Journal of the Egyptian Society of Parasitology, Vol.43, No.2, August 2013
J. Egypt. Soc. Parasitol., 43(2), 2013: 517 – 526

FATTY ACIDS CONTENTS IN *BIOMPHALARIA ALEXANDRINA* DURING THE COURSE OF INFECTION WITH *SCHISTOSOMA MANSONI*

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Abstract

The study examined the effects of larval trematode infection on the neutral lipid and phospholipid content of *Biomphalaria alexandrina* infected with *Schistosoma mansoni*. Uninfected snails were used as matched controls. As determined by qualitative high-performance silica gel thin-layer chromatography (HPTLC), the major neutral lipids present in the whole bodies and digestive gland-gonad complexes in both infected and uninfected snail populations were free sterols, free fatty acids, and triacylglycerols, and the major polar lipids were phosphatidylcho-line and phosphatidyl ethanolamine. Quantitative analysis by HPTLC with visible and UV scanning reflectance densitometry showed the snail's digestive gland lipid level was found to be almost halved in 20 days post infection; a more then 80% reduction being visible after the subsequent 40 and 60 days.

Key Words: Egypt, *Biomphalaria alexandrina*, fatty acids, *Schistosoma mansoni*

Introduction

Schistosoma mansoni infections in *Biomphalaria* snails alter the quality and quantity of lipids in this snail host (Massa *et al*, 2007). For instance, Thompson (1997) found that *B. glabrata* infected with *S. mansoni* had significantly higher levels of triacylglycerols in the digestive gland-gonad complex (DGG) than that of uninfected control snails. By the using of the *B. glabrata-S. mansoni* model, Muller *et al*. (2000) found that at 8 weeks post-infection, the concentrations of the free sterols and triacylglycerols in the infected DGG increased about two times compared to the uninfected control snails. Thompson *et al*. (1991) analyzed neutral lipids and phospholipids in the DGGs of uninfected *B. glabrata* and infected with *S. mansoni* found reduced levels of phosphatidylcholine and several other phospholipids in the DGGs infected snails. Fried *et al*.(1989) determined the effects of larval *E. caproni* infection on the neutral lipid content of the DGG in *B. glabrata* and found that the triacylglycerols, steryl esters, and free fatty acids were elevated in control DGGs compared to those in infected ones. Perez *et al.* (1994) analyzed the phospholipids in the DGG of *B. glabrata* infected with *E. caproni* and found that the mean weight percentage of phosphatidylserine was 1.5 times greater in the DGG of infected vs uninfected snails.

Tunholi-Alves*et al*.(2011) studied the

effect of *Biomphalaria glabrata* exposure to different doses (5 & 50) of *Echinostoma paraensei* miracidia on the total levels of cholesterol and triglycerides circulating in the hemolymph and the neutral lipids in the digestive gland-gonad (DGG) complex. The snails were dissected one, two, three and four weeks after infection to collect the hemolymph and DGG tissue, to measure the levels of cholesterol and triglycerides in the hemolymph and neutral lipids in the tissue. The hemolymph showed a similar order of variation for both substrates tested in the first week after infection. The lipids reduced levels in the infected snails indicated intense use of these substrates both by the intermediate host and the parasite, suggesting its probable participation in the energy metabolism and structural construction of the developing larval stages. Alterations in the profile of neutral lipids in the DGG were also found. The results obtained indicate that in this model, the lipid metabolism depends on the miracidial dose used (Fried *et al*, 2001).

Beers *et al*. (1995) analyzed the effects of *E. caproni* infection on *B. glabrata*. An analysis of the snails fed a hen's egg yolk-lettuce mixed diet showed that the triacylglycerols were significantly reduced in the DGGs of infected snails, whereas the free sterols were significantly elevated in the DGGs of infected snails. The triacylglycerol level was reduced but not significantly different in the DGGs of infected vs uninfected snails. DGGs with daughter rediae of *E. caproni* were used as samples, but snails the infection was pre-patent. The conflicting of some results in the previous studies on lipid content in *Biomphalaria* infected with *S. mansoni*, initiates this research to examine using high-performance TLC (HPTLC) analysis on infected snails and matched controls. So, this study focus on neutral lipid and phospholipid analysis of *B. alexandrina* infected with *S. mansoni* under conditions of controlled miracidial infection and snail diet.

