



Fatty Acid Composition of Fat Body and Malpighian Tubules of the Tenebrionid Beetle, *Zophobas atratus*: Significance in Eicosanoid-Mediated Physiology

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ABSTRACT. Total and phospholipid fatty acid composition of fat body and Malpighian tubules from two larval stages, pupae and adults, of *Zophobas atratus* were analyzed. Saturated and unsaturated C16 and C18 fatty acids were major components and varied by life stage and tissue source. Eicosanoid-precursor fatty acids, including 20:3n-6, 20:4n-6 and 20:5n-3, were present in low quantities and varied by life stage and tissue source. 20:3n-6 was always present in the lowest proportions, indicating that eicosanoids derived from 20:4n-6 and 20:5n-3 (the 2- and 3-series) are likely to be of greater physiological significance in this insect. Fatty acid composition of *Z. atratus* fat body and Malpighian tubules was independent of diet, suggesting that this insect controls its fatty acid composition to meet the needs of individual tissues and ontogenetic constraints. Copyright © 1996 Elsevier Science Inc. COMP BIOCHEM PHYSIOL 115B;4:429–437, 1996.

KEY WORDS. Eicosanoid, prostaglandin, fatty acid, PUFA, immunity, water regulation, fat body, Malpighian tubule

INTRODUCTION

Eicosanoids are oxygenated biologically active metabolites of three polyunsaturated fatty acids (C20 PUFA), namely 20:3n-6, 20:4n-6 and 20:5n-3. When appropriately analyzed, eicosanoids have been detected in every animal tissue examined (2,33,34). Although most of our information on eicosanoid actions stems from their clinical significance in mammals (8,9,18,21), recognition of the biological significance of eicosanoids in invertebrates is also rapidly increasing (6,11,16,31,32,34,40). Eicosanoids mediate cellular immune responses to bacterial infections in larvae of the tobacco hornworm, *Manduca sexta* (19,43), and the beetle, *Zophobas atratus* (20). One group of eicosanoids, prostaglandins, modulate basal fluid secretion rates in Malpighian tubules from adult females of the mosquito, *Aedes aegypti* (26), and from the ant, *Formica polyctena* (52). Prostaglandins are also found in Malpighian tubules from adult mealworm beetles, *Tenebrio molitor* (14), where they are suspected to similarly regulate fluid secretion. Eicosanoids have been shown to regulate thermoregulatory sweating in the desert cicada, *Tibicen dealbatus* (48), and they are involved with the reproductive behavior and egg-laying behavior in several insect species (38,45).

The presence and biological significance of eicosanoids

in insects has stimulated considerable research into the presence of eicosanoid-precursor PUFA in insect tissue lipids (33,44). Despite an extensive literature on insect fatty acid compositions, based largely on gas chromatographic analyses during the 1960s and 1970s (7,44), little information on the presence and metabolism of C20 PUFA in insect tissue lipids is available (35,44,50). The information we do have indicates that terrestrial insects depart from the usual patterns of fatty acid compositions in animals by often maintaining very low proportions (<1.0%) of C20 PUFA in their cellular lipids. Several theoretical and practical issues are associated with the low proportions of C20 PUFA in insect tissue lipids. On the practical side, the low abundance of these acids means that they have been overlooked in most analyses (7,44), and special attention must be given to analytical protocols when looking for them. On the theoretical side, there are many biochemical issues: do insects actively maintain low proportions of C20 PUFA in their cellular lipids, or do the low proportions result from environment constraints, such as diet? Are there local pools of C20 PUFA within complex cellular lipids? Do specific tissues maintain unique fatty acid compositions? Are there ontogenetic correlates to fatty acid compositions?

With a view to gaining a more detailed understanding of insect eicosanoid systems, we approached some of the above questions by conducting detailed analyses of total fatty acids (TL) and phospholipid (PL) fatty acids of fat body and Malpighian tubules of larvae, pupae and adults of the beetle *Z.*

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atratus. As noted in a preliminary analysis (20), very low proportions of C20 PUFA occur in the tissue lipids of this insect. Here, we report substantial differences in fatty acid profiles of fat body and Malpighian tubules; moreover, we show that tissue fatty acid profiles change during post-embryonic development.

MATERIALS AND METHODS

Organisms

Cultures of the tenebrionid beetle *Z. atratus* were started from larvae obtained from a local pet store. All beetles used in these experiments were in the second or third generation from culture initiation. This species has an indeterminate number of larval instars, so immature stages were characterized by weight. Small larvae (third or fourth instar) weighed ca. 0.1 g. Medium larvae (fifth or sixth instar) weighed ca. 0.3 g. Pupae used were 1–2 weeks post-pupation, and adults used were 1–2 weeks post-eclosion. All insects were unsexed. All stages were reared on a diet consisting of six parts rolled oats to one part unbleached flour containing 5% brewer's yeast. Water was provided by damp paper towels. Insects were held in open plastic rearing trays in incubators at $27 \pm 1^\circ\text{C}$ with a relative humidity (RH) of $70 \pm 10\%$ in constant darkness.

