The predominance of polyunsaturated fatty acids in the butterfly *Morpho peleides* before and after metamorphosis

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Abstract We hypothesized that the polyunsaturated fatty acids of the butterfly were probably derived from the diet and that there might be a great loss of body fat during metamorphosis. To substantiate these hypotheses, we analyzed the fatty acid composition and content of the diet, the larva, and the butterfly *Morpho peleides*. Both the diet and the tissues of the larva and butterfly had a high concentration of polyunsaturated fatty acids. In the diet, linolenic acid accounted for 19% and linoleic acid for 8% of total fatty acids. In the larva, almost 60% of the total fatty acids were polyunsaturated: linolenic acid predominated at 42% of total fatty acids, and linoleic acid was at 17%. In the butterfly, linolenic acid represented 36% and linoleic acid represented 11% of total fatty acids. The larva had a much higher total fatty acid content than the butterfly (20.2 vs. 6.9 mg). Our data indicate that the transformation from larva to butterfly during metamorphosis drastically decreased the total fatty acid content. There was bioenhancement of polyunsaturated fatty acids from the diet to the larva and butterfly. This polyunsaturation of membranes may have functional importance in providing membrane fluidity useful in flight.— Wang, Y., D. S. Lin, L. Bolewicz, and W. E. Connor. **The predominance of polyunsaturated fatty acids in the butterfly *Morpho peleides* before and after metamorphosis. *J. Lipid Res.* 2006.** 47: **530–536.**

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Insects and vertebrates share many common metabolic pathways. Although lipid metabolism in vertebrates has been well studied, there are fewer studies in insects. Insects may be useful models that can facilitate our general understanding of biology (1). For most insects, there is a dietary requirement for polyunsaturated fatty acids (2, 3). Using an analysis of the fatty acid composition of seven insect orders, Thompson (4) identified possible phylogenetic trends. It was also observed that in the order Lepidoptera, most of the species had a high content of polyunsaturated fatty acids. The linolenic acid of the 49 species analyzed varied from 0.1% to 51.0% of total fatty acids, with a mean of 22.2%. These trends provided insights into potential relationships between insect orders. The fatty acid composition of many insect species has been analyzed in the past (5, 6). However, in view of the multitude of insect species that exist, most knowledge of the fatty acid compositions of insects is derived from studies of a relatively small number of insect species.

The butterfly of the order Lepidoptera undergoes metamorphosis from larva to butterfly. The larva feed on the leaves of the plants and then spin a cocoon. The butterfly feeds on flower nectar, which is available later in the year. We found no information about the fatty acid composition and fat content of the butterfly before and after metamorphosis and no information about how the diet of the larva might influence its fatty acid composition.

On a recent trip, we visited the Butterfly Farm at Chaa Creek, Belize, in Central America. Chaa Creek is a tourist attraction and hostel for travelers in Belize. As we have developed an interest in the evolutionary patterns of fatty acids, having previously studied snails and slugs (7), we saw the potentialities of butterfly research and discussed our ideas with the scientific director, Mike Green. We decided to collaborate on fatty acid and sterol research. At the Butterfly Farm, the butterfly *Morpho peleides*, or blue morpho, is raised in a controlled environment. The sole food of these butterfly larva is the leaves of the rain forest tree *Pterocarpus*, on which the butterfly lays its eggs. Because the larva grow on this sole food source and the butterfly expends energy for metamorphosis and later flight with limited food intake, we hypothesized that diet must play an important role in the fatty acid composition of the larva, and the butterfly may have similar or even enhanced polyunsaturated fatty acid composition as the larva, but with lower body fat and body weight. To test these hypotheses, we measured the food consumption of the larva on a daily basis and analyzed the fatty acid composition and content of the diet as well as the larva. After the larva had undergone metamorphosis, we examined the fatty acid composition and content of the butterfly, which had emerged and which had a beautiful blue color.

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METHODS

Both butterfly larva and rain forest tree leaves were collected at the Butterfly Farm at Chaa Creek. The larva were individually raised in glass jars and fed only the leaves of the *Pterocarpus* tree, which is the preferred diet of this species. We measured the amount of leaves consumed over a 24 h period. The larva and leaves were frozen and transported by air to the United States. Both larva and leaves were stored at −70°C. Adult blue morpho butterflies at the end of life were later collected from the Oregon Zoo in Portland. All weights were wet weight.