Materials and Methods

Snail infection: Group of 90 juvenile *Biomphalaria alexandrina* (3-5mm in diameter) was exposed individually to 10 newly hatched miracidia of *Schistosoma mansoni* obtained from Schistosome Biological Supply Project, Theodor Bilharz Research Institute, in vials containing 3ml dechlorinated water for 3-4 hours. After that, snails were gathered in 3 aquaria containing dechlorinated water and food was presented as dry oven lettuce leaves and water was changed three times a week. Exposed snails were used 10, 20 & 30 days post miracidial exposure in HPTLC studies. Another group of uninfected (50 snails) cross-matched snails served as a control was prepared for HPTLC analysis as previously described.

Snails in G1 (infected) and G2 (control) were divided into two subgroups. In the first subgroup, whole bodies of snails were obtained by carefully removing the shells with needles and forceps. Digestive gland-gonad complexes (DGG) were removed from the remaining viscera and placed into test tubes containing 10 ml chloroform: methanol (1:1) mixture, covered with a lid and

placed in a refrigerator (5°C) until needed. Three DGG were pooled per sample.

In the second subgroups, hemolymph was obtained by cracking the shell in a Petri dish, tipping the dish, and collecting the hemolymph from the edge with a pipette. Hemolymph from three snails was pooled to prepare one sample of about 100µl. Plasma (hemolymph minus hemocytes) was obtained after centrifuging the hemolymph for 6 min at 5000×*g* and separating the top layer (plasma) from the pellet (hemocytes and debris). The pellet was discarded.

Lipids were extracted from the digestive gland (Folch *et al,* 1957) using a chloroform: methanol (1:1) mixture, then separated into fractions of triglycerids (triacetin) and phospholipids by thin-layer chromatography on silica gel in a mixture of hexane: ether: acetic acid (73:25:2). Chromatograms were developed in iodine vapour. Separated fractions of lipids were exposed to alkaline methanolysis, and fatty acid methyl esters were separated by gas-liquid chromatography using a Pye 104 model 24 gas chromatograph. Identification of individual fatty acids was made by comparing the relative retention time of standard fatty acids and their mass spectra, revealed by gas chromatograph-mass spectrometry. The fatty acid content was estimated as the area under a peak computed by triangulation. The data were expressed as the percentage of the sum of all fatty acids of the given lipid fraction. The plasma was processed by the same procedure as the digestive gland.

Gas chromatography (GC): FAMEs were separated and quantified by capillary gas chromatography. The chromatography system consisted of a Hewlett Packard gas chromatograph (model 6890), a flame ionization detector, and Hewlett-Packard Chem Station software. The injection port and the detector temperatures were 270°C & 280°C, respectively. The split ratio was 1:20. The flow rates of compressed air and hydrogen were 300 mL/min and 30 mL/min, respectively. Helium was used as carrier gas (2.8 mL/min). The oven temperature was programmed at a rate of 6.5°C/min from 130°C (1 min hold) to 170°C, increased to a rate of 2.75°C/min to 215°C, again increased to a rate of 40°C/min to 230°C, was held for 12 min. Total fatty acids levels and spectra of FAMEs are obtained by HP 3365 Chem Station computer program. Chemical structures of FAMEs were determined by analyses of spectra and by comparing obtained spectra with the spectra of authentic standards (Sigma-Aldrich Chemicals). Individual FAME was identified by comparison with the chromatographic behaviors of authentic standards (Sigma-Aldrich Chemicals).

Statistical analysis: Analyses were performed using SPSS (12.0). Percentages of fatty acids were tested by analysis of variance (ANOVA) and comparisons between means were performed with Tukey's test. The differences between means were considered to be significant whenever $P < 0.05$.

Results

The results are shown in tables (1, 2, 3 & 4).

