# Energy Restriction Dilutes the Changes Related to Dietary Fat Type in Membrane Phospholipid Fatty Acid Composition in Rats

Ming C. Cha and Peter J.H. Jones

**To investigate liver cell membrane phospholipid (PL) fatty acid (FA) composition in response to the consumption of different types of dietary fat and graded levels of energy intake, rats were fed for 10 weeks on a diet containing either fish oil, safflower oil, or beef tallow. Within each dietary fat group, subgroups were either provided free access to food or energy-restricted to 85% or 70% of the ad libitum intake by reducing the dietary carbohydrate content while keeping other macronutrient intakes constant. Higher ($P < .05$) proportions of docosahexaenoic acid, linoleic acid, and monounsaturated FA were observed in the membrane PL of the fish oil, safflower oil, and beef tallow groups, respectively, resembling the FA composition in the diets. However, such modifications of dietary FA composition in membrane PL FA were influenced by body energy status. The higher docosahexaenoic acid and total n-3 FA content in phosphatidylcholine (PC), sphingomyelin (SPH), and phosphatidylserine (PS) of the ad libitum fish oil group compared with the other dietary groups no longer existed when energy supply was restricted. Therefore, reducing energy intake tended to dilute the changes of membrane PL FA composition occurring as a function of dietary FA composition. These data suggest that the influence of dietary fat type on cellular structure and perhaps function becomes increasingly important with progressively positive energy balance.**


THE FATTY ACID (FA) composition of the cell membrane determines its structure and fluidity and may play a critical role in the control of many metabolic processes.[1] Evidence has shown that the modification of membrane phospholipid (PL) composition can influence cellular division,[2,3] membrane protein function,[4-6] and receptor-mediated signal transmission.[7] Endothelial cell membrane PL enriched with n-6 polyunsaturated FA (PUFA) showed a dramatic increase in cellular triglyceride content compared with those enriched with n-3 PUFA.[8]

Membrane FA composition can be modified through changes in dietary FA composition.[9] Infants fed a formula without arachidonic acid and docosahexaenoic acid showed a reduction in the FAs in erythrocyte membrane PL.[10] Feeding rats a diet containing an equal quantity of either n-3 or n-6 PUFA for 1 week increased the unsaturated to saturated FA ratio of adipocyte membrane PL in the n-6 PUFA diet group but not in the n-3 PUFA group.[11]

Changes in membrane FA composition subsequent to energy restriction have also been noted. Food restriction resulted in a significant increase of essential FAs in microsomal membrane while attenuating the level of docosapentaenoic and docosahexaenoic acids.[12] Thus, membrane FA composition is determined independently by either qualitative fat intake or total body energy balance. Recently, we have demonstrated that tissue FA composition,[13] as well as cholesterol and FA biosynthesis,[14] are influenced by an interaction between the dietary FA composition and energy intake level. In considering the importance of membrane lipid FA composition in the regulation of enzyme activity involving cellular lipid metabolism,[1,4,15] we were interested to see whether a similar interactive effect of dietary FA composition and energy restriction also affects membrane FA composition. The outcome from such an investigation provides insight into the possible mechanisms defining the role of qualitative FA intake in the maintenance of optimal cellular function consistent with health.

The objective of the present study was therefore to examine the combined effect of dietary fat type and graded levels of energy intake on liver cell membrane PL FA composition. To examine the action of an energy deficit exclusively without confounding by a decreased FA intake, energy restriction in the present study was achieved by removing carbohydrate from the diets while keeping the intake of fat and other macronutrients constant.