The fatty acid composition of butterfly larva, leaves, and butterfly was analyzed by methods described previously (8). All samples were ground carefully, and the total lipids were extracted with chloroform-methanol (2:1; with added 3,5-di-*tert*-butyl-4-hydroxytoluene for antioxidation). Aliquots of the lipid extracts were saponified by incubation with 2 ml of 6% ethanolic KOH for 1 h at 37°C. After the addition of water, neutral sterols were removed by hexane extraction. The aqueous phase was acidified, and the fatty acids were recovered by hexane extraction. Methyl esters were prepared by heating the dried extract with 1 ml of 10% boron trifluoride (BF_3) in methanol for 10 min at 100°C in tightly sealed tubes with Teflon-coated screw caps. All solvent evaporation was carried out under a gentle stream of nitrogen to prevent lipid peroxidation. Fatty acid methyl esters were analyzed by GC (Perkin-Elmer, Norwalk, CT) on an apparatus equipped with a 30 meter Supelco SP 2330 fused silica capillary column (column temperature of 185–190°C) attached to a HP 85 computer/3390A integrator. Results are reported as percentage total fatty acids by weight. A mixture of fatty acid standards was run daily for calibration purposes.

To analyze the total fatty acid contents of the leaves, larva, and adult butterfly, a known amount of internal standard (C-17, heptadecaenoic acid) was added to each sample analyzed, so that the mass of individual fatty acids could be calculated after GC analysis. The GC system was the same as that described above.

To analyze the fatty acid compositions of lipid classes, another aliquot of lipid extract was subjected to TLC using the solvent system hexane-chloroform-diethyl ether-acetic acid (80:10:10:1.5, v/v), and the lipid classes (phospholipids, free fatty acids, triglycerides, and cholesteryl esters) were separated. The TLC bands of each lipid class were scraped into the centrifuge tubes and heated (100°C) for 10 min in BF3 for phospholipids and free fatty acids and for 45 min in BF3, benzene, and methanol for triglyceride and cholesteryl esters. Fatty acid methyl esters were also analyzed with GC, as described above.

To quantify the fatty acid content in major lipid pools, lipid extracts were subjected to TLC to separate into different lipid classes as described above. A known quantity of heptadecaenoic acid was added to each sample before methylation and GC analysis. The total fatty acid content of each lipid pool was thus calculated (phospholipid, triglyceride, and cholesteryl ester).

Statistical analyses

Means and standard deviations were calculated to characterize the fatty acid composition (as a percentage of total fatty acids) in larva and butterflies and *Pterocarpus* leaves. Differences between the fatty acid composition means were ascertained by use of the appropriate t-test. We used Bonferroni inequality to control the overall α-level. Data were analyzed using SPSS for Windows (release 11.0.1, November 15, 2001; SPSS, Inc., Chicago, IL).

RESULTS

The fatty acid composition of the leaf of the *Pterocarpus* tree (the sole diet of the larva) is presented in **Table 1** as percentages of total fatty acids. There was a high content of polyunsaturated fatty acids (32.8%): 18.9% of total fatty acids as linolenic acid (18:3 n-3) and 7.5% as linoleic acid (18:2 n-6). The two major saturated fatty acids were stearic acid (18:0) at 26.7% and palmitic acid (16:0) at 25% of total fatty acids. Oleic acid (18:1 n-9) contributed only 4.5% of the total fatty acids. Among the four lipid classes, phospholipids had the highest polyunsaturated fatty acid content, with a similar fatty acid composition as for total fatty acids. Phospholipid fatty acids constitute the chief membrane fatty acids.

The fatty acid composition of the larva was quite different from that of the diet (**Table 2**). More than 50% of the larval fatty acids were polyunsaturated. Linolenic

TABLE 1. Fatty acid composition of the butterfly diet, the leaves of the *Pterocarpus* tree

		Lipid Classes			
Fatty Acids	Total Fatty Acids	Phospholipids	Free Fatty Acids	Triglycerides	Sterol Esters
Saturated					
14:0	1.82 ± 0.10	1.73 ± 0.47	1.95 ± 1.13	3.33 ± 0.73	3.44 ± 0.60
16:0 palmitic	25.00 ± 0.80	24.31 ± 3.38	30.63 ± 3.65	18.48 ± 1.28	12.72 ± 3.41
18:0 stearic	26.7 ± 0.83	17.91 ± 3.42	19.66 ± 1.78	10.40 ± 2.59	14.08 ± 3.80
Monounsaturated					
16:1 (n-7)	1.42 ± 0.54	1.75 ± 0.55	1.68 ± 0.52	3.89 ± 1.37	6.34 ± 5.80
18:1 (n-9)/(n-7) oleic	4.48 ± 0.38	4.86 ± 0.32	11.69 ± 2.07	20.27 ± 4.53	8.31 ± 2.91
18:1 (n-9) *trans*	1.62 ± 0.63	0.14 ± 0.21	1.36 ± 0.21	3.04 ± 3.46	1.62 ± 1.37
Polyunsaturated					
16:2 (n-4)	3.30 ± 0.58	1.60 ± 0.40	0.26 ± 0.20	1.05 ± 1.39	1.47 ± 1.51
18:2 (n-6) linoleic	7.52 ± 0.17	9.61 ± 2.47	12.86 ± 1.34	4.14 ± 3.32	3.10 ± 1.70
18:3 (n-3) α-linolenic	18.89 ± 0.44	21.23 ± 4.17	11.09 ± 1.41	1.95 ± 1.82	1.35 ± 1.00
Total saturated	56.54 ± 1.72	47.82 ± 2.12	56.32 ± 2.04	41.50 ± 4.41	39.99 ± 6.59
Total monounsaturated	8.24 ± 0.91	7.52 ± 0.48	15.87 ± 2.07	29.46 ± 6.60	19.04 ± 5.48
Total polyunsaturated	32.83 ± 0.29	34.99 ± 2.10	26.22 ± 2.60	15.62 ± 4.04	13.32 ± 3.80
Total (n-6)	9.84 ± 0.18	11.49 ± 2.37	13.31 ± 1.50	8.84 ± 2.60	6.12 ± 2.31
Total (n-3)	19.56 ± 0.52	21.41 ± 4.18	11.77 ± 1.60	4.10 ± 1.73	3.52 ± 1.68
(n-6)/(n-3)	0.50 ± 0.02	0.57 ± 0.23	1.15 ± 0.20	2.41 ± 1.04	1.93 ± 0.82