Table 1: Fatty acid contents in phospholipids of *B. alexandrina* infected with *S. mansoni* (expressed as % of total phospholipid fatty acids).

Fatty Acids	Control Mean* ± S.E. **	Exposed snails to miracidia after		
		10 days	20 days	30 days
		Mean* ± S.E. **	Mean* ± S.E. **	Mean* ± S.E. **)
C12:0	0.29 ± 0.03a	-	-	0.10 ± 0.01a
C14:0	1.70 ± 0.11a	-	-	1.50 ± 0.11a
C15:0	0.47 ± 0.14a	0.43 ± 0.13a	0.70 ± 0.26a	0.80 ± 0.04a
C16:0	20.95 ± 1.24a	20.30 ± 1.24a	24.06 ± 1.23b	30.02 ± 1.29c
C17:0	0.46 ± 0.04a	0.50 ± 0.04a	2.54 ± 0.18b	0.50 ± 0.02a
C18:0	8.68 ± 0.75a	9.01 ± 0.85a	11.72 ± 0.94a	11.41 ± 0.96a
∑SFA	32.55 ± 1.42a	30.24 ± 1.41a	39.02 ± 1.75b	44.33 ± 1.95c
C16:1ω7	3.60 ± 0.35a	7.12 ± 0.69b	14.70 ± 1.16c	3.95 ± 0.32a
C18:1ω9	35.46 ± 1.40a	33.11 ± 1.48a	18.13 ± 1.19b	13.02 ± 1.21c
C20:1ω9	0.31 ± 0.02a	0.38 ± 0.03a	0.52 ± 0.05a	3.12 ± 0.31
∑MUFA	39.37 ± 1.62a	40.41 ± 1.85a	33.35 ± 1.39b	20.09 ± 1.23c
C18:2ω6	16.02 ± 1.12a	20.10 ± 1.28b	9.03 ± 0.87c	13.05 ± 1.22d
C18:3ω3	3.06 ± 0.29a	2.33 ± 0.25a	3.84 ± 0.32a	5.82 ± 0.53b
C20:2ω6	1.02 ± 0.11a	1.11 ± 0.09a	1.13 ± 0.08a	2.11 ± 0.22b
C20:4ω6	3.70 ± 0.31a	3.92 ± 0.29a	7.09 ± 0.72b	8.07 ± 0.82b
C20:5ω3	3.76 ± 0.35a	2.02 ± 0.18b	6.60 ± 0.74c	5.03 ± 0.49d
C22:2ω6	-	-	-	1.50 ± 0.09
∑PUFA	27.56 ± 1.35a	29.33 ± 1.26a	27.59 ± 1.25a	35.58 ± 1.44b

Means averages of 3 replicates of 1 sample. Values = mean ± S.E. **Means with same letter in each row do not significantly differ from each other, *P*> 0.05. SFA: Saturated Fatty Acids, MUFA: Monounsaturated Fatty Acids, PUFA: Polyunsaturated Fatty Acids

Table 2: Fatty acid contents in neutral lipids of *B. alexandrina* infected with *S. mansoni* (expressed as % of total neutral lipids fatty acids).

