Fatty Acids in the Lipids of *Drosophila* Heads: Effects of Visual Mutants, Carotenoid Deprivation and Dietary Fatty Acids

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Lipids of *Drosophila* heads were extracted and separated by high-performance thin-layer chromatography. Fatty acid compositions of major phospholipids as well as of triglycerides were analyzed by gas-liquid chromatography. Proportions of the major fatty acids (14:0, 16:0, 16:1, 18:0, 18:1, 18:2, 18:3) varied depending on the lipid analyzed. Docosahexaenoic acid (22:6), common in vertebrate photoreceptors and brain, and arachidonic acid (20:4), a precursor of eicosanoids, were lacking. A comparison of the fatty acid composition of the diet *vs.* the head suggested that *Drosophila* can desaturate but may not be able to elongate fatty acid carbon chains. Fatty acid analyses were carried out after the following visual system alterations: i) the transduction mutant where *no r*eceptor *p*otential results from a deficit in phospholipase C; ii) an allele of *ey*es *a*bsent; iii) the mutant *o*uter *r*habdomeres *a*bsent which lacks visual pigment and rhabdomeres in the predominant type of compound eye receptor, rhabdomeres 1 through 6; and iv) carotenoid deprivation which reduces opsin and rhabdomere size. We also evaluated aging by comparing newly-emerged *vs.* aged wild-type flies. Alterations in fatty acid composition based on some of these manipulations were found. Based on comparisons between flies reared on media differing in C_{16} and C_{18}, there is an indication that diet readily affects tissue fatty acid composition.
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In previous studies (1,2) we have quantified the fatty acids of phospholipids (PL) and triglycerides (TG) of *Drosophila* heads. Our primary interest in the fatty acids is based in their importance as structural components of the PL of visual membranes, which, in the fly, are tightly packed microvillar organelles called rhabdomeres. In addition, fatty acids are of interest in such membranes, whose main function is signal transduction, since they can serve as precursors of signalling molecules.

The strategy of genetic dissection (3), comparing strains with *vs.* without identified visual structures or processes in *Drosophila,* helped us to dissect the fatty acids of the visual system. In the present study, we analyzed: i) *o*uter *r*habdomeres *a*bsent *(ora)* an opsin mutant which selectively eliminates the predominant rhabdomeres, 1 through 6, (R1–6) of the compound eye (3); ii) *ey*es *a*bsent *(eya)* which eliminates the compound eyes and reduces the optic lobes; and iii) *no r*eceptor *p*otential *(norpA)* whose phenotype results from a phospholipase C (PLC) deficiency (4,5). We also utilized carotenoid deprivation which lowers opsin in all receptors (6) and makes the rhabdomeres smaller (7). Moreover, we compared samples from aged *vs.* control heads and samples derived from animals reared on diets with different fatty acid compositions.

MATERIALS AND METHODS

Animals. Stocks of *Drosophila melanogaster* were raised at room temperature under cyclic lighting on a standard diet known to be adequate for visual receptor development (8). In the medium of agar, brewer's yeast, molasses and corn meal, the compounds most relevant to visual development were the yellow pigment (zeaxanthin) in corn meal and a supplement of β-carotene (0.13 mg/mL), both precursors of rhodopsin's chromophore. Flies were deprived of carotenoids by rearing on Sang's medium (9); replete controls were raised on Sang's medium supplemented with β-carotene at 0.13 mg/mL. Importantly, the diets differed in fatty acid composition (Table 1).

Wild type (Oregon-R) was used as the normal control fly. Our *norpA* stock was $norpA^{EE5}$, an effective allele (10), originally from Prof. Seymour Benzer's laboratory at the California Institute of Technology (Pasadena, CA). An allele of *ey*es *a*bsent *(eya)* was obtained from Prof. Terry McGuire at Rutgers University (New Brunswick, NJ); these flies lack the compound eyes but not the simple eyes (ocelli) (11). We used the well-studied *o*uter *r*habdomeres *a*bsent *(ora)* mutant allele of the R1–6 opsin gene in which the outer rhabdomeres (R1–6) are selectively deleted (12); R1–6 rhabdomeres comprise the predominant photoreceptive organelle in the compound eye. For *ora,* as well as the wild type controls in the *ora* experiments, flies were aged in cyclic lighting one week or more because this manipulation insured a better deletion of *ora*'s rhabdomeres (12). For all other types, flies were newly-eclosed, that is, newly emerged from the pupa case.