Values shown are percentages of total fatty acids (means ± SD, n = 6).

TABLE 2. Fatty acid composition of the butterfly larva

Fatty Acids	Total Fatty Acids	Lipid Classes			
		Phospholipids	Free Fatty Acids	Triglycerides	Sterol Esters
Saturated					
14:0	0.49 ± 0.14	1.03 ± 0.51	0.32 ± 0.08	0.41 ± 1.25	4.21 ± 1.25
16:0 palmitic	18.15 ± 3.87	12.70 ± 2.22	12.00 ± 1.05	21.27 ± 2.62	19.13 ± 2.07
18:0 stearic	12.23 ± 4.22	16.44 ± 4.19	13.96 ± 1.35	6.12 ± 0.93	12.79 ± 2.64
Monounsaturated					
16:1 (n-7)	0.68 ± 0.26	0.57 ± 0.27	0.35 ± 0.13	0.81 ± 0.31	3.66 ± 1.81
18:1 (n-9)/(n-7) oleic	6.81 ± 2.68	9.89 ± 2.14	7.08 ± 1.10	8.18 ± 0.61	13.30 ± 1.84
18:1 (n-9) *trans*	0.19 ± 0.30	0.15 ± 0.25	1.38 ± 0.33	0.25 ± 0.23	1.96 ± 1.44
Polyunsaturated					
16:2 (n-4)	0.23 ± 0.19	0.19 ± 0.14	0.08 ± 0.01	0.06 ± 0.04	0.35 ± 0.28
18:2 (n-6) linoleic	16.74 ± 1.89	16.13 ± 2.92	18.84 ± 1.85	14.01 ± 0.77	10.88 ± 2.06
18:3 (n-3) α-linolenic	41.71 ± 9.36	30.40 ± 3.21	40.77 ± 1.10	46.37 ± 3.50	20.82 ± 3.78
Total saturated	32.26 ± 5.58	33.91 ± 3.37	28.37 ± 2.14	28.98 ± 2.90	40.44 ± 4.59
Total monounsaturated	7.78 ± 2.78	10.93 ± 2.20	9.45 ± 0.98	9.30 ± 0.83	19.41 ± 3.81
Total polyunsaturated	60.28 ± 6.82	49.56 ± 3.12	60.98 ± 1.68	61.09 ± 3.38	34.59 ± 5.94
Total (n-6)*	17.35 ± 2.16	17.86 ± 2.86	19.64 ± 1.85	14.54 ± 0.68	12.38 ± 2.01
Total (n-3)*	42.39 ± 8.32	31.03 ± 3.26	41.05 ± 1.03	46.43 ± 3.48	21.53 ± 4.02
(n-6)/(n-3)	0.44 ± 0.17	0.58 ± 0.14	0.48 ± 0.05	0.31 ± 0.03	0.58 ± 0.05

Values shown are percentages of total fatty acids (means ± SD, n = 6).
*Includes small quantities at 20:4 (n-6) and 20:5 (n-3) as discussed in the text.

acid accounted for 41.7% of the total fatty acids, and linoleic acid contributed 16.7% of the total. The two saturated fatty acids, stearic and palmitic acids, were much lower, at 12.2% and 18.2%, respectively. A comparison of the fatty acid composition of the leaves and larva showed a great increase in polyunsaturated fatty acids (linolenic and linoleic acids) and decreased saturated fatty acids (stearic and palmitic acids) in larva compared with the dietary fatty acids of the leaves (**Fig. 1**).

In both the triglyceride and free fatty acid fractions, there were the same high proportions of linolenic acid as in the total fatty acids (41–46%). Triglyceride fatty acids represent the storage form of fatty acids. The linolenic acid content in phospholipids was somewhat lower (30%). The linoleic acid levels were similar. Small quantities of arachidonic acid (1.2%) and eicosapentaenoic acid (0.31%) were present in the phospholipid fraction. The sterol esters had a lower polyunsaturated fatty acid content and a higher saturated fatty acid content.