Fatty Acids	Control Mean* ± S.E. **	Exposed snails to miracidia after		
		10 days	50 days	40 days
		Mean* ± S.E. **	Mean* ± S.E. **	Mean* ± S.E. **
C10:0	0.66 ± 0.06a	-	-	1.10 ± 0.10b
C12:0	0.74 ± 0.07a	0.12 ± 0.01a	0.57 ± 0.05a	2.03 ± 0.20b
C13:0	0.71 ± 0.07a	0.20 ± 0.02a	3.12 ± 0.28b	3.75 ± 0.28b
C14:0	4.04 ± 0.38a	2.00 ± 0.21b	4.24 ± 0.39a	4.63 ± 0.36a
C15:0	1.38 ± 0.12a	1.60 ± 0.12a	2.19 ± 0.20b	1.62 ± 0.14a
C16:0	19.85 ± 1.25a	22.08 ± 1.25b	24.21 ± 1.32b	27.90 ± 1.33c
C17:0	0.68 ± 0.05a	2.30 ± 0.28b	3.00 ± 0.25c	1.68 ± 0.14d
C18:0	7.36 ± 0.68a	4.33 ± 0.29b	10.26 ± 0.90c	7.08 ± 0.68a
∑SFA	35.42 ± 1.43a	32.63 ± 1.120a	47.59 ± 2.23b	49.79 ± 2.30b
C16:1ω7	7.21 ± 0.65a	5.47 ± 0.56b	6.18 ± 0.58c	7.49 ± 0.71a
C18:1ω9	16.99 ± 1.20a	36.20 ± 2.16b	17.60 ± 1.25a	7.84 ± 0.75c
C20:1ω9	6.14 ± 0.55a	4.10 ± 0.35b	0.73 ± 0.08c	2.28 ± 0.24d
∑MUFA	30.34 ± 1.42a	45.47 ± 2.21b	24.51 ± 1.31c	17.61 ± 1.20d
C18:2ω6	11.84 ± 0.96a	15.56 ± 1.10b	12.10 ± 0.85a	10.50 ± 0.85a
C18:3ω3	7.56 ± 0.68a	2.10 ± 0.16b	10.92 ± 0.80c	15.40 ± 1.18d
C20:2ω6	1.94 ± 0.11a	0.71 ± 0.07b	0.54 ± 0.06b	1.64 ± 0.12a
C20:3ω6	1.31 ± 0.10a	-	-	-
C20:4ω6	5.92 ± 0.57a	1.48 ± 0.10b	1.94 ± 0.11b	3.53 ± 0.32c
C20:5ω3	4.70 ± 0.41a	2.10 ± 0.32b	2.18 ± 0.20b	2.33 ± 0.22b
C22:2ω6	-	-	-	0.20 ± 0.02
C22:6ω3	1.86 ± 0.10a	-	-	-
∑PUFA	35.13 ± 1.48a	21.95 ± 1.29b	27.68 ± 1.39c	33.60 ± 1.38a

Table 3: Fatty acid contents in total body lipids of *B.alexandrina* infected with *S. mansoni* (expressed as % of total body lipids fatty acids).

Fatty Acids	Control Mean* ± S.E. **	Exposed snails to miracidia after		
		10 days	20 days	30 days
		Mean* ± S.E. **	Mean* ± S.E. **	Mean* ± S.E. **
C12:0	-	0.98 ± 0.08a	-	1.84 ± 0.14a
C13:0	1.67 ± 0.09a	0.63 ± 0.56b	1.63 ± 0.10a	1.60 ± 0.12a
C14:0	5.67 ± 0.52a	2.35 ± 0.25b	5.86 ± 0.49a	10.58 ± 0.92c
C15:0	1.45 ± 0.09a	1.18 ± 0.13a	1.77 ± 0.13a	1.51 ± 0.12a
C16:0	20.06 ± 1.22a	16.43 ± 1.06b	26.48 ± 1.35c	33.01 ± 0.30d
C17:0	1.84 ± 0.10a	0.90 ± 0.82b	0.43 ± 0.38b	1.80 ± 0.16a
C18:0	8.34 ± 0.75a	8.65 ± 0.75a	10.70 ± 0.95b	7.27 ± 0.68a
∑SFA	39.40 ± 1.52a	31.12 ± 1.42b	46.87 ± 1.85c	57.61 ± 2.04d
C16:1ω7	5.64 ± 0.48a	11.88 ± 0.95b	8.52 ± 0.74c	5.10 ± 0.48a
C18:1ω9	10.46 ± 0.94a	24.20 ± 1.23b	12.60 ± 1.04a	10.26 ± 0.92a
C20:1ω9	2.64 ± 0.21a	2.70 ± 0.21a	0.80 ± 0.72b	1.97 ± 0.18c
C22:1ω9	2.81 ± 0.22a	1.50 ± 0.11b	-	-
∑MUFA	21.60 ± 1.35a	40.28 ± 1.42b	21.92 ± 1.26a	17.33 ± 1.10c
C18:2ω6	16.43 ± 1.48a	9.37 ± 0.64b	13.11 ± 1.16c	7.22 ± 0.72d
C18:3ω3	8.67 ± 0.72a	11.00 ± 1.14b	11.72 ± 0.98c	5.17 ± 0.56d
C20:2ω6	2.54 ± 0.22a	2.32 ± 0.34a	0.42 ± 0.36b	3.40 ± 0.39c
C20:4ω6	0.81 ± 0.08a	1.92 ± 0.21b	1.47 ± 0.14b	4.40 ± 0.32c
C20:5ω3	6.86 ± 0.47a	1.13 ± 0.08b	5.60 ± 0.49a	3.46 ± 0.35c
C22:2ω6	3.25 ± 0.22a	2.41 ± 0.12a	1.47 ± 0.10b	2.20 ± 0.23a
C22:6ω3	2.12 ± 0.23	-	-	-
∑PUFA	39.98 ± 1.53a	28.15 ± 1.34b	31.68 ± 1.42c	25.50 ± 1.32d