TABLE 1

Percentages of Fatty Acids in Medium

Fatty acid	Regular diet (mole %)	Sang's diet (mol %)
16:0	74.37	61.13
18:0	13.61	20.49
18:1	10.06	18.38
18:2	1.96	trace[a]
18:3	trace	trace

[a]Less than 1%.

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Abbreviations: DHA, docosahexaenoic acid; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol-*bis*(β-aminoethyl ether) *N,N,N',N'*-tetraacetic acid; *eya, ey*es *a*bsent; GLC, gas-liquid chromatography; HPTLC, high-performance thin-layer chromatography; *norpA, no r*eceptor *p*otential; *ora, o*uter *r*habdomeres *a*bsent; PC, phosphatidylcholine; PC_r, a likely PC analogue migrating to the right of PC in HPTLC; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PL, phospholipids; PLC, phospholipase C; PS, phosphatidylserine; R1–6, rhabdomeres 1 through 6; TG, triglyceride; UV, ultraviolet.

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Extractions. Glass vials with flies were plunged into liquid nitrogen. When flies were frozen, shaking vials vigorously separated heads from bodies, and 100 heads were sorted quickly with a brush at room temperature. Heads were then put into (usually) 1 mL of ice cold 0.32 M sucrose with 50 mM Tris-HCl buffer (pH = 7.4) and ethylenediaminetetraacetic acid (EDTA) (1 mM) and homogenized with a tightly fitted Teflon pestle. Then, 4 vols of chloroform/methanol (2:1, vol/vol) was added, followed by vortexing. In order to facilitate the recovery of the anionic PL, 50 μL of 40 mM ethylene glycol-*bis*(β-aminoethyl ether) *N,N,N′,N′*-tetraacetic acid (EGTA) was added to the tube, followed by revortexing. The tubes were centrifuged at 1500 $\times$ *g* for 5 min, and the organic phase was recovered and passed through a mini-column containing anhydrous Na_2SO_4. For more complete extraction of the acidic PL, a second extraction with 2 vol of chloroform/methanol (2:1, vol/vol) with 0.4% HCl was performed. The second organic extract was neutralized with one drop of 4 N NH_4OH before combining with the first one, and the extract was then evaporated to dryness.

High-performance thin-layer chromatography (HPTLC) and gas-liquid chromatography (GLC). The lipids were resuspended in chloroform/methanol (2:1, vol/vol). Three separate aliquots of the samples were spotted onto 10 $\times$ 10 cm HPTLC plates (Silica gel, 60, E. Merck, Darmstadt, Germany). These plates had previously been dipped into a solution containing 1% potassium oxalate with 2 mM EDTA and then diluted with methanol in the ratio of 3:2 (vol/vol). Data from the three batches were averaged, and standard deviations were calculated.

The procedures for HPTLC separation of PL have been described (13). Briefly, plates were developed in the first solvent system containing chloroform/methanol/acetone/29% ammonium hydroxide (70:40:10:10, by vol). After drying, plates were exposed to HCl vapor for 3 min for hydrolysis of the alkenyl ether linkages and were further developed in a second dimension using a solvent system containing chloroform/methanol/acetone/acetic acid/0.1 M ammonium acetate (70:30:27.5:2.25:5, by vol). After development, the plates were sprayed with 2,7-dichlorofluorescein, and the lipid spots were identified under a ultraviolet (UV) lamp. Individual PL or TG (together with silica gel) were scraped into test tubes. Prior to transmethylation, a known amount of heptadecanoic acid (17:0) methyl ester was added to each sample as an internal standard and for quantification of the PL in the sample.