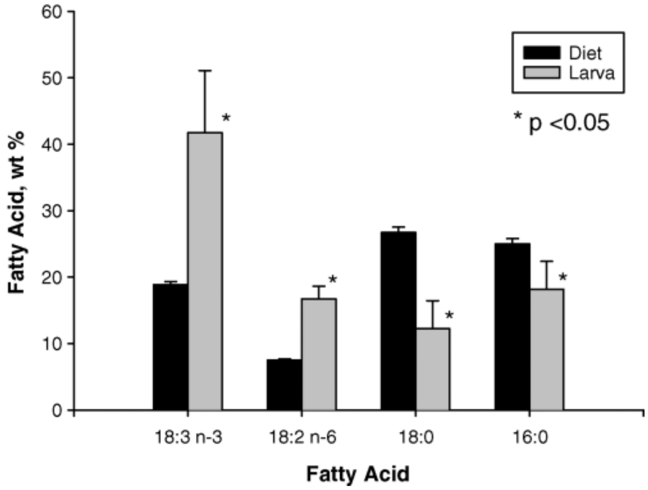

Fig. 1. Comparison of the fatty acid composition of the diet and the larva. Values shown are means ± SD.

The fatty acid composition of adult butterflies is depicted in **Table 3**. Like the larva, they had a high content of polyunsaturated fatty acids (49% of total fatty acids). Linolenic acid continued to be high, at 35.7% of total fatty acids. Linoleic acid content was lower (10.8% vs. 16.7%; $P < 0.002$) and oleic acid was higher (16.1% vs. 6.8%; $P < 0.012$) than in larva. The fatty acid composition of phospholipids, free fatty acids, and triglycerides was similar to the composition of the total fatty acids. Again, sterol esters had the lowest polyunsaturated fatty acids and higher saturated fatty acids. The fatty acid composition of larva and butterflies in general was similar except that the larva had higher linoleic acid and lower oleic acid than adult butterflies (**Fig. 2**). Like the larva, the phospholipids of the adult butterflies contained arachidonic acid (0.97%) and eicosapentaenoic acid (1.19%).

From the amount of leaves consumed by the larva, we calculated the total dietary intake of fatty acids by the larva (**Table 4**). The larva consumed 1.24 mg of fatty acids per day (0.65 mg saturated, 0.14 mg monounsaturated, and 0.45 mg polyunsaturated). They consumed 0.17 mg of linoleic acid and 0.28 mg of linolenic acid per day.

The fatty acid content both in mg/whole body and mg/mg body weight is presented in **Table 5**. The transformation from larva to butterfly caused a significant decrease in fatty acids in terms of both total body fatty acid and fatty acid per unit of body weight. In total body fatty acid content, there was a 65% decrease. Both saturated and polyunsaturated fatty acids decreased 68–70%. Monounsaturated fatty acids decreased 30%. In terms of fatty acid per unit of body weight, there was a 53% decrease in total fatty acid per unit of body weight. Saturated and polyunsaturated fatty acids decreased 54% and 60%, respectively, and monounsaturated fatty acids remained unchanged.

TABLE 3. Fatty acid composition of the adult butterfly

Fatty Acids	Total Fatty Acids	Lipid Classes			
		Phospholipids	Free Fatty Acids	Triglycerides	Sterol Esters
Saturated					
14:0	0.18 ± 0.16	0.18 ± 0.08	0.37 ± 0.06	0.63 ± 0.36	26.99 ± 16.82
16:0 palmitic	20.36 ± 8.98	13.91 ± 3.9	16.09 ± 5.1	18.28 ± 6.94	11.01 ± 14.04
18:0 stearic	8.17 ± 5.17	10.18 ± 3.10	9.69 ± 1.36	3.12 ± 1.72	2.64 ± 1.36
Monounsaturated					
16:1 (n-7)	3.77 ± 2.97	1.97 ± 1.92	2.11 ± 0.92	2.85 ± 1.11	3.08 ± 3.82
18:1 (n-9)/(n-7) oleic	16.10 ± 6.93	21.81 ± 6.19	15.69 ± 6.36	20.70 ± 17.72	12.75 ± 7.24
18:1 (n-9) *trans*	0.44 ± 031	0.26 ± 0.1	0.42 ± 0.32	0.86 ± 1.18	1.41 ± 1.83
Polyunsaturated					
16:2 (n-4)	0.29 ± 0.47	0.48 ± 0.43	0.05 ± 0.04	0.30 ± 0.45	2.49 ± 4.73
18:2 (n-6) linoleic	10.82 ± 2.95	12.64 ± 2.30	12.08 ± 2.78	7.39 ± 4.78	2.72 ± 2.17
18:3 (n-3) α-linolenic	35.76 ± 11.97	34.09 ± 5.31	36.28 ± 7.60	35.39 ± 22.56	9.56 ± 8.75
Total saturated	29.76 ± 6.14	24.89 ± 3.63	28.20 ± 2.70	25.55 ± 2.50	48.29 ± 17.58
Total monounsaturated	20.75 ± 9.15	24.67 ± 6.00	19.19 ± 6.06	26.72 ± 21.04	23.42 ± 11.43
Total polyunsaturated	48.98 ± 13.49	49.91 ± 5.64	51.48 ± 7.22	46.13 ± 23.37	22.39 ± 8.29
Total (n-6)*	11.50 ± 3.05	13.78 ± 2.15	13.06 ± 2.42	9.46 ± 2.10	6.48 ± 1.74
Total (n-3)*	36.86 ± 11.87	35.44 ± 5.51	38.16 ± 22.10	35.76 ± 22.10	12.84 ± 9.13
(n-6)/(n-3)	0.32 ± 0.08	0.40 ± 0.09	0.35 ± 0.06	0.71 ± 0.96	0.86 ± 0.77