Determination of Fat Body and Malpighian Tubule Fatty Acid Compositions

Individual insects were anesthetized by chilling on ice for 5 min. Fat body tissue and Malpighian tubules were removed and separately suspended in 2 ml of chloroform:methanol (2:1, v/v) containing 50 μl of 2% butylated hydroxytoluene to minimize autoxidation of PUFAs. Total lipids and phospholipids were isolated as described in Stanley-Samuelson *et al.* (41,42). Each sample was homogenized and separated into two portions. The total lipid portion was directly transmethylated by refluxing in acidified methanol for 90 min. After cooling, the fatty acid methyl esters (FAME) were extracted three times in hexane, dried over sodium sulfate, concentrated to dryness with nitrogen and then made up to 100 μl with hexane. The remaining total lipid fraction was concentrated to dryness with nitrogen, dissolved in a small amount of hexane, applied to thin-layer chromatographic plates (20 \times 20 cm, 0.25-mm silica gel G plates, Sigma Chemical Co., St Louis, MO) and developed in hexane:ethyl ether:acetic acid (80:20:1 v/v/v). Phospholipids remain at the origin. These were removed by scraping the band into 15-ml Teflon-lined screw cap reaction tubes and transmethylating as described previously. Twenty insects were used to prepare five replicate analyses of the four *Zophobas* life stages we examined.

Chromatographic Analysis

The fatty acid methyl esters were chromatographed on a Hewlett-Packard 5890 Series II gas chromatograph (San

Fernando, CA) equipped with a Supelcowax 10 capillary column (30 m by 0.25 mm, 0.25 μm film thickness, Supelco, Inc., Bellefonte, PA) and a flame ionization detector. Data acquisition and processing were done on a Zeos 486 PC (Zeos International, St. Paul, MN) with Hewlett-Packard Series II Chemstation software. The analyses were conducted using temperature programming at $2^\circ\text{C}/\text{min}$ from 150 to 250°C with an initial 2 min hold. Helium was the carrier gas at 0.6 ml/min. Components were tentatively identified by comparing their retention times to those of authentic standards (Sigma Chemical Co.).

Fatty acid identifications were confirmed by gas chromatography-mass spectrometry. The fatty acid methyl esters were analyzed on a Hewlett-Packard 5890 Series II gas chromatograph interfaced with a Hewlett-Packard 5971 electron impact mass selective detector operated at 70 eV. Separations were performed on a Supelcowax 10 capillary column identical to the one above but programmed at 1°C per min from 170 to 220°C . Chromatographic conditions included a 45-sec splitless injection, a 2-min initial hold period and the use of ultrapure helium as the carrier gas at 1 ml/min. Retention times and total ion mass spectra of fatty acid methyl esters were compared with authentic standards from Sigma Chemical or by comparison with published electron impact mass spectra (17,29).

Statistical Analyses

Compositional analyses of fatty acids were conducted as previously described. Area counts obtained from electronic integration were summed and converted into percent values. For ANOVA, percent values (as proportions) were converted into arcsin square root transformations. Reported means and variances are on the untransformed values. Summary statistics and analysis of variance were conducted using Statgraphics Plus, Version 6 (Statistical Graphics Corp., Rockville, MD).

RESULTS

The fatty acid compositions of TL and PL from fat body and Malpighian tubules of small and medium larvae, pupae and adults of *Z. atratus* are presented in Tables 1–4. Several observations apply to all life stages. First, as expected in animal tissue lipids, the quantitatively major components were 16:0, 16:1, 18:0, 18:1, and 18:2n-6. In addition to these five components, 18 other saturated and unsaturated fatty acids were detected in all samples. Second, although 18:0 is present in many insects at less than 5%, it accounted for 10–18% of the fatty acids present in each lipid class of *Z. atratus*. Third, the major polyunsaturated component, 18:2n-6, occurred in much higher proportions of PL fatty acids (>40%), compared with TL fatty acids ($\leq 25\%$). Fourth, eicosanoid-precursor PUFA were present in low, but readily measurable, proportions in all life stages.