Conditions for the analysis have been described previously (13). Briefly, conversion of the glycerolipid acyl groups to their methyl esters was achieved by base-catalyzed methanolysis. We added 2 mL of 0.5 M NaOH in anhydrous methanol (sodium methoxide) to the sample. After incubation at room temperature for 10 min, the mixture was partitioned into two phases by adding 4 mL of chloroform and 1.5 mL of water. After phase separation, the organic layer was removed and filtered through a mini-column of anhydrous Na_2SO_4. The organic solvent was evaporated to dryness under nitrogen, and the fatty acid methyl esters were redissolved in hexane. Fatty acid methyl esters were separated using a Supelco (Bellefonte, PA) SP2330 0.2 μm capillary column, 30 m long and 0.25 mm inside diameter, with helium as the carrier gas. Column temperature was programmed from 140 to 195°C at 4°C/min. Analysis was with a HP 5890 gas-liquid chromatograph (Hewlett-Packard, Avondale, PA) equipped with a flame-ionization detector. Recoveries of fatty acids from phospholipids after methanolysis had been verified to be over 95% (13).

RESULTS

Experiments carried out in this study involved *Drosophila* that were reared either on a diet based on natural products such as yellow cornmeal, molasses and yeast, the regular diet (8), or a more defined medium which was useful for deprivation or supplementation with vitamin A, the Sang's diet (9). Importantly, the diets differed in fatty acid composition as shown in Table 1. For example, the Sang's diet had a considerably higher proportion of 18:1 and a slightly lower proportion of 18:2 as compared with the regular diet.

Figure 1 shows a typical GLC tracing depicting the fatty acids from phosphatidylethanolamine (PE) in *eya* together with the internal standard (17:0). The most conspicuous finding was the absence of polyunsaturated fatty acids with chain lengths longer than 18 carbons, an observation in good agreement with that reported by Yoshioka *et al.* (14). Despite the presence in the diets of trace amounts of the essential fatty acids with 18:2 and 18:3 acyl chains, substantial proportions of these fatty acids are present in the PL of *Drosophila* heads. Since the fatty acids of *Drosophila* heads showed a substantial proportion of 16:1 which was not found in either food, it is concluded that flies are capable of desaturating 16:0. When we omitted the 17:0 standard, our GLC tracings did not reveal detectable levels of odd-numbered fatty acids, which is in disagreement with the data of Yoshioka *et al.* (14).

During the analysis of PL and TG by GC, it is possible to quantify the mass of the lipids obtained. The relative

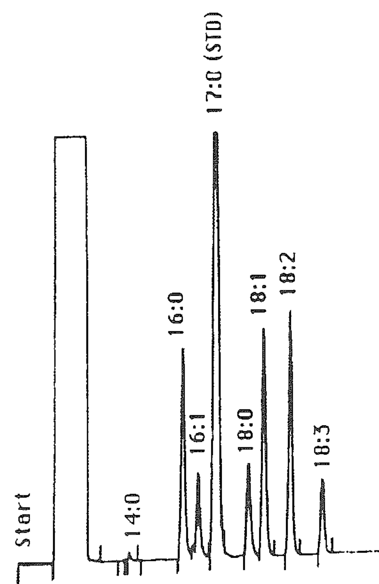

FIG. 1. A typical gas-liquid chromatogram, in this case from the *eyes absent* mutant for the phospholipid phosphatidylethanolamine. Fatty acid peaks at 14:0, 16:0, 16:1, 18:0, 18:1, 18:2 and 18:3 are labeled. The high peak at 17:0 is due to the standard (STD); when the STD is omitted (data not shown), there is virtually no reading at this location.

recovery of the standard (17:0 methyl ester), which had been added in known amount to samples from specified numbers of fly heads, should be inversely proportional to the quantity of each lipid in each strain. Data in Table 2 are calculated to represent the amount of each specified lipid for each experimental fly type studied (*eya, ora, norpA* and vitamin A deprived). PE, phosphatidylcholine (PC), a lipid which migrates to the right of PC on the HPTLC plate (PC_r), phosphatidylinositol (PI), phosphatidylserine (PS) and TG were analyzed. We divided the recovery of standard (averaged from the three samples partitioned from each 100-head batch) from each appropriate control strain by the recovery for each experimental strain. Lipid extracts were prepared with *eya* and *norpA* (*vs.* newly-eclosed wild-type controls), *ora* (aged to insure rhabdomere loss *vs.* aged wild-type controls) and carotenoid deprived and replete (control) wild-type reared on the same food (Sang's medium without *vs.* supplemented with β-carotene, respectively). As expected, *eya* shows the largest decrease in lipid content since the mutation eliminates the compound eyes entirely and reduces the optic lobes. Also, as expected, vitamin A deprived flies have lower PL since the rhabdomeres are reduced (7). We expected that *ora* would have lower PL since this mutant lacks the R1-6 rhabdomeres. Surprisingly, there were actually higher PL ratios in this mutant, suggesting that there may be a compensatory increase in PL in parts of the head other than the eyes. With the exception of a decrease in PC and PC_r, other membrane PL in *norpA* were not different from those of the wild type. This finding was expected because the *norpA* flies were newly emerged, while rhabdomere diminution is only realized in aged mutants (15). On the other hand, there was a large increase in TG in *norpA* as compared to controls. The cause for this increase is not clear.