Values shown are percentages of total fatty acids (means ± SD, n = 6).
*Includes small quantities at 20:4 (n-6) and 20:5 (n-3) as discussed in the text.

There were significant changes in both body weight and fatty acid content in the triglyceride pool during metamorphosis (**Table 6**). Body weight decreased 40%. The triglyceride pool decreased 84%. The phospholipid and cholesteryl ester pools did not change.

DISCUSSION

In this study, we describe a butterfly species that contains a large amount of the polyunsaturated fatty acids linoleic and linolenic acids. High concentrations of these polyunsaturated fatty acids are also found in the leaf of the *Pterocarpus* tree, the sole food of the larva. Our results suggest that the diet of the larva contributes significantly to the high content of polyunsaturated fatty acids in the butterfly. Dietary effects on the fatty acid composition of tissues has been shown in several other insect species (9–12). In silkworm, Unni et al. (13) observed that the fatty acid composition of the diet affected its growth. For most insects, there is a dietary requirement for polyunsaturated fatty acids (2, 3). However, in the report by Blomquist et al. (14), certain insect species, such as cockroach (*Periplaneta americana*), termite (*Zootermopsis angusticollis*), and cricket (*Acheta domesticus*), demonstrated de novo synthesis of linoleic acid from acetate. We found no data, however, on the de novo synthesis of 18:3 n-3 in insects.

Comparing the fatty acids of the diet and the larva in our study, there was a relative increase in the two essential fatty acids, n-6 and n-3, and a reciprocal decrease in two saturated fatty acids (palmitic and stearic acids). The possibility exists that the larva can convert saturated or monounsaturated fatty acids in the diet to the two essential fatty acids (n-6 and n-3).

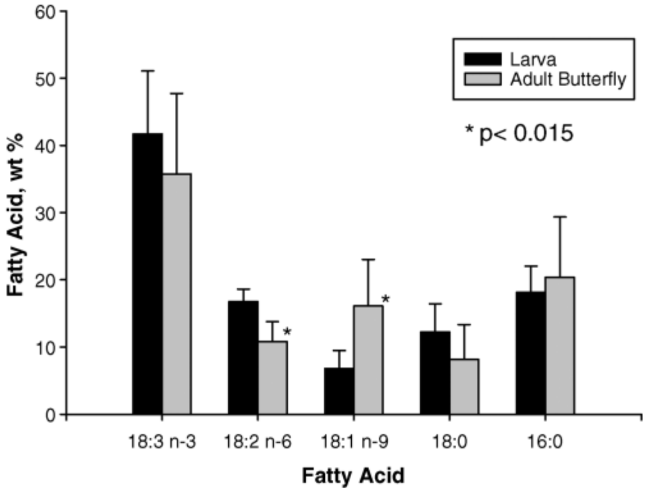

Fig. 2. Comparison of the fatty acid composition of the larva and the adult butterfly. Values shown are means ± SD.

TABLE 4. Dietary consumption of fatty acids by the larva

Fatty Acids	Consumption
	mg/day
Saturated	
14:0	0.01 ± 0.01
16:0	0.37 ± 0.31
18:0	0.24 ± 0.16
Monounsaturated	
16:1 n-7	0.02 ± 0.01
18:1 n-9	0.09 ± 0.10
18:1 *trans*	0.02 ± 0.03
Polyunsaturated	
16:2 n-4	0.02 ± 0.02
18:2 n-6	0.17 ± 0.18
18:3 n-3	0.28 ± 0.22
Total saturated	0.65 ± 0.51
Total monounsaturated	0.14 ± 0.15
Total polyunsaturated	0.58 ± 0.47
Total n-6	0.21 ± 0.20
Total n-3	0.36 ± 0.28