## MATERIALS AND METHODS

### *Animals and Diets*

Seventy-two male Sprague-Dawley rats (209 ± 6.5 g) purchased from Charles River (Montreal, Quebec, Canada) were housed individually in stainless steel hanging cages with a 12-hour light-dark cycle at 22° ± 1°C environment. After habituation to a commercial rat chow diet for 7 days, the rats were randomized into 3 dietary fat groups and fed a 20% fat (wt/wt) diet containing either fish oil, safflower oil, or beef tallow as the fat source. The beef tallow was supplemented with 1% safflower oil to maintain an adequate intake of linoleic acid. The FA composition of the dietary fats is shown in Table 1. Animals within each fat group either had free access to the control diet (n = 8) or were energy-restricted to 85% (n = 8) or 70% (n = 8) of the ad libitum daily intakes. The control diet contained 15% casein, 20% fat, and 55% carbohydrate with an adequate amount of vitamins and minerals. To supply equal quantities of all nutrients except carbohydrate in the 85% and 70% energy restriction diets compared with the control diet, the amount of protein, fat, and other micronutrients was proportionally increased through systematic removal of carbohydrate[13] as indicated in Table 2. Body weight was recorded weekly. At the end of the 10-week feeding period, the animals were anesthetized by carbon dioxide gas and then killed. The livers were collected, weighed, immediately frozen in liquid $N_2$ after adding a homogenization buffer (0.25 mol/L sucrose and 10 mmol/L HEPES, pH 7.5), and then stored at −80°C until further analysis.

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**Table 1. Major FA Composition of the Experimental Fats (% wt/wt)**

| FA | Fish Oil | Safflower Oil | Beef Tallow |
|---|---|---|---|
| 14:0 | 9.6 | — | 4.1 |
| 16:0 | 18.7 | 7.1 | 27.7 |
| 16:1(n-7) | 13.7 | — | 6.0 |
| 18:0 | 5.8 | 2.0 | 14.0 |
| 18:1(n-9) | 11.5 | 20.8 | 45.5 |
| 18:1(n-7) | 2.5 | — | — |
| 18:2(n-6) | 2.0 | 68.7 | 1.9 |
| 18:3(n-3) | 2.3 | 1.1 | 0.2 |
| 20:1(n-9) | 1.1 | 0.3 | 0.6 |
| 20:4(n-6) | 1.3 | — | — |
| 20:5(n-3) | 16.6 | — | — |
| 22:4(n-6) | 0.9 | — | — |
| 22:6(n-3) | 14.0 | — | — |

NOTE. Missing data indicate that the FA was not detectable.

### *Membrane Preparation and FA Analysis*

Liver cell plasma membranes were prepared according to the method of Fleischer and Kervina.[16] The livers were homogenized in the homogenization buffer and centrifuged at 1,400 × *g* for 15 minutes. The pellet was suspended in a high-density sucrose solution (1.6 mol/L sucrose, 10 mmol/L HEPES, and 1 mmol/L $MgCl_2$, pH 7.5), overlayered with the homogenization buffer, and centrifuged at 70,900× *g* for 70 minutes in an SW-28 rotor using an LE-80K ultracentrifuge (Beckman Palo Alto, CA). The band at the interface between the 2 layers was collected and washed twice with a washing buffer (0.25 mol/L sucrose, 10 mmol/L HEPES, and 1 mmol/L EDTA, pH 7.5). The pellet was resuspended in a buffer (1.45 mol/L sucrose, 10 mmol/L HEPES, and 1 mmol/L EDTA, pH 7.5), top-loaded with the homogenization buffer, and centrifuged at 68,400 × *g* for 60 minutes. The plasma membrane appeared as a band at the 0.25 mol/L:1.45 mol/L sucrose interface. The membrane fraction acquired by this method is believed to have 97% purity.[16] Total lipid was extracted from the membrane by procedures described previously.[17] Individual PLs, including phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), and sphingomyelin (SPH), were separated by 1-dimensional thin-layer chromatography (TLC) as described by Holub and Skeaff.[18] PLFA methyl esters were prepared using $BF_3$:hexane:methanol (7:6:7) reagent and separated by a gas-liquid chromatograph (model 5890; Hewlett Packard, Palo Alto, CA) equipped with a 30-m × 0.2-mm SP2330 column (Supelco, Bellefonte, PA), flame ionization detectors, and automated injection. FA methyl ester peaks were identified using authenticated standards (Supelco). PLFA composition analysis was performed in duplicate following TLC separation.

### *Statistics*

Data were analyzed by a 2-way ANOVA using a SAS general linear model program (SAS Version 6; SAS Institute, Cary, NC). Mean values were separated by Fisher's protected least-significant difference (LSD) procedure. Differences between means were considered significant at a *P* level less than .05. Data are expressed as the mean ± SEM.