The fatty acid profiles of TL and PL prepared from fat body and Malpighian tubules of small and medium weight

TABLE 1. Fatty acid percent composition (as methyl esters) of fat body and Malpighian tubules from small larvae of *Zophobas atratus*

Fatty acid	TL fat body	PL fat body	TL Malp. tub.	PL Malp. tub.
14:0	0.49 ± 0.03	0.13 ± 0.03	0.63 ± 0.06	0.55 ± 0.24
15:0	0.06 ± 0.00	0.05 ± 0.01	0.11 ± 0.02	0.13 ± 0.07
16:0	29.83 ± 1.66	19.76 ± 1.34	28.37 ± 1.49	13.27 ± 2.60
16:1	1.18 ± 0.20	0.63 ± 0.13	1.24 ± 0.23	0.67 ± 0.12
17:0	0.15 ± 0.01	0.18 ± 0.02	0.19 ± 0.02	0.33 ± 0.11
17:1	0.09 ± 0.01	0.12 ± 0.03	0.14 ± 0.04	0.20 ± 0.06
18:0	15.01 ± 4.70	11.20 ± 0.99	11.46 ± 1.96	16.01 ± 1.37
18:1	41.74 ± 3.25	26.32 ± 2.61	42.42 ± 0.92	25.43 ± 1.64
18:2	10.61 ± 0.44	39.38 ± 2.62	13.99 ± 0.73	40.47 ± 4.39
19:0	0.08 ± 0.07	0.07 ± 0.02	0.06 ± 0.01	0.09 ± 0.01
18:3n-3	0.18 ± 0.08	0.38 ± 0.18	0.21 ± 0.02	0.27 ± 0.15
20:0	0.24 ± 0.02	0.30 ± 0.12	0.29 ± 0.02	0.52 ± 0.28
20:1	0.12 ± 0.06	0.12 ± 0.10	0.11 ± 0.02	0.14 ± 0.08
20:2	0.06 ± 0.02	0.33 ± 0.44	0.23 ± 0.21	0.26 ± 0.07
20:3n-6	Tr*	0.01 ± 0.00	Tr	0.03 ± 0.01
20:4n-6	0.02 ± 0.01	0.20 ± 0.08	0.09 ± 0.04	0.21 ± 0.11
20:5n-3	Tr	0.04 ± 0.01	0.01 ± 0.01	0.06 ± 0.02
20:5n-3	0.04 ± 0.01	0.22 ± 0.03	0.10 ± 0.07	0.15 ± 0.02
22:0	0.03 ± 0.01	0.26 ± 0.10	0.19 ± 0.06	0.68 ± 0.47
22:1	0.01 ± 0.00	0.11 ± 0.02	0.03 ± 0.01	0.21 ± 0.05
23:0	0.03 ± 0.01	0.12 ± 0.03	0.04 ± 0.01	0.14 ± 0.05
24:0	0.01 ± 0.00	0.07 ± 0.02	0.06 ± 0.04	0.17 ± 0.05

Values are means ± SD.

*Trace, <0.01%.

larvae were similar (Tables 1 and 2). Oleic acid (18:1) was the major TL component and 18:2n-6 was the major PL component, both at about 40% of their respective lipid class. Although generally similar otherwise, fat body PL were higher in 16:0 (ca. 20% of PL fatty acids), compared with Malpighian tubule PL (ca. 11–13% of PL fatty acids).

The fatty acid profiles of TL and PL from fat body and Malpighian tubules of pupae are shown in Table 3. Compared with larval fat body TL patterns, the pupal fat body had slightly higher proportions of 16:1 (ca. 4% vs 1%), considerably lower proportions of 18:0 (ca. 5% vs 17%) and about double the proportions of 18:2n-6 (ca. 22% vs 11%). Pupal fat body PL fatty acid patterns were similar to the larval PL fat body patterns.

The fatty acid profiles of pupal Malpighian tubules were considerably different from their larval analogues. TL from pupal Malpighian tubules were higher in 16:1 (ca. 4% vs 1%), lower in 18:1 (ca. 31% vs 43%) and higher in 18:2n-6 (ca. 25% vs 16%) compared with larval preparations. The PL fatty acid patterns also differed. Preparations from pupae were higher in 16:0 (ca. 16% vs 11%), lower in 18:0 (ca. 10% vs 15%) and slightly higher in 18:2 (ca. 48% vs 45%).

The fatty acid compositions of TL and PL prepared from fat body and Malpighian tubules of adults (Table 4) were generally similar to the patterns from pupae, with some differences. The main differences were in Malpighian tubules. Compared with pupae, adult TL were considerably higher in 18:0 (ca. 17% vs 7%) and lower in 18:1 (ca. 31% vs 43%) and 18:2n-6 (ca. 20% vs 25%). The PL patterns in

adult and pupal Malpighian tubule preparations were similar.

Potential differences in eicosanoid-precursor PUFA were analyzed by plotting the proportions of PL 20:3n-6, 20:4n-6 and 20:5n-3 as histograms for each life stage and tissue (Figs. 1 and 2). In fat body, 20:4n-6 and 20:5n-3 were predominant in larval preparations, whereas they were less predominant in pupal and adult preparations. Total fat body PUFA content also varied by life stage (Fig. 3), with small and medium larvae having greater proportions of total PUFA than did pupae and adults. In Malpighian tubules, the total PUFA content was less than in fat body and did not vary by life stage. However, some differences in individual PUFA were noted in the Malpighian tubule PL preparations, with the small larvae and adults having significantly less 20:3n-6 than 20:4n-6 and 20:5n-3 (Fig. 2).