The acyl group profiles of individual PL (PS, PI, PE, PC, PC_r) and TG in heads of newly-eclosed wild-type *Drosophila* reared on the regular diet are shown in Table 3. Although subtle differences could be found among the PL, the overall profiles were similar with high proportions of 18:1, 18:2 and, in some cases, 18:3, the latter two being very low in the regular food (Table 1). On the other hand, the acyl group profile of TG is in many ways different from that of PL. Similar to the TG acyl profiles of mammalian systems, the hallmark of the TG profile of *Drosophila* heads is the near absence of 18:0. On the other hand, the TG in *Drosophila* heads showed a high proportion of 14:0, a fatty acid which was below detection level in the food. An unknown lipid localized to the right of PC on the HPTLC plates (PC_r) has a greater proportion of the short chain saturated fatty acids (14:0 and 16:0) than PC. These data suggest that this newly resolved PL spot is part of the PC species. One conspicuous finding is that there are no fatty acid chains longer than 18 carbons in the PL and TG of *Drosophila* heads, confirming and extending the observations of Yoshioka *et al.* (14).

Since PE is the predominant PL present in the *Drosophila* head, we compared the acyl group profile of PE for different types of *Drosophila* (newly-eclosed wild-type, newly-eclosed *norpA*, newly-eclosed *eya*, aged wild-type, aged *ora*, and carotenoid replete and deprived wild-type, with only the latter two being reared on Sang's medium). As shown in Table 4, all types fed the regular diet showed similar fatty acid profiles. That is, aging and mutant condition did not change the profile of acyl groups in PE. Results from PI, not presented, are much like those for PE. The most striking diffrences result from comparing the flies fed the regular diet *vs.* the Sang's medium. Flies reared on Sang's medium showed significantly lower

TABLE 2

Ratio of Lipid in Experimental *vs.* Control Heads

Lipid[a]	*eya*[b]	*ora*[c]	*norpA*[d]	Vit. A deprived[e]
PE	0.46	1.09	1.08	0.76
PC	0.65	1.38	0.69	0.65
PC_r	—	—	0.62	—
PI	0.56	2.00	0.96	0.65
PS	0.58	1.30	1.08	0.52
TG	—	—	2.54	—

[a] PE, phosphatidylethanolamine; PC, phosphatidylcholine; PC_r, lipid fraction migrating to the right of PC; PI, phosphatidylinositol; PS, phosphatidylserine; TG, triglyceride.

[b] *eyes absent*, control is wild-type, both newly-eclosed, regular diet.

[c] *outer rhabdomeres absent*, control is wild-type, both aged, regular diet.

[d] *no receptor potential*, control is wild-type, both newly-eclosed, regular diet.

[e] Control is vitamin A replete, both wild type, Sang's diet.

TABLE 3

Percentages of Fatty Acids in the Lipids of Heads of Newly-Eclosed Wild-Type Flies Reared on Regular Diet