Table 4: Total fatty acid contents *in B. alexandrina* infected with *S. mansoni*

Fatty Acids	Control Mean* ± S.E. **	Exposed snails to miracidia after		
		10 days	20 days	30 days
		Mean* ± S.E. **	Mean* ± S.E. **	Mean* ± S.E. **
∑SFA	32.55 ± 1.42a	29.24 ± 1.31a	39.22 ± 1.65b	45.32 ± 1.75c
	35.42 ± 1.33a	30.43 ± 1.50a	48.39 ± 2.33b	50.89 ± 2.50b
	39.00 ± 1.42a	30.15 ± 1.42b	47.67 ± 1.65c	58.81 ± 2.14d
∑MUFA	40.37 ± 1.42a	40.41 ± 1.75a	34.45 ± 1.49b	20.09 ± 1.23c
	31.34 ± 1.32a	45.57 ± 2.30b	23.31 ± 1.21c	17.61 ± 1.21d
	24.30 ± 1.35a	40.28 ± 1.42b	20.82 ± 1.22a	18.33 ± 1.12c
∑PUFA	28.56 ± 1.25a	28.33 ± 1.36a	27.59 ± 1.23a	36.58 ± 1.45b
	36.13 ± 1.28a	20.95 ± 1.39b	27.68 ± 1.32c	34.60 ± 1.36a
	40.18 ± 1.43a	26.15 ± 1.24b	31.68 ± 1.42c	24.50 ± 1.22d

Discussion

As a source of energy, neutral lipids are extremely important in pulmonate snails during the course of schistosome infection (Duncan *et al*, 1987; Stuart and Ballantyne, 1996; Kobucar *et al*, 1997). Triacylglycerols, the component lipids, are initially hydrolyzed to free fatty acids which are, in turn, oxidized in the fatty acid cycle within the mitochondrial matrix (Voogt, 1984; Tripathi and Singh, 2002). Fatty acids detected in total body lipids, phospholipid, and neutral lipid fractions from infected and uninfected *B. alexandrina* include C10:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0 SFAs; C16:1ω7, C18:1ω9, C20:1ω9 MUFAs & C18:2ω6, C18:3ω3, C20:2ω6, C20: 3ω6, C20:4ω6, C20:5ω3, C22:2ω6,

C22:6ω3 PUFAs. Fatty acids are very common in marine and freshwater mollusks (Pollero *et al*, 1983).

Lipids have many functions in biological systems, energetic and structural. In snails it has been identified that the lipids are involved in the animals survival under the physiological stress conditions, such as long food restriction or when the snails are parasitized, when the carbohydrates reserves are quickly depleted and the lipids are consumed more frequently and changes in different kinds of lipids are observed (Storey, 2002; Giokas *et al*, 2005; Bandstra, 2006a). They are structural components of biological membranes and alterations on number and composition of thelipids may happen when changes in the metabolic state of the snails occur in response to stress factors (Stuart *et al*, 1998a, b).