TABLE 5. Fatty acid content of the larva and butterfly

Fatty Acids	Larva (n = 6)	Butterfly (n = 6)	Differences		Larva (n = 6)	Butterfly (n = 6)	Differences	
	mg/whole body		*mg/body*	%	*μg/mg body wt*		*μg/mg*	%
Saturated								
14:0	0.1 ± 0.1	0.03 ± 0.03^{a}	−0	−73	0.2 ± 0.4	0.1 ± 0.1^{b}	−0.1	−65
16:0	3.3 ± 1.4	1.4 ± 1.4^{c}	−6.3	−81	7.8 ± 2.8	4.1 ± 2.8^{b}	−3.7	−47
18:0	2.1 ± 0.9	0.3 ± 0.54^{b}	−1.8	−85	4.4 ± 2.1	1.1 ± 0.5^{d}	−3.3	−76
Monounsaturated								
16:1 n-7	0.1 ± 0.1	0.3 ± 0.3	+0.2	+242	0.2 ± 0.1	0.8 ± 0.6^{b}	+0.6	+370
18:1 n-9	1.5 ± 0.5	0.9 ± 0.6	−0.6	−41	3.2 ± 0.7	2.8 ± 1.2	−0.4	−12
18:1 *trans*	0.1 ± 0.1	0.03 ± 0.05^{b}	−0.1	−75	0.3 ± 0.3	0.1 ± 0.1	−0.2	
Polyunsaturated								
16:2	0.1 ± 0.1	0.02 ± 0.02	—	−77	0.2 ± 0.2	0.1 ± 0.1	−0.1	−69
18:2 n-6	3.3 ± 1.1	0.7 ± 0.7^{c}	−0.3	−78	7.1 ± 2.0	2.2 ± 1.5^{c}	+5	−69
18:3 n-3	8.7 ± 3.1	2.8 ± 3.3^{a}	−0.6	−68	18.5 ± 4.5	7.9 ± 6.7^{a}	−10.6	−57
Total saturated	6.2 ± 2.3	2.0 ± 1.7^{d}	−0.4	−68	13.4 ± 44.6	6.1 ± 3.0^{a}	−7.3	−54
Total monounsaturated	1.7 ± 0.6	1.2 ± 0.9	−0.5	−30	3.7 ± 0.9	3.7 ± 1.7	0	+1.3
Total polyunsaturated	12.2 ± 4.2	3.6 ± 4.1^{d}	−8.6	−70	26.0 ± 6.7	10.4 ± 8.2^{d}	−15.6	+60
Total n-6	3.4 ± 1.2	0.7 ± 0.8^{c}	−2.6	−78	7.3 ± 2.1	2.3 ± 1.5^{c}	−5	−68^{c}
Total n-3	8.8 ± 3.1	2.8 ± 3.3^{a}	−6.0	−68	18.7 ± 4.6	8.0 ± 6.7^{a}	−10.7	−57
Total fatty acids	20.2 ± 7.0	6.9 ± 6.6^{a}	−13.3	−66	43.0 ± 11.9	20.2 ± 12.2^{a}	−22.85	−53

[a] $P < 0.01$, butterfly versus larva.
[b] $P < 0.05$, butterfly versus larva.
[c] $P < 0.001$, butterfly versus larva.
[d] $P < 0.005$, butterfly versus larva.

Alternatively, if the butterfly larva are incapable of synthesizing essential fatty acids from the diet, these opposing changes could be explained by retention of the essential fatty acids at the expense of saturated fatty acids. Incidentally, we know the intake of linoleic and linolenic acids by the larva: 0.17 mg/day for linoleic acid and 0.28 mg/day for linolenic acid (Table 4). The pool size of linoleic acid was 3.3 mg, and the pool size of linolenic acid was 8.7 mg. With the assumption that the butterfly larva are incapable of synthesizing essential fatty acids, we estimated from these data that the turnover times for linoleic and linolenic acids were 34.5 and 51.2 days, respectively.

A unique aspect of this butterfly is its high content of linolenic acid, which was 41% of the total fatty acids. Thompson (4) reported the fatty acid composition of seven insect orders. In the order Lepidoptera, he found a high content of linolenic acid in most of the species analyzed. In *Drosophila melanogaster* larvae, fatty acids in the membrane affected membrane fluidity (15). The high polyunsaturated fatty acid content of the membranes of the butterfly may make possible its flying ability. In our previous study, we found that docosahexaenoic acid (22:6 n-3) was most concentrated in sperm tail, and we postulated that such polyunsaturation was required for sperm mobility (16). We detected only small quantities of 20:4 n-6 and 20:5 n-3, presumably synthesized by the larva or butterfly from 18:2 n-6 and 18:3 n-3 respectively. In *Eurygaster integriceps*, it was reported that elongation of C18 to C20 occurred (17). Studying the changes in the fatty acid composition of *Drosophila* during development and aging with a fat-free diet, Green and Geer (18) examined the fatty acid composition changes in different stages. Important for the interpretation of our study, there were no essential fatty acids (18:2, 18:3) found at any stage of development. This study indicated an inability to synthesize essential fatty acids. In a previous study, we found both arachidonic acid (20:4 n-6) and eicosapentaenoic acid (20:5 n-3) in slugs and snails, but no docosahexaenoic acid (22:6 n-3) (7). However, 22:5 n-3 was present, suggesting that elongation to the next step (24:5) did not occur in slugs and snails.