To assess the influence of diet on insect tissue fatty acid compositions, we analyzed the fatty acids associated with total lipid extracts of the beetle culture medium (Table 5). The fatty acid profile of the culture medium was different from the insect tissue fatty acid profiles, indicating that the insect fatty acid patterns do not directly mirror the fatty acid patterns of their food sources.

DISCUSSION

Eicosanoids mediate nodulation, a cellular immune response to bacterial infections, in *Z. atratus* (20). We tested the eicosanoid hypothesis by treating *Z. atratus* larvae with

TABLE 2. Fatty acid percent composition (as methyl esters) of fat body and Malpighian tubules from medium size larvae of *Zophobas atratus*

Fatty acid	TL fat body	PL fat body	TL Malp. tub.	PL Malp. tub.
14:0	0.50 ± 0.03	0.16 ± 0.04	0.59 ± 0.13	0.55 ± 0.09
15:0	0.06 ± 0.01	0.08 ± 0.02	0.20 ± 0.11	0.29 ± 0.05
16:0	30.15 ± 0.99	20.12 ± 2.18	26.75 ± 3.66	11.35 ± 0.64
16:1	1.12 ± 0.23	0.66 ± 0.19	0.99 ± 0.39	0.87 ± 0.13
17:0	0.16 ± 0.01	0.16 ± 0.02	0.21 ± 0.03	0.33 ± 0.03
17:1	0.11 ± 0.01	0.12 ± 0.03	0.15 ± 0.05	0.42 ± 0.26
18:0	16.99 ± 5.20	11.69 ± 1.81	9.65 ± 4.54	15.23 ± 0.68
18:1	38.93 ± 4.00	26.49 ± 2.63	43.30 ± 4.95	24.41 ± 1.50
18:2	11.12 ± 0.86	38.14 ± 2.65	16.91 ± 4.80	44.60 ± 1.30
19:0	0.04 ± <0.01	0.05 ± 0.01	0.06 ± 0.02	0.09 ± 0.02
18:3n-3	0.28 ± 0.02	0.39 ± 0.04	0.29 ± 0.04	0.39 ± 0.07
20:0	0.23 ± 0.02	0.41 ± 0.43	0.29 ± 0.03	0.23 ± 0.01
20:1	0.11 ± 0.01	0.16 ± 0.05	0.13 ± 0.03	0.06 ± 0.01
20:2	0.05 ± 0.02	0.14 ± 0.05	0.04 ± 0.02	0.23 ± 0.09
20:3n-6	0.02 ± 0.01	Tr*	0.05 ± 0.06	0.02 ± 0.02
20:4n-6	0.02 ± 0.01	0.06 ± 0.03	0.05 ± 0.03	0.12 ± 0.05
20:3n-3	Tr	0.01 ± 0.01	Tr	Tr
20:5n-3	0.04 ± 0.01	0.25 ± 0.08	0.08 ± 0.04	0.12 ± 0.03
22:0	0.02 ± 0.01	0.34 ± 0.35	0.13 ± 0.09	0.20 ± 0.04
22:1	0.01 ± <0.01	0.11 ± 0.02	0.04 ± 0.02	0.24 ± 0.05
23:0	0.02 ± 0.01	0.20 ± 0.19	0.03 ± 0.01	0.15 ± 0.08
24:0	0.01 ± <0.01	0.26 ± 0.31	0.06 ± 0.02	0.12 ± 0.04

Values are means ± SD.

*Trace, <0.01%.

TABLE 3. Fatty acid percent composition (as methyl esters) of fat body and Malpighian tubules from pupae of *Zophobas atratus*