## RESULTS

The body and liver weight of rats fed diets varying by fat source and energy intake level are shown in Table 3. Dietary fat type had no effect on body weight. Graded levels of energy restriction proportionally decreased ($P < .01$) the body weight gain across dietary fat groups. Different fat consumption resulted in varied liver weight gains ($P < .01$). Liver weight was higher ($P < .01$) in the fish oil group compared with the other 2 fat groups when the animals had free access to food. Energy restriction decreased ($P < .02$) liver weight in all dietary fat groups.

The major FA composition of liver cell membrane PC in animals fed diets varying by fat source and energy intake level is shown in Table 4. Different fat intake influenced ($P < .03$) the content of linoleic, arachidonic, and docosahexaenoic acids, as well as monounsaturated and n-6 unsaturated FA, without affecting other FAs. The proportion of docosahexaenoic acid was higher in animals fed the fish oil diet consumed ad libitum and at 85% of ad libitum intake compared with the groups fed other fats. However, such differences were not observed when less food was supplied. In contrast, more monounsaturated FAs were found in the beef tallow–fed animals. Energy restriction had no effect on the content of almost all FAs in PC, with the exception of linoleic acid, in beef tallow–fed animals, the proportion of which was decreased ($P = .05$) in response to food restriction. There was a trend for increasing total saturated FA content in PC when energy intake was restricted, although such increases did not reach statistical significance.

The major FA composition of liver cell membrane PE in rats fed diets differing by fat type and energy intake level is shown in Table 5. The type of dietary fat modified ($P < .01$) the concentration of linoleic and docosahexaenoic acids, as well as total monounsaturated and n-6 polyunsaturated FA, in PE. Fish oil feeding resulted in greater docosahexaenoic acid incorporation into PE as compared with the other fats. Similarly, more ($P < .05$) linoleic acid and n-6 FA were found in safflower oil–fed rats. A higher ($P < .05$) proportion of monounsaturated FA was observed in beef tallow–fed animals. Energy restriction had no effect on any of the FAs in PE.

The major FA composition of liver cell membrane PI in rats fed diets varying by FA composition and energy intake level is shown in Table 6. Different fat intakes significantly influenced ($P < .05$) linoleic acid and saturated, monounsaturated, and n-6 FA content in PI, while not influencing other FAs. Unlike the composition in the diet, docosahexaenoic acid content was similar in the fish oil–fed group compared with the other dietary fat groups. In addition, total saturated FA content was higher in the ad libitum and 85% ad libitum fish oil groups versus the other oil groups. In contrast, safflower oil feeding was associated with higher linoleic acid and n-6 FA content, whereas more monounsaturated FA was observed in beef tallow–fed animals,

**Table 2. Composition of the Experimental Diets (g/100 g)**

| Component | Energy Intake Group | | |
|---|---|---|---|
| | 100% | 85% | 70% |
| Casein | 15 | 17.6 | 21.4 |
| Fat | 20 | 23.5 | 28.6 |
| Cornstarch | 45 | 32.7 | 15 |
| Sucrose | 10 | 11.8 | 14.3 |
| Cellulose | 5 | 8.6 | 13.6 |
| AIN-93M-MX | 3.5 | 4.1 | 5 |
| AIN-93-VM | 1 | 1.2 | 1.4 |
| L-Cystine | 0.18 | 0.21 | 0.26 |
| Choline bitartrate | 0.25 | 0.29 | 0.36 |
| *t*-Butylhydroquinone | 0.004 | 0.005 | 0.006 |

Abbreviations: AIN, American Institute of Nutrition; MX, mineral mix; VM, vitamin mix.

**Table 3. Body and Liver Weight (g) of Rats Fed Diets Varying in Fat Source and Energy Intake Level**

| Parameter | Energy Intake Group | Dietary Fat | | |
|---|---|---|---|---|
| | | Fish Oil | Safflower Oil | Beef Tallow |
| Body weight | 100% | 571.1 ± 10.2 | 572.8 ± 12.7 | 555.9 ± 26.0 |
| | 85% | 532.9 ± 8.2* | 552.3 ± 7.5 | 519.9 ± 11.9* |
| | 70% | 468.0 ± 11.0*† | 493.5 ± 4.4*† | 475.8 ± 8.3*† |
| Liver weight | 100% | 22.8 ± 0.9[a] | 19.8 ± 0.9[b] | 19.2 ± 1.5[b] |
| | 85% | 18.2 ± 0.6* | 17.7 ± 0.9 | 16.5 ± 0.7* |
| | 70% | 16.1 ± 0.5[a]* | 14.1 ± 0.7[ab]*† | 12.9 ± 0.8[b]*† |

NOTE. Values are the mean ± SEM (n = 8). Means with different superscript letters in the same row are significantly different ($P < .05$, Fisher's protected LSD). For each parameter in the same colunn: *significantly different *v* 100% group and †significantly different *v* 85% group.

which generally reflected the FA composition in the respective diet. No effect of energy deficit was detected for any of the FAs investigated.

