hosts on the high-fat diet may have resulted from a combination of both host ingesta and worms feeding on an altered gut mucosa.

Alterations in phospholipid constituents observed between the two worm populations were not as dramatic as those seen in the neutral lipids, and significant differences in the worm content of phosphatidylcholine and phosphatidylethanolamine were seen only at week 2 p.i. Phospholipids, being structural components of membranes, are probably less subject to dietary influences than is a neutral lipid such as triacylglycerol, which serves as a storage or depot lipid in intestinal parasites.

This is the first study that has examined the influence of host dietary lipids on the chemical constituents of any helminth, and it is therefore not possible to generalize our findings to other worm species. There is considerable basic information on various chemical constituents in larval and adult trematodes, cestodes, and nematodes (see the review in Fried and Haseeb 1996). Information from the aforementioned review should be helpful to future investigators who wish to examine the question as to how host dietary nutrients influence the chemical constituents of helminths.

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