Phospholipids and sterols have a structured role in the membrane systems of the cell. The ratio of polyunsaturated to saturated fatty acids in phospholipids has a significant effect on the biophysical properties of biological membranes (19). In both the leaf and the butterfly, the fatty acid composition of phospholipids was similar to that

TABLE 6. Changes of body weight and fatty acid content in major lipid classes during metamorphosis of the butterfly

Variable	Larva	Butterfly	*P*
Body weight (mg)	471.7 ± 138.5 (6)	282.8 ± 129.8 (6)	<0.035
Fatty acid (mg)			
Phospholipid	2.63 ± 1.56 (4)	1.76 ± 1.11 (5)	NS
Triglyceride	20.46 ± 7.46 (4)	3.25 ± 1.32 (5)	<0.001
Cholesteryl ester	0.66 ± 0.31 (4)	1.77 ± 1.33 (5)	NS

Values in parentheses are numbers of animals. NS, not significant.

of the total fatty acids. In larva phospholipids, however, the linolenic acid content was slightly lower than that of the leaf (30.4% vs. 41.7%).

Triglycerides represent the largest store of metabolic energy in insects. The major storage site for triglycerides in insects is usually the fat body (3). The triglyceride of the leaves had a much lower polyunsaturated fatty acid content than did the total fatty acids. In contrast, the polyunsaturated fatty acids in triglyceride of both larva and adult butterflies were similar to the composition of the total fatty acids. This suggested that, in contrast to mammals, whose dietary lipid mostly is triglyceride, the butterfly probably derived its polyunsaturated fatty acids mostly from phospholipids in the diet, which have much higher levels of polyunsaturated fatty acids than triglyceride.

In a separate but related study, we found a mixture of different sterols present in the leaves, larva, and butterfly (W. E. Connor, Y. Wang, M. Green, and D. L. Lin, unpublished data). The fatty acid composition of the sterol esters represented a group of sterols. The polyunsaturated fatty acids of sterol esters were much higher in larva and butterfly than in the leaf. This is not surprising, because sterol esters are formed either from free fatty acids (esterification) or from phospholipids (*trans*-esterification). Both phospholipid and free fatty acid classes had higher polyunsaturated fatty acid levels in larva and butterfly than in the leaf.

In our study, the butterfly larva had a higher linoleic acid content and a lower oleic acid content than the butterfly. This difference may be attributable to the fatty acid metabolism in the butterfly. During metamorphosis in a fly, D'Costa and Birt (20) found that the saturates vary according to one pattern and the unsaturates according to another. Their data suggested that the activity of desaturase systems varied during metamorphosis. This suggestion was supported by the data of Canavoso. (2), which showed the reduced incorporation of acetate to monoenes during the pupal period in some insects. In our study, the transformation from larva to butterfly drastically decreased the total fatty acid content. Although the butterfly had a lower body weight (283 mg) than larva (471 mg), this did not entirely explain the loss of fatty acid. There was also a decrease in fatty acid per unit weight. Because fatty acid is the most efficient source of energy (21), the larva must have lost considerable tissue fat during metamorphosis and the butterfly during flight as a result of energy expenditure. The drastic decrease of triglyceride observed in our study is consistent with this view, whereas total phospholipid did not change. It is interesting that saturated and polyunsaturated fatty acids decreased to the same degree (68–70%), whereas less monounsaturated fatty acid was lost, only 30%. The n-6 fatty acids lost a little more than n-3 fatty acid (78% vs. 67%). The lesser loss of monounsaturated fatty acid suggested the possibility that the adult butterfly can synthesize oleic acid from other substrate.

In conclusion, given a rare opportunity, we were able to measure the fatty acid intake and content of two life forms (larva and adult butterfly) of the butterfly species *M. peleides*. This butterfly contains high levels of polyunsaturated fatty acids (linolenic and linoleic acids). Nearly 60% of fatty acids in larva and 50% of fatty acids in the butterfly were polyunsaturated. The polyunsaturated fatty acids in the diet were also high (27% of total fatty acids). The daily intake of polyunsaturated fatty acids of the larva was equal to 8% of the total tissue polyunsaturated fatty acids. Diet was a significant contributor to the polyunsaturation of fatty acids of the butterfly. In metamorphosis from larva to butterfly, there was the loss of a large amount of fatty acids, probably as a result of energy expenditures. The fatty acid content of the butterfly was only approximately one-third of that of the larva (6.9 vs. 20.2 mg). Restricted to a single dietary source, which provided a high polyunsaturated content, the butterfly ultimately acquired phospholipid membranes that were polyunsaturated. These more fluid membranes may facilitate flight.