The major FA composition of liver cell membrane PS in rats fed diets varying by FA composition and energy content is shown in Table 7. Among the FAs investigated, dietary fat composition influenced ($P < .05$) linoleic acid and monounsaturated and n-6 FA content in membrane PS. However, such changes in FA composition in PS in response to dietary FA composition were dependent on the energy intake level. Differences in the level of linoleic acid and monounsaturated FA between the ad libitum–fed fish oil group and other fat groups fed ad libitum could not be detected when energy intake was restricted.

The major FA composition of liver cell membrane SPH in rats consuming diets varying by fat type and energy intake is shown in Table 8. Dietary FA composition influenced ($P < .05$) the proportion of almost all FAs investigated in SPH. Fish oil feeding was associated with a higher proportion of docosahexaenoic acid as compared with the other oils, while more monounsaturated FA was found in animals fed the beef tallow diet. The responses of arachidonic and docosahexaenoic acids and n-6 FA to changes in the dietary fat source were also dependent on the energy intake level. The difference in the content of these FAs in response to the selection of dietary fat type in ad libitum–fed animals was not observed when the energy supply was restricted to 70% of the ad libitum intake. No effect of energy restriction was found on the level of any FA in SPH, with the exception of linoleic acid in beef fat–fed rats, the proportion of which decreased with heightened energy deficiency.

The proportion of n-3 FAs of liver cell membrane PC, PE, PS, PI, SPH, and total PL in rats fed different dietary fats at graded levels of energy intake are depicted in Fig 1. Fish oil feeding resulted in an increased ($P < .05$) proportion of total n-3 FA in

**Table 4. PC FA Composition in Liver Cell Membrane of Rats Fed Diets Varying in Fat Source and Energy Intake Level (% wt/wt)**

| FA | Energy Intake Group | Dietary Fat | | |
|---|---|---|---|---|
| | | Fish Oil | Safflower Oil | Beef Tallow |
| 18:2(n-6) | 100% | 2.9 ± 0.3[a] | 6.6 ± 1.4[b] | 5.9 ± 0.8[b] |
| | 85% | 2.4 ± 0.3[a] | 8.0 ± 1.1[b] | 4.0 ± 0.9[a] |
| | 70% | 2.3 ± 0.2[a] | 5.9 ± 1.0[b] | 3.1 ± 0.5[a]* |
| 20:4(n-6) | 100% | 7.2 ± 1.6 | 12.7 ± 4.7 | 11.1 ± 2.5 |
| | 85% | 7.0 ± 1.7[a] | 19.9 ± 5.4[b] | 14.7 ± 3.5[ab] |
| | 70% | 5.6 ± 1.6 | 13.4 ± 5.1 | 10.2 ± 4.5 |
| 22:6(n-3) | 100% | 6.9 ± 1.7[a] | 1.3 ± 0.3[b] | 2.6 ± 0.7[b] |
| | 85% | 5.6 ± 1.9[a] | 1.7 ± 0.5[b] | 2.7 ± 0.7[ab] |
| | 70% | 3.7 ± 1.4 | 1.4 ± 0.5 | 2.1 ± 1.0 |
| ΣSaturated | 100% | 62.1 ± 2.9 | 67.0 ± 4.4 | 63.3 ± 3.5 |
| | 85% | 66.6 ± 3.6 | 56.2 ± 4.0 | 63.7 ± 4.3 |
| | 70% | 73.3 ± 3.1 | 69.4 ± 5.9 | 68.1 ± 5.1 |
| ΣMonounsaturated | 100% | 7.6 ± 1.0[a] | 5.4 ± 1.2[a] | 13.1 ± 1.6[b] |
| | 85% | 8.9 ± 1.2[a] | 4.4 ± 0.5[b] | 13.6 ± 1.5[c] |
| | 70% | 8.4 ± 0.8[a] | 4.2 ± 0.2[b] | 14.2 ± 1.9[c] |
| Σ(n-6) | 100% | 11.2 ± 2.0 | 17.8 ± 6.3 | 18.2 ± 3.5 |
| | 85% | 9.7 ± 2.1[a] | 28.9 ± 6.6[b] | 19.7 ± 4.3[b] |
| | 70% | 8.9 ± 1.9 | 19.8 ± 6.1 | 14.0 ± 5.3 |
| P/S ratio | 100% | 0.4 ± 0.1 | 0.5 ± 0.1 | 0.4 ± 0.1 |
| | 85% | 0.4 ± 0.1 | 0.6 ± 0.2 | 0.4 ± 0.1 |
| | 70% | 0.3 ± 0.1 | 0.4 ± 0.1 | 0.4 ± 0.1 |