Fatty acid	PS[a]	PI[b]	PE[c]	PC[d]	PC_r[e]	TG[f]
			mole %			
14:0	3.0[g] (0.3)	—	0.6 (0.1)	2.0 (0.01)	6.8 (0.9)	23.0 (1.0)
16:0	9.4 (0.1)	17.6[h]	18.1 (0.6)	24.8 (1.0)	39.0 (3.1)	21.3 (1.0)
16:1	3.0 (0.2)	6.6	7.3 (0.3)	11.5 (0.6)	—	26.7 (1.0)
18:0	11.9 (0.5)	10.8	11.0 (0.2)	8.0 (1.7)	14.6 (1.2)	0.8 (0.3)
18:1	32.5 (0.2)	25.3	24.2 (0.6)	24.4 (1.8)	18.3 (2.9)	22.6 (1.0)
18:2	24.5 (0.1)	29.6	28.8 (0.7)	26.5 (1.8)	17.1 (4.4)	3.2 (0.5)
18:3	15.8 (0.2)	10.1	10.2 (0.5)	3.0 (0.2)	—	0.3 (0.1)

[a] Phosphatidylserine, averaged from three experiments.

[b] Phosphatidylinositol, averaged from four experiments.

[c] Phosphatidylethanolamine, averaged from four experiments.

[d] Phosphatidylcholine, averaged from three experiments.

[e] Lipid fraction migrating to the right of phosphatidylcholine, one experiment.

[f] Triglyceride, averaged from 2 experiments.

[g] Values averaged from 3 samples (± standard deviation).

[h] Values for PI averaged from 2 samples, no error listed.

TABLE 4

Percentages of Fatty Acids in Phosphatidylethanolamine in Heads of All Fly Types Studied

Fatty acid	Young wild-type[a]	Young *norpA*[b]	Young *eya*[c]	Aged wild-type[a]	Aged *ora*[d]	Vitamin A replete[e]	Vitamin A deprived[e]
				mole %			
16:0	18.1[f] (0.6)	19.4[g]	16.8 (0.5)	17.2 (0.4)	15.4 (0.8)	16.9 (0.2)	16.3 (0.8)
16:1	7.3 (0.3)	6.6	12.0 (0.2)	11.4 (0.6)	13.0 (0.5)	30.0 (0.4)	27.9 (1.7)
18:0	11.0 (0.2)	16.3	8.9 (0.5)	8.9 (0.5)	10.4 (0.3)	5.3 (0.02)	6.4 (0.4)
18:1	24.2 (0.6)	26.5	28.3 (0.9)	25.0 (1.0)	25.0 (2.1)	41.4 (1.0)	41.4 (2.0)
18:2	28.8 (0.7)	24.2	21.9 (0.4)	27.0 (0.3)	25.1 (1.1)	4.9 (0.4)	6.1 (0.5)
18:3	10.2 (0.5)	6.7	12.2 (0.8)	10.5 (0.6)	11.2 (0.9)	1.5 (0.04)	2.0 (0.2)

[a] Regular diet; "young" = newly-eclosed; "aged" = one week post-eclosion.
[b] *no* receptor *p*otential, regular diet, newly-eclosed.
[c] *ey*es *a*bsent, regular diet, newly-eclosed.
[d] *o*uter *r*habdomeres *a*bsent, regular diet, one week post-eclosion.
[e] Sang's diet, newly-eclosed.
[f] Values averaged from three samples (± standard deviation).
[g] Values for young *norpA* averaged from two samples, no error listed.

TABLE 5

Percentages of Fatty Acids in Triglyceride in Heads of All Fly Types Studied

Fatty acid	Young wild-type[a]	Young *norpA*[b]	Young *eya*[c]	Aged wild-type[a]	Aged *ora*[d]	Vitamin A replete[e]	Vitamin A deprived[e]
				mole %			
14:0	23.0[f] (1.0)	23.2 (3.6)	18.4 (1.1)	15.9[g]	26.8	21.1 (3.1)	16.4 (1.2)
14:1	2.1 (0.1)	2.4 (0.3)	1.9 (0.1)	1.6	2.5	—	—
16:0	21.3 (1.0)	22.5 (1.2)	12.0 (0.4)	12.7	10.5	12.9 (0.6)	9.4 (0.4)
16:1	26.7 (1.0)	23.5 (0.5)	15.4 (0.4)	16.7	13.2	11.6 (0.5)	7.7 (0.5)
18:0	0.8 (0.3)	0.8 (0.1)	—	—	—	—	—
18:1	22.6 (1.0)	24.0 (2.0)	49.4 (1.3)	50.1	44.6	51.9 (4.1)	63.1 (0.8)
18:2	3.2 (0.5)	3.3 (0.4)	2.9 (0.1)	3.2	2.5	2.5 (0.1)	3.3 (0.2)
18:3	0.3 (0.5)	0.5 (0.1)	—	—	—	—	—

[a–f] As in Table 4.
[g] Values for aged wild-type and aged *ora* averaged from two samples, no error listed.

proportions of 18:2 and 18:3 and higher proportions of 16:1 and 18:1 than those raised on the regular food; recall that the Sang's medium had higher 18:1 (Table 1).