Fatty acid	TL fat body	PL fat body	TL Malp. tub.	PL Malp. tub.
14:0	1.06 ± 0.64	0.08 ± 0.05	1.63 ± 0.57	0.34 ± 0.06
15:0	0.29 ± 0.18	0.15 ± 0.07	0.51 ± 0.10	0.21 ± 0.05
16:0	29.23 ± 2.27	20.46 ± 1.36	27.15 ± 1.90	15.88 ± 1.71
16:1	4.50 ± 0.80	1.54 ± 0.50	4.39 ± 0.64	1.46 ± 0.19
17:0	0.65 ± 0.14	0.56 ± 0.13	0.65 ± 0.09	0.64 ± 0.11
17:1	0.53 ± 0.09	0.28 ± 0.14	0.53 ± 0.08	0.53 ± 0.20
18:0	4.81 ± 0.95	11.07 ± 1.47	6.74 ± 1.18	10.18 ± 0.86
18:1	35.40 ± 3.41	17.73 ± 1.43	31.31 ± 1.34	19.93 ± 2.20
18:2	22.37 ± 4.02	44.88 ± 3.86	25.43 ± 1.10	48.30 ± 2.34
19:0	0.11 ± 0.06	0.12 ± 0.04	0.14 ± 0.05	0.14 ± 0.07
18:3n-3	0.32 ± 0.08	0.21 ± 0.04	0.31 ± 0.06	0.27 ± 0.02
20:0	0.18 ± 0.09	0.47 ± 0.52	0.37 ± 0.28	0.55 ± 0.46
20:1	0.08 ± 0.03	0.26 ± 0.34	0.06 ± 0.01	0.07 ± 0.04
20:2	0.10 ± 0.13	0.57 ± 0.59	0.10 ± 0.09	0.08 ± 0.07
20:3n-6	0.01 ± 0.01	0.01 ± 0.01	0.03 ± 0.02	0.03 ± 0.01
20:4n-6	0.03 ± 0.02	0.07 ± 0.05	0.05 ± 0.03	0.08 ± 0.03
20:3n-3	Tr*	0.01 ± <0.01	0.01 ± 0.03	0.02 ± 0.02
20:5n-3	0.01 ± 0.01	0.03 ± 0.02	0.04 ± 0.03	0.09 ± 0.03
22:0	0.09 ± 0.07	1.07 ± 1.47	0.29 ± 0.21	0.49 ± 0.46
22:1	0.02 ± 0.01	0.06 ± 0.02	0.06 ± 0.04	0.36 ± 0.19
23:0	0.12 ± 0.14	0.25 ± 0.35	0.10 ± 0.08	0.19 ± 0.10
24:0	0.07 ± 0.03	0.12 ± 0.10	0.10 ± 0.07	0.20 ± 0.07

Values are means ± SD.

*Trace, <0.01%.

TABLE 4. Fatty acid percent composition (as methyl esters) of fat body and Malpighian tubules from adults of *Zophobas atratus*

Fatty acid	TL fat body	PL fat body	TL Malp. tub.	PL Malp. tub.
14:0	1.53 ± 0.43	0.19 ± 0.05	1.84 ± 0.18	0.40 ± 0.13
15:0	0.31 ± 0.14	0.22 ± 0.04	0.68 ± 0.20	0.22 ± 0.09
16:0	31.75 ± 1.19	20.91 ± 1.18	25.77 ± 1.71	9.99 ± 1.31
16:1	2.87 ± 1.54	1.78 ± 0.47	2.16 ± 0.79	0.94 ± 0.17
17:0	0.54 ± 0.11	0.46 ± 0.16	2.79 ± 3.14	0.61 ± 0.15
17:1	0.42 ± 0.13	0.18 ± 0.08	0.37 ± 0.11	0.72 ± 0.37
18:0	8.24 ± 2.94	10.68 ± 0.79	17.17 ± 3.45	15.43 ± 0.57
18:1	31.85 ± 2.74	19.36 ± 1.39	25.01 ± 1.62	20.19 ± 0.76
18:2	21.32 ± 1.03	44.57 ± 1.69	19.75 ± 0.93	49.41 ± 1.40
19:0	0.11 ± 0.01	0.10 ± 0.02	0.27 ± 0.09	0.13 ± 0.05
18:3n-3	0.37 ± 0.09	0.35 ± 0.13	1.31 ± 1.38	0.35 ± 0.09
20:0	0.21 ± 0.06	0.20 ± 0.04	0.81 ± 0.29	0.33 ± 0.07
20:1	0.10 ± 0.04	0.07 ± 0.03	0.56 ± 0.29	0.05 ± 0.02
20:2	0.10 ± 0.05	0.28 ± 0.08	0.39 ± 0.24	0.11 ± 0.10
20:3n-6	0.01 ± 0.01	Tr*	Tr	0.01 ± 0.01
20:4n-6	0.04 ± 0.02	0.11 ± 0.06	Tr	0.09 ± 0.05
20:3n-3	0.01 ± 0.01	0.02 ± 0.01	Tr	0.02 ± 0.03
20:5n-3	0.01 ± <0.01	0.02 ± 0.01	Tr	0.03 ± 0.01
22:0	0.06 ± 0.03	0.27 ± 0.06	0.41 ± 0.20	0.37 ± 0.24
22:1	0.03 ± 0.02	0.07 ± 0.02	0.13 ± 0.09	0.18 ± 0.04
23:0	0.09 ± 0.05	0.06 ± 0.01	0.27 ± 0.21	0.20 ± 0.05
24:0	0.03 ± 0.01	0.10 ± 0.03	0.31 ± 0.29	0.21 ± 0.12

Values are means ± SD.
*Trace, <0.01%.