NOTE. Values are the mean ± SEM (n = 7-8). Means with different letter superscript in the same row are significantly different ($P < .05$, Fisher's protected LSD). For each FA in the same column, *significantly different *v* 100% group.

**Table 5. PE FA Composition in Liver Cell Membrane of Rats Fed Diets Varying in Fat Source and Energy Intake Level (% wt/wt)**

| FA | Energy Intake Group | Dietary Fat | | |
|---|---|---|---|---|
| | | Fish Oil | Safflower Oil | Beef Tallow |
| 18:2(n-6) | 100% | 1.2 ± 0.3[a] | 4.4 ± 0.8[b] | 2.1 ± 0.4[a] |
| | 85% | 1.3 ± 0.4[a] | 6.0 ± 0.9[b] | 1.9 ± 0.5[a] |
| | 70% | 1.1 ± 0.3[a] | 4.8 ± 0.8[b] | 1.3 ± 0.2[a] |
| 20:4(n-6) | 100% | 5.3 ± 1.0 | 14.4 ± 3.9 | 12.1 ± 2.4 |
| | 85% | 5.6 ± 1.1[a] | 19.2 ± 3.8[b] | 11.8 ± 2.8[b] |
| | 70% | 5.6 ± 1.6[a] | 18.4 ± 3.9[b] | 11.5 ± 2.8[b] |
| 22:6(n-3) | 100% | 10.2 ± 2.6[a] | 2.0 ± 0.6[b] | 4.3 ± 1.7[b] |
| | 85% | 10.3 ± 2.0[a] | 3.9 ± 1.1[b] | 3.5 ± 0.9[b] |
| | 70% | 7.9 ± 2.2[a] | 4.0 ± 1.1[ab] | 2.9 ± 0.9[b] |
| ΣSaturated | 100% | 74.3 ± 5.3 | 72.4 ± 6.4 | 73.3 ± 5.0 |
| | 85% | 70.9 ± 6.8 | 63.2 ± 6.2 | 69.4 ± 5.6 |
| | 70% | 76.9 ± 6.6 | 66.9 ± 5.9 | 76.6 ± 3.9 |
| ΣMonounsaturated | 100% | 3.7 ± 0.7[a] | 5.7 ± 1.3[ab] | 7.3 ± 0.9[b] |
| | 85% | 2.9 ± 0.5[a] | 5.6 ± 1.3[a] | 8.9 ± 1.6[b] |
| | 70% | 2.4 ± 0.3[a] | 4.2 ± 0.5[ab] | 6.7 ± 0.2[b] |
| Σ(n-6) | 100% | 6.8 ± 1.2[a] | 19.8 ± 4.8[b] | 14.6 ± 2.8[ab] |
| | 85% | 7.3 ± 1.3[a] | 27.1 ± 4.7[b] | 14.2 ± 2.9[a] |
| | 70% | 7.1 ± 1.9[a] | 24.5 ± 4.9[b] | 13.3 ± 3.2[a] |
| P/S ratio | 100% | 0.3 ± 0.1 | 0.3 ± 0.1 | 0.3 ± 0.1 |
| | 85% | 0.4 ± 0.2 | 0.6 ± 0.2 | 0.3 ± 0.1 |
| | 70% | 0.3 ± 0.2 | 0.5 ± 0.1 | 0.2 ± 0.1 |

NOTE. Values are the mean ± SEM (n = 6-8). Means with different letter superscript in the same row are significantly different ($P < .05$, Fisher's protected LSD).

