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Fatty acid composition of *Biomphalaria glabrata* (Gastropoda: Planorbidae) maintained at low, ambient, and high temperatures as determined by GC-MS

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ABSTRACT

The fatty acid composition of *Biomphalaria glabrata* snails maintained at 17 (cold), 24 (ambient), and 31 °C (warm) was determined by using gas chromatography-mass spectrometry (GC-MS). Snails were necropsied following two weeks of maintenance at the above temperatures and the whole snail bodies were pooled to provide samples that were later analyzed. Palmitic (16:0), eicosanoic (20:1), stearic (18:0), α-linoleic (18:3 n-3), and linoleic (18:2 n-6) acids comprised 67% of the total fatty acids in the snails maintained at 31 ºC. α-Linoleic (18:3 n-3), palmitic (16:0), eicosanoic (20:1), stearic (18:0), and linoleic acids (18:2 n-6) comprised 66% of the fatty acids of the snails maintained at 24 ºC. Eicosanoic (20:1), stearic (18:0), linoleic (18:2 n-6), oleic (18:1), and α-linoleic acid (18:3 n-3) comprised 63% of the fatty acids of the snails maintained at 17 ºC. No significant differences (ANOVA) were found in any of the individual fatty acids as a function of maintaining the snails at the three different temperatures. However, there were significant differences in the total combined unsaturated versus combined saturated fatty acid content among the snails maintained at the three different temperatures.

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INTRODUCTION

Biomphalaria glabrata (Say, 1816) is a medically important snail often used in research on *Schistosoma mansoni*, a trematode that causes the debilitating disease schistosomiasis. Recent concerns of global climate change leading to the spread of snail borne infections such as schistosomiasis make studies of environmental effects on *B. glabrata* a priority. The effects of temperature on various biological characteristics of *B. glabrata*, such as survival, growth, and fecundity, have been previously described [1]. High performance thin layer chromatography (HPTLC) has been used to examine the effects of temperature on the neutral lipid content of *B. glabrata*, and the results of the study showed significant differences in triacylglycerol content of snails maintained at 14, 28, and 32 °C for up to two weeks [2]. There is no study that has examined the fatty acid content in *B. glabrata* in response to temperature change.

The effects of seasonal temperature variations on the composition of fatty acids in *Cepaea nemoralis*, a pulmonate land snail, have been previously studied [3]. No significant differences in fatty acid composition as a function of temperature changes were found, and it was concluded that

environmental temperature changes did not affect the fatty acid composition of these land snails [3]. Other environmental conditions have been found to affect fatty acid composition in aquatic snails. Significant differences were found in the fatty acid composition of *B. glabrata* fed hen's egg yolk versus those fed leaf lettuce [4]. While no differences were found in the fatty acids in the pulmonate land snail *Cepaea nemoralis* [3], the purpose of our study was to examine possible differences in the fatty acids in a representative aquatic pulmonate snail, *B. glabrata*.

Therefore, we examined the fatty acid composition of *B. glabrata* maintained at three temperatures, 17, 24, and 31 °C. We also compared total unsaturated and saturated fatty acids in the snails maintained at these three temperatures for a period of 2 weeks.

EXPERIMENTAL

Sample preparation

Sexually mature *B. glabrata* snails ranging from 9.7 to 16.3 mm (NMRI strain) were obtained from Dr. Fred A. Lewis, Head, Schistosomiasis Laboratory, Biomedical Research Institute (Rockville, MD, USA). The snails were maintained in 1 L mason jars containing artificial spring water (ASW) [5] and were fed boiled leaf lettuce *ad libitum*. The water and food in the snail cultures were changed every other day. The snails were maintained at five per culture and the cultures were kept at 17, 24, and 31 °C. Snails were exposed to a 12 hr light and 12 hr dark cycle.

The snails were necropsied 2 weeks after the initiation of the temperature maintenance. At necropsy the shells were removed and discarded and the whole snail bodies were pooled to obtain samples weighing from 313 to 407 mg; each sample consisted of 2 to 4 snails, and a total of four pools were maintained at 31 °C, three pools at 24 °C, and three pools at 17 °C.

As previously described [2], the samples were homogenized in a 7 mL Pyrex Tenbroeck tissue grinder (No. 08-414-10B, Fisher Scientific, Pittsburgh, PA, USA) with 3 mL of chloroform-methanol (2:1, v/v). These extracts were filtered through cotton wool and were then treated with 0.75 mL of Folch wash (0.88% KCl). The lipid-containing lower phase was separated and evaporated just to dryness under a stream of nitrogen at room temperature. The total lipid samples were dissolved in 5 mL of chloroform-methanol (2:1, v/v) and transferred into a 25 mL round bottom flask, and the solvent removed by rotary evaporation at 50 ºC. The lipids were converted into fatty acid methyl esters by treatment with 10 mL of 1% methanolic sodium methoxide solution overnight at ambient temperature. The fatty acid methyl esters were extracted in a 100 mL separatory funnel using 40 mL of petroleum ether (40-60 ºC), and the extract was dried by passing through anhydrous sodium sulfate. The solvent was removed by rotary evaporation at 40 °C, and the residue was dissolved in 1 mL of petroleum ether. Five µL of each test solution was injected into the gas chromatography-mass spectrometry (GC-MS) instrument using a 10 µL syringe.