eicosanoid biosynthesis inhibitors and then recording the ability of experimental larvae to form nodules in response to bacterial injections. Relative to control larvae, the capability of experimental larvae to form nodules was severely impaired, and we suggested that eicosanoids mediate one or more crucial steps on the overall nodulation process (20). We also used standard protocols (24,39) to assess the ability of *Z. atratus* tissue preparations to biosynthesize eicosanoids

from radioactive precursors. We found that fat body preparations were competent to biosynthesize prostaglandins and a lipoxygenase product tentatively identified as one or more isomers of hydroxyeicosatetraenoic acid (20). The idea that eicosanoids exert a crucial biological role in immunity and that one of the insect immune tissues is competent to synthesize eicosanoids animates more detailed investigations into the *Z. atratus* eicosanoid system. In this article we report our investigation into a fundamental element of eicosa-

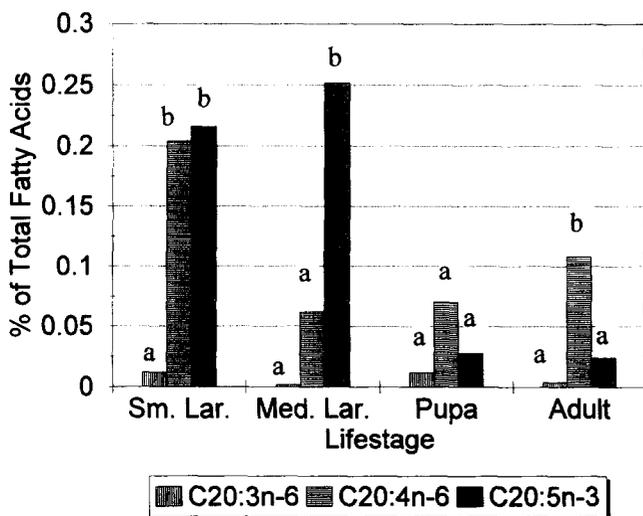


FIG. 1. Fat body phospholipid PUFA composition of *Z. atratus* as a function of life stage. Bars for each mean at each life stage with the same letter are not statistically different ($P > 0.05$).

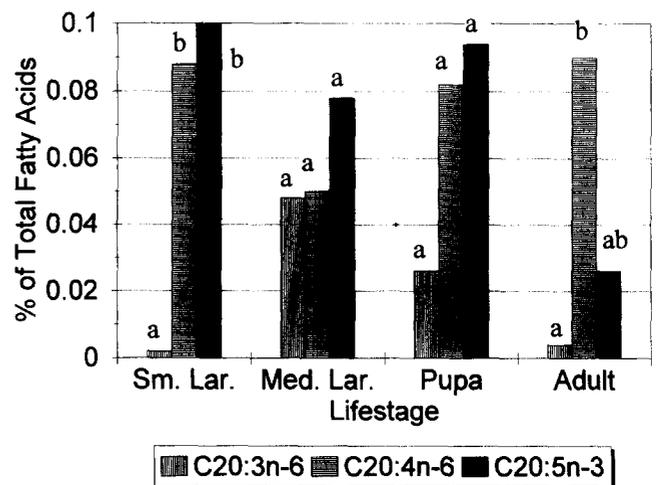


FIG. 2. Malpighian tubule phospholipid PUFA composition of *Z. atratus* as a function of life stage. Bars for each mean at each life stage with the same letter are not statistically different ($P > 0.05$).

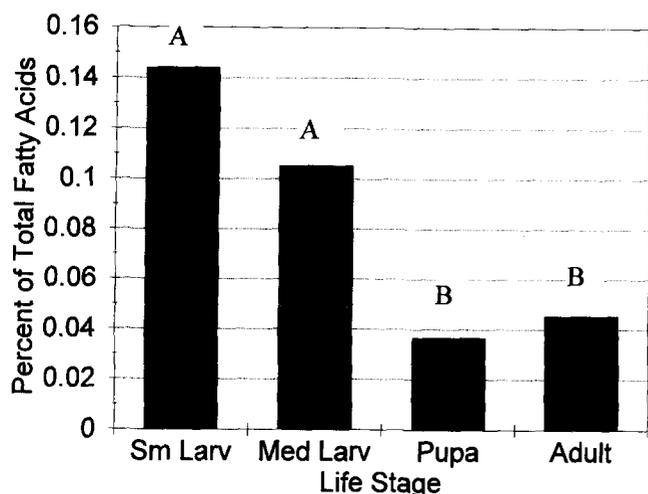


FIG. 3. Mean total phospholipid fat body PUFA content by life stage. Bars for each mean at each life stage with the same letter are not statistically different ($P > 0.05$).

noid biosynthesis, namely the presence of eicosanoid precursor fatty acids in *Z. atratus* tissues.