The lipid contents in the snails assayed after 10 days post infection were evidently smaller than those in the control. The lipid contents became clearly lower as the infection proceeded. For example, the digestive gland of those snails examined after 20 days post infection showed extensive reduction as a result of the activities of the sporocysts and cercariae present; the content of lipid was much lower than that in the control. Similar patterns were observed for lipid content in the digestive gland of the snails examined after 30 days post infection.

The present results revealed that the qualitatively most important fatty acids in gastropods are similar in *B. alexandrina* and fatty acid profiles of the snail conform to the common pattern, characteristic of gastropods in general (Pollero *et al*, 1981; 1983; Storey, 2002; Giokas *et al*, 2005). In the present analyses, C16:0, C18:0, C18:1ω9 and C18:2ω6 acids were major components. Small proportions of C10:0, C12:0, C13:0, C22:2ω6, and C22:6ω3 acids were also found. Similar findings were also reported in other works (Dembitsky *et al*, 1992; Abad *et al*, 1995; Misra *et al*, 2002; Fried *et al*, 2001; Go *et al*, 2002). Temperature, food availability, and metabolic and physiological activities can affect the lipid and fatty acids composition of mollusks (Misra *et al*, 1985; Abad *et al*, 1995; 2002). In this study, the statistical analyses revealed significant differences in ∑SFA, ∑MUFA&∑PUFA levels in *B. alexandrina*.

Mollusks stored lipids for reproductive purposes (Bandstra *et al*, 2006a); however, they provide energy during winter, when carbohydrate reserves are depleted (Bandstra *et al*, 2006b). So, fatty acid composition of snail, neutral lipids in particular, is dictated by snail's metabolic activities which were affected by the presence of schistosome parasite within the snails. The parasites obtain energy predominantly by catabolism of carbohydrates (Barret and Saghir, 1999), so they deplete the hosts' carbohydrate reserves at the onset of infection, while the snails draw energy by decomposing lipids. This is very plausible, particularly in view of the fact that lipids are virtually the sole energy source in numerous fasting or hibernating animals. A parasitic infection confronts an organism with a situation resembling that of fasting (Dun-

kan *et al*, 1987). The loss of lipids in the digestive gland of the parasite infected snails is evidence that the snails mobilize energy resources accumulated in that form to compensate for the loss of carbohydrates in these parasites.

The triacylglycerols store SFAs for energy purposes and they also may be temporary PUFAs reservoir (Ackman, 1983; Napolitano *et al*, 1992), which could be transferred to structural lipids or directed to specific metabolic pathways. In contrast, phospholipid fractions of mollusks show considerably less seasonal variation to maintain structural integrity of the cell as compared to neutral lipids (Chu and Greaves, 1991).

The loss of lipids in the digestive gland of the infected snails points at mobilization of lipid energy reserves to compensate for the deficiency of carbohydrates, used by these parasites. As a source of energy, neutral lipids are extremely important in pulmonate snails (Duncan *et al*, 1987; Stuart and Ballantyne, 1996; Kobucar *et al*, 1997). The results showed that the digestive glands parasitized by *S. mansoni* are reduced in amount of neutral (storage) lipids. Humiczewska and Rajsk, (2005) reported that carbohydrate levels were greatly reduced during a parasitic infection that agreed with others (Jarusiewicz *et al.,* 2006).

Conclusion

Schistosomiasis is still to be complicated unsolved health problem encountering medical, socio-economic and behavioral parameters worldwide. The severity of risk factors for schistosomiasis infection depends basically on the concentration of cercariae population coming out of snails in natural water bodies. Besides, freshwater snails serve as intermediate host for risky zoonotic trematodes.

The outcome results showed that the loss of lipids in the digestive gland of the infected snails point at mobilization of lipid energy reserves to compensate for the deficiency of carbohydrates, used by these parasites and the concentration of triacylglycerols in the infected digestive gland-gonad complex was significantly less than that of the uninfected.

Acknowledgements

This study was kindly financial support by the Internal Research Project No. 98C, Theodor Bilharz Research Institute. The experiments described in this work comply with the current research laws of Egypt.

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