Fatty acid analysis by GC-MS

The analysis of the fatty acid methyl esters was carried out by using a Shimadzu (Columbia, MD, USA) QP5000 GC-MS system equipped with an Omegawax 250 fused silica capillary column (30 m x 0.25 mm id, 0.25 um film thickness; Supelco, Bellefonte, PA, USA) and quadrupole mass spectrometer operated in total ion current (TIC) mode. The carrier gas was helium with a flow rate of 0.8 mL/min. The temperature gradient program of the column was as follows: isothermal at 160 ºC for 5 min, increased to 240 ºC at 4 ºC/min, and isothermal at 240 ºC for 25 min (50 min total analysis time). The injection port temperature was 240 ºC. The GC peaks were identified by comparison with the retention times of fatty acid methyl ester standards (Supelco 37 component FAME mixture, Catalog No. 18919-1AMP) and by the National Institute of Standards and Technology (NIST; Gaithersburg, MD, USA) MS library.

Quantitative results were determined by area normalization, in which the percentage of each component was calculated from its peak area divided by the total peak area [4]. ANOVA from Microsoft Excel was used to determine statistical significance among snail samples maintained at the different temperatures, with $P < 0.05$ considered as significant.

RESULTS AND DISCUSSION

Results are presented in Table 1. Palmitic (16:0), eicosanoic (20:1), stearic (18:0), a-linoleic (18:3 n-3), and linoleic acid (18:2 n-6) comprised 67% of the total fatty acids in the snails maintained at 31 ℃. α-Linoleic (18:3 n-3), palmitic (16:0), eicosanoic (20:1), stearic (18:0), and linoleic acid (18:2 n-6) comprised 66% of the fatty acids of the snails maintained at 24 ℃. Eicosanoic (20:1), stearic (18:0), linoleic (18:2 n-6), oleic (18:1), and α-linoleic acid (18:3 n-3) comprised 63% of the fatty acids of the snails maintained at 17 ℃. However, no significant differences (ANOVA) in the content of free fatty acids among the *B. glabrata* snails at the three temperatures were found in this study, as can be seen in Table 1, Column 1, Entries 1-15. There were significant differences in the total combined unsaturated and combined saturated fatty acid content among the snails maintained at these three temperatures; the snails maintained at 31 ℃ had a significantly lower combined unsaturated fatty acid content and higher combined saturated fatty acid content when compared to the other two snail groups (see Column 1 of Table 1, Entries 16 and 17).

The major finding in our study is the lack of significant differences in any of the individual fatty acids of the *B. glabrata* snails maintained at the three temperatures. A previous study using HPTLC found differences in the triacylglycerol fraction of these snails as a function of temperature [2]. The difference between that study and ours is that the former examined neutral lipid classes, whereas ours examined the fatty acid content of the total lipid fraction. If we preparatively isolated the neutral lipid fractions and examined the fatty acid content of the triacylglycerol fraction, perhaps we may observe differences in fatty acid composition as a function of temperature.

In the snails maintained at 31 ℃, we found significantly higher levels of the combined saturated fatty acids and significantly lower levels

Table 1. Fatty acid composition (area%) of snails maintained at 17, 24, and 31 ℃.

Entry No.	Fatty acids	Mean ± SE of 17 ℃ samples (n = 3)	Mean ± SE of 24 ℃ samples (n = 3)	Mean ± SE of 31 ℃ samples (n = 4)
1	Myristic acid (14:0)	1.6 ± 0.3	1.5 ± .3	1.5 ± 0.2
2	Pentadecanoic acid (15:0)	-	0.8[a]	1.0 [a]
3	Palmitic acid (16:0)	10.9 ± 1.9	13.2 ± 1.9	16.2 ± 1.5
4	Palmitoleic acid (16:1)	1.1 ± 0.2	0.9[a]	-
5	Heptadecanoic acid (17:0)	1.3 ± 0.2	1.3 ± 0.2	1.5 ± 0.3
6	Stearic acid (18:0)	14.1 ± 2.3	12.5 ± 2.6	13.3 ± 1.3
7	Oleic acid (18:1)	11.7 ± 1.6	9.6 ± 1.9	10.0 ± 1.1
8	Linoleic acid (18:2n-6)	12.2 ± 0.7	12.5 ± 1.3	11.4 ± 1.0
9	α-Linoleic acid (18:3n-3)	11.2 ± 3.5	16.2 ± 5.2	11.9 ± 3.0
10	Eicosanoic acid (20:1)	15.6 ± 2.5	13.1 ± 2.5	15.9 ± 1.7
11	Eicosodienoic acid (20:2n-6)	4.7 ± 0.5	5.3 ± 0.6	5.3 ± 0.4
12	Eicosotrienoic acid (20:3n-6)	1.43[a]	1.1[a]	0.7
13	Eicosotrienoic acid (20:3n-3)	1.30[a]	1.3 ± 0.1	1.0 ± 0.1
14	Arachidonic acid (20:4n-6)	6.2 ± 2.4	5.7 ± 1.6	4.4 ± 1.0
15	Unidentified	9.5 ± 1.4	7.4 ± 0.9	7.7 ± 0.7
16	Total saturated fatty acids[b]	27.9 ± 4.7	29.3 ± 5.2	33.5 ± 3.3
17	Total unsaturated fatty acids[b]	65.3 ± 3.4	65.5 ± 4.4	60.6 ± 2.9

[a]Not enough samples gave values, so no standard error (SE) could be calculated.
[b]$P < 0.001$.

of the combined unsaturated fatty acids compared to the snails maintained at 24 ºC and 17 ºC. This decrease in the combined saturated fatty acids with lower environmental temperatures is crucial for normal cell function because it increases the membrane fluidity [6].

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