Our results document the overall fatty acid compositions of TL and PL prepared from two *Z. atratus* tissues, Malpighian tubules and fat body. These data are in accord qualitatively with the general background on animal lipids (33,35,44). The commonly observed components, 16:0, 16:

TABLE 5. Fatty acid percent composition (as methyl esters) of diet used to rear *Zophobas atratus*

Fatty acid	Percent composition of total lipids
14:0	0.23
15:0	0.05
16:0	18.94
16:1	0.36
17:0	0.10
17:1	0.07
18:0	2.12
18:1	29.12
18:2	44.70
19:0	0.04
18:3n-3	2.17
20:0	0.18
20:1	0.84
20:2	0.14
20:3n-6	Tr*
20:4n-6	0.01
20:3n-3	0.03
20:5n-3	Tr
22:0	0.15
22:1	0.52
23:0	0.04
24:0	0.19

*Trace, <0.01%.

1, 18:0, 18:1 and 18:2n-6, were present in both lipid fractions prepared from Malpighian tubules and fat body. As expected, the major polyunsaturated, 18:2n-6, occurs in greater relative abundance in PL, compared with TL.

On the practical issue of overlooking eicosanoid-precursor PUFA, we note that these components were found in greater abundance in PL than in TL. Stanley-Samuelson and Dadd (35) suggested this is a general pattern in animal lipids because these components are usually associated with biomembranes rather than storage sites. This biochemical arrangement may, in part, explain why the C20 PUFA are easily overlooked in routine analyses. The fatty acid moieties in triacylglycerols, the quantitatively major lipid fraction in insects, contain very low proportions of C20 PUFA and can render the C20 PUFA almost undetectable with ordinary flame ionization gas chromatography. However, even when PL are purified by preparative thin-layer chromatography before analysis, the C20 PUFA can be overlooked because they make up such small proportions of PL fatty acids. For example, 20:3n-6 was present only as a trace in PL from larval fat body (Table 2). These fatty acids were similarly present in only trace amounts in last-instar larvae (20). Thus, without special attention, it may appear that C20 PUFA do not occur in most terrestrial insects when in fact they do.

C20 PUFAs are generally associated with the sn-2 position of cellular PLs. Eicosanoids are biosynthesized from free fatty acids, and it is generally thought that the first and rate-limiting step in eicosanoid biosynthesis is the release of substrate from PLs (4,5). Phospholipase A₂ (PLA₂) is responsible for hydrolyzing eicosanoid precursor PUFAs from cellular PLs. Several PLA₂s are specific for arachidonate-associated PLs; this specificity is taken to indicate the regulatory role of some PLA₂s in eicosanoid biosynthesis. Compared with their mammalian counterparts, insect intracellular PLA₂s have received very little attention. Bitsch and her colleagues described a PLA₂ in reproductive tissues of the firebrat, *Thermobia domestica* (28). This PLA₂ appears to modulate eicosanoid biosynthesis (1). We described intracellular PLA₂s in preparations of *M. sexta* fat body and hemocytes (30,50). The hemocyte PLA₂ exhibited a marked preference for arachidonyl-containing substrate, again indicating the enzyme may regulate eicosanoid biosynthesis. Although still scant, this limited information indicates intracellular PLA₂s may regulate eicosanoid biosynthesis in insects and other invertebrates.

The often very low proportions of C20 PUFA in terrestrial insect cellular PL stands in contrast to the relatively high proportions of these components in mammals and aquatic animals, including aquatic insects. Many aquatic insects have close to a third of their tissue fatty acids as C20 PUFA (12). But some terrestrial insects appear to have localized pools of C20 PUFA as well. Arachidonic acid (20:4n-6) accounts for about 25% of the phosphatidylcholine

fatty acids prepared from spermatophores of the cricket *Teloeoryllus commodus* (37). More recently, we found that certain predatory insects, including the tiger beetle, *Cicindela circumpecta*; the Robber fly *Asilus* sp. (51); the ant, *Myrmica incompleta* and the fly, *Microdon albicomatus* (41) have higher proportions of C20 PUFA. It is clear that insects are not, as a class, defined by low proportions of eicosanoid-precursor PUFA. Do most terrestrial insects, then, actively maintain these low proportions?

The fatty acid pattern of the *Z. atratus* diet is considerably different from the beetle tissue fatty acid profiles. This suggests that *Z. atratus* expresses a selective fatty acid incorporation and remodeling system. We made similar observations on hemocytes from the tobacco hornworm, *M. sexta* (10,23), where circulating hemocytes had lower proportions of C20 PUFA than in the hemolymph or in the culture medium. Moreover, when hemocytes were allowed to incorporate radioactive 20:4n-6 or 20:5n-3 into cellular PL, a steady transfer of these components out of cellular PL followed. These findings suggest that the low proportions of C20 PUFA result from controlled processes within the insects and not from constraints imposed by their environments.