The acyl group profiles for TG for the same fly types are presented in Table 5. In contrast with the acyl profile for PE, TG fatty acids tend to show large differences with age and mutant condition. The Sang's medium flies showed strikingly high 18:1. Interestingly, 18:1 was also very high in *eya* flies, which might imply that it is correspondingly low in the visual system which *eya* flies lack. Finally, the aged flies have a higher proportion of 18:1 and lower proportions of 16:0 and 16:1 in the TG (Table 5). Recall that we had originally decided to study aged wild-type flies as controls in the experiment with aged *ora* since *ora* loses its rhabdomeres as a function of age (12).

DISCUSSION

One of our primary findings is that the fatty acid content of *Drosophila* heads can readily be altered by the diet. Large differences in fatty acid composition for PL and TG were observed by comparing flies reared on the Sang's diet (for vitamin A manipulations) *vs.* the regular diet. Our data are in line with the statement by Turunen (16) that "the tissue fatty acid composition often is quite sensitive to dietary changes." A comparison of the fatty acids from the food *vs.* those from *Drosophila* suggests that the flies cannot elongate the fatty acids to make ≥20 carbon compounds but they can desaturate the fatty acids, *e.g.,* from 16:0 to 16:1.

Our results are in general agreement with those reported by Yoshioka *et al.* (14) who determined the fatty acid composition of PI and PC in *Drosophila.* Both studies concur in that there are no long chain polyunsaturated fatty acids in the tissue PL. The small discrepancies can well be attributed to differences in the diet since the diet used by Yoshioka *et al.* had less 16:0, did have 16:1 and had higher 18:0, 18:1 and 18:2. They obtained higher values of C_{16} fatty acids and lower values of C_{18} fatty acids in PI and the reverse for PC compared to ours. Their analysis showed the presence of small amounts of C_{15} and C_{17} fatty acids, but we did not find them in our study. On the other hand, we observed small amounts of 18:3 while they did not.

It would be interesting to determine whether dietary supplementation with long chain fatty acids may have any effects on the fatty acids of the visual system. In a preliminary study, R. Sapp and W. S. Stark (unpublished) found that supplementation of the regular food with menhaden oil (containing a high proportion of polyunsaturated fatty acids) had no obvious effect on visual receptor ultrastructure. On the other hand, D.-M. Chen and W. S. Stark (unpublished) showed a small increase in electroretinographic sensitivity of R1–6 from this supplementation, especially in the near UV region of the spectrum. These data suggest that the fly model will prove useful to elucidate the relationship between dietary fatty acids and visual function.

Vertebrate visual receptors contain high proportions of the long chain polyunsaturated fatty acids, especially 22:6 and 20:4 (17). Such high levels of long chain fatty acids in vertebrate visual receptors are of substantial interest (18). Although the function of 22:6 (DHA) in vertebrate photoreceptors is not known (19,20), the conventional wisdom is that unsaturated fatty acids contribute to membrane fluidity which could facilitate interactions of the molecules of the signal transduction cascade in the planar disk membranes of the rod outer segment. A defect in metabolism or transport of DHA in poodles afflicted with progressive rod-cone degeneration suggests that DHA is essential for visual receptor maintenance in vertebrates (21). *Drosophila* may prove helpful in determining the function of DHA since DHA is absent in the lipids of flies whose vision is intact. In contrast to the fly, the cabbage butterfly *Pieris brassicae* incorporates 20:5n-3 (eicosapentaenoic acid) into a variety of lipids including PI (22). Insects provide a wide natural diversity in diet potentially providing a wealth of comparative biochemistry. For instance, some insects are "phytophagous," meaning that they feed on plants, and, in many cases, the insect-host relationship is very specific, while others feed on meat or blood, which are rich sources of long chain fatty acids.

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