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REFERENCES

1. Law, J. H., and M. A. Wells. 1989. Insects as biochemical models. *J. Biol. Chem.* **264:** 16335–16338.
2. Canavoso, L. E., Z. E. Jouni, K. J. Karnas, J. E. Penington, and M. A. Wells. 2001. Fat metabolism in insects. *Annu. Rev. Nutr.* **21:** 23–46.
3. Kerkurt, G. A., and L. I. Gilbert, editors. 1985. Comprehensive Insect Physiology, Biochemistry and Pharmacology. Pergamon, Oxford, UK.
4. Thompson, S. N. 1973. A review and comparative characterization of the fatty acid composition of seven insect orders. *Comp. Biochem. Physiol.* **45B:** 467–482.
5. Fast, P. G. 1964. Insect lipids: a review. *Mem. Entomol. Soc. Can.* **37:** 1–50.
6. Gilbert, L. I. 1967. Lipid metabolism and function in insects. *Adv. Insect Physiol.* **4:** 69–211.
7. Zhu, N., X. Dai, D. S. Lin, and W. E. Connor. 1994. The lipids of slugs and snails: evolution, diet and biosynthesis. *Lipids.* **29:** 869–875.
8. Anderson, G. J., W. E. Connor, J. D. Corliss, and D. S. Lin. 1989. Rapid modulation of the n-3 docosahexaenoic acid levels in the brain and retina of the newly hatched chick. *J. Lipid Res.* **30:** 433–441.
9. Stark, W. S., T. N. Lin, D. Brackbahn, J. S. Christianson, and G. Y. Sun. 1993. Fatty acids in the lipids of Drosophila heads: effects of visual mutants, carotenoid deprivation and dietary fatty acids. *Lipids.* **28:** 345–350.
10. Kiyashko, S. I., B. J. Audrey, N. Tetsuya, I. S. Vassilii, and W. Eitaro. 2004. Fatty acid composition of aquatic insect larvae *Siticto chisonomus pictulus* (Diptera: Chironomidae): evidence of feeding upon methanotrophic bacteria. *Comp. Biochem. Physiol.* **139:** 705–711.
11. Sushchirk, N. N., M. I. Gladyshev, A. V. Moskvichova, D. N. Makhutova, and G. J. Kalachova. 2003. Comparison of fatty acid composition in major lipid classes of the dominant benthic invertebrates of the Yenisei River. *Comp. Biochem. Physiol.* **134:** 111–122.
12. Barlow, J. S. 1966. Effects of diet on the composition of body fat in *Lucillia sericata. Nature.* **212:** 1478–1479.
13. Unni, B. G., A. C. Kakoty, D. Khanibos, P. R. Bhattacharya, M. G. Pathak, K. Pilla, R. Pillai, A. Choudhury, P. C. Jaikia, and A. C. Ghosh. 1966. Lipid and fatty acid composition of muga silkworm, *Dutheraea assana*, host plants in relation to silkworm growth. *J. Lipid Mediat. Cell Signal.* **13:** 295–300.
14. Blomquist, G. J., L. A. Dwyer, A. J. Chu, R. O. Ryan, and M. De

Renobales. 1982. Biosynthesis of linoleic acid in a termite, cockroach and cricket. *Insect Biochem.* **12:** 349–353.

15. Miller, R. R., Jr., A. O. Dare, M. L. Moore, R. G. Kooser, and B. W. Geer. 1993. Long-chain fatty acids and ethanol affect the properties of membranes in *Drosophila melanogaster* larvae. *Biochem. Genet.* **31:** 113–131.
16. Connor, W. E., D. S. Lin, D. P. Wolf, and M. Alexander. 1998. Uneven distribution of desmosterol and docosahexaenoic acid in the heads and tails of monkey sperm. *J. Lipid Res.* **30:** 1404–1411.
17. Bashan, M., H. Akbas, and K. Yurdakoc. 2002. Phospholipid and triacylglycerol fatty acid composition of major life stages of sunn pest, *Eurygaster integriceps* (Heteroptera: Surtelleridae). *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **132:** 375–380.
18. Green, P. R., and B. W. Geer. 1979. Changes in the fatty acid composition of *Drosophila melanogaster* during development and aging. *Arch. Int. Physiol. Biochim.* **87:** 485–491.
19. Robb, R., R. Hammond, and L. L. Bieber. 1972. Temperature-dependent changes in sphingosine composition and composition of fatty acids of glycerophosphatides from *Musca domestica* larvae. *Insect Biochem.* **2:** 131–136.
20. D'Costa, M. A., and L. M. J. Burt. 1966. Changes in the lipid content during the metamorphosis of the blowfly, *Lucilia. J. Insect Physiology.* **12:** 1377–1394.
21. Candy, D. J., A. Becker, and G. Wegener. 1997. Coordination and integration of metabolism in insect flight. *Comp. Biochem. Physiol.* **1173:** 475–482.