Most of our information on insect fatty acid compositions derive from analyses of total or fractionated lipid extracts of whole insects (7,44). Analyses at this level leave open the possibility that insects sequester certain fatty acids in particular tissue or lipid class pools that go unnoticed. The spermatophore lipids from the cricket *T. commodus* (37) illustrate the point. Moreover, Zinkler (53) showed that specific PL classes prepared from retinas of the butterfly, *Deilephila elpenor*, maintained about 40% of the phosphatidylethanolamine fatty acids as 20:5n-3. These findings support the idea that certain tissues and particular lipid fractions within tissues serve as localized pools of C20 PUFA. However, sequesterizations of this sort do not always occur. In our analysis of PL from whole heads, thoraces and individual tissues within the abdomens of mealworm beetles, *Tenebrio molitor*, we found very low proportions of C20 PUFA (13). Upon more detailed analyses of Malpighian tubules from this species, we found that selected PL fractions, including phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine/inositol and cardiolipin, exhibited no more C20 PUFA than were seen in previous analyses (14). So far, there is no evidence of relatively rich C20 PUFA pools in these beetles.

Fat body and Malpighian tubules of all life stages of *Z. atratus* maintain slightly different fatty acid profiles. For example, in adults, 16:0 made up about 21% of fat body PL and about 10% of Malpighian tubule PL. Also in adults, 18:2 comprised about 44% of fat body PL and about 49% of Malpighian tubule PL. Such differences in tissue-specific fatty acid profiles have been seen in other insects, including the beetle *T. molitor* (13), the cricket *T. commodus* (37),

the mosquito *Aedes aegypti* (27) and the moth *M. sexta* (22). These findings argue that individual tissues and cell systems are competent to develop and maintain their own fatty acid profiles, presumably in response to local needs.

Z. atratus underwent a number of changes in fatty acid profiles during development. For example, 18:2n-6 increased from about 40% of PL prepared from Malpighian tubules of small larvae to about 50% of matching preparations from pupae and adults. We noted changes in 16:0, 18:0 and 18:1 as well. The proportions of C20 PUFA also changed during development (Figs. 1-3). In fat body PL, 20:5n-3 decreased from about 0.2% in larvae to about 0.03% in pupae and about 0.02% in adults. Similarly, 20:4n-6 decreased from about 0.2% in small larvae to 0.05% in large larvae and pupae; 20:4n-6 was present at about 0.1% in adults. The proportions of C20 PUFA in Malpighian tubules changed much less during development. These findings are consistent with a number of studies, all showing that fatty acid profiles of whole insects and specific insect tissues are altered during the development from egg to adult (44).

Given the very small proportions of C20 PUFA in the tissue lipids of most terrestrial insects, the availability of these components could exert a limitation on the potential for eicosanoid biosynthesis. However, C20 PUFA can be biosynthesized from their C18 counterparts via elongation/desaturation pathways. The crickets *T. commodus* and *Acheta domesticus*, the cockroach *Periplaneta americana* and the waxmoth *Galleria mellonella* produce 20:3n-6 and 20:4n-6 from 18:2n-6 (15,44,46,47). Moreover, the waxmoth, *G. mellonella*, is able to produce 20:5n-3 from 18:3n-3 via similar elongation/desaturation pathways. These pathways are present in all vertebrates that have been so studied, with the exception of the domestic cat, and they probably occur in most insect species other than mosquitoes (3,44). The significance of these pathways in eicosanoid biosynthesis is that despite the very low relative proportions of C20 PUFA in insect tissues, insects are probably able to produce C20 PUFA as required. Our data on *Z. atratus* support this idea, because intermediates in the elongation/desaturation pathways were present in the tissues we analyzed. For example, PL from fat body and Malpighian tubules have small proportions of 18:3n-3 and 20:3n-3, both intermediates in synthesis of 20:5n-3. The slightly higher proportions of 20:5n-3 indicate this is the terminal step in the pathway (36,45).

The expression of desaturation pathways has also been shown to be regulated in *G. mellonella* (46). When waxmoth larvae were reared on diets rich in PUFA, no evidence for elongation/desaturation of C18 components was found; however, when reared on fatty acid free diets that provided no unsaturated components, considerable elongation/desaturation activity was found (46). Furthermore, in the present study, the proportions of C20 PUFA in fat body of *Z. atratus* steadily declined through development. Both points suggest

that insects are able to respond to immediate needs by up- or down-regulating these biosynthetic pathways.

Our analyses of the tissue fatty acid compositions of *Z. atratus* also provide insight into the potentials for eicosanoid biosynthesis. We note that 20:3n-6 was present in very low, nearly catalytic, proportions of tissue PL, whereas 20:4n-6 and 20:5n-3 were in relatively higher abundance. This indicates that these two components are more readily available for eicosanoid biosynthesis. Eicosanoids are defined in terms of the particular C20 PUFA from which they are derived. We surmise that eicosanoids derived from 20:4n-6 and 20:5n-3 (the 2- and 3-series) are likely to be of greater physiological importance than those derived from 20:3n-6 (the 1-series). Future direct investigations of eicosanoid biosynthesis is required to clarify this point.

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