**Table 6. PI FA Composition in Liver Cell Membrane of Rats Fed Diets Varying in Fat Source and Energy Intake Level (% wt/wt)**

| FA | Energy Intake Group | Dietary Fat: Fish Oil | Safflower Oil | Beef Tallow |
|---|---|---|---|---|
| 18:2(n-6) | 100% | 2.2 ± 1.4 | 4.2 ± 0.6 | 1.2 ± 0.3 |
| | 85% | 0.8 ± 0.5[a] | 5.6 ± 0.7[b] | 2.5 ± 0.6[a] |
| | 70% | 1.9 ± 1.4[ab] | 3.7 ± 0.4[a] | 1.7 ± 0.5[b] |
| 20:4(n-6) | 100% | 7.7 ± 2.7 | 17.9 ± 5.9 | 18.3 ± 2.9 |
| | 85% | 7.3 ± 3.8 | 20.8 ± 4.0 | 16.9 ± 3.1 |
| | 70% | 13.7 ± 3.8 | 22.7 ± 5.3 | 20.6 ± 5.1 |
| 22:6(n-3) | 100% | 5.3 ± 1.8 | 3.8 ± 2.3 | 5.0 ± 1.7 |
| | 85% | 2.8 ± 1.3 | 5.5 ± 4.3 | 3.1 ± 0.8 |
| | 70% | 9.8 ± 3.1 | 3.9 ± 0.1 | 2.2 ± 0.5 |
| ΣSaturated | 100% | 78.6 ± 6.7[a] | 70.5 ± 5.2[ab] | 62.5 ± 5.9[b] |
| | 85% | 82.9 ± 6.7[a] | 61.5 ± 5.4[b] | 65.1 ± 3.6[b] |
| | 70% | 64.5 ± 7.8 | 68.6 ± 5.1 | 66.6 ± 4.9 |
| ΣMonounsaturated | 100% | 1.6 ± 0.2 | 4.1 ± 1.2 | 7.5 ± 3.1 |
| | 85% | 2.1 ± 1.0[a] | 1.8 ± 0.4[a] | 10.9 ± 2.9[b] |
| | 70% | 1.9 ± 1.0 | 1.8 ± 0.5 | 7.2 ± 3.3 |
| Σ(n-6) | 100% | 10.0 ± 3.1 | 22.2 ± 6.1 | 20.1 ± 3.4 |
| | 85% | 8.2 ± 4.6[a] | 26.9 ± 3.8[b] | 20.4 ± 3.2[ab] |
| | 70% | 15.9 ± 4.4 | 27.2 ± 5.7 | 22.8 ± 5.1 |
| P/S ratio | 100% | 0.3 ± 0.1 | 0.4 ± 0.1 | 0.5 ± 0.1 |
| | 85% | 0.2 ± 0.1 | 0.6 ± 0.2 | 0.4 ± 0.1 |
| | 70% | 0.6 ± 0.2 | 0.5 ± 0.1 | 0.4 ± 0.1 |

NOTE. Values are the mean ± SEM (n = 4-8). Means with different letter superscript in the same row are significantly different ($P < .05$, Fisher's protected LSD).

**Table 7. PS FA Composition in Liver Cell Membrane of Rats Fed Diets Varying in Fat Source and Energy Intake Level (% wt/wt)**

| FA | Energy Intake Group | Dietary Fat: Fish Oil | Safflower Oil | Beef Tallow |
|---|---|---|---|---|
| 18:2(n-6) | 100% | 1.1 ± 0.2[a] | 6.9 ± 1.3[b] | 2.8 ± 0.9[a] |
| | 85% | 1.2 ± 0.8 | 2.7 ± 0.7 | 2.0 ± 0.5 |
| | 70% | 1.4 ± 0.8 | 4.4 ± 1.5 | 1.4 ± 0.2 |
| 20:4(n-6) | 100% | 3.6 ± 0.5 | 12.5 ± 5.6 | 11.3 ± 3.9 |
| | 85% | 1.7 ± 0.02[a] | 10.25 ± 3.7[b] | 11.2 ± 2.2[b] |
| | 70% | 3.7 ± 1.4 | 15.2 ± 5.5 | 11.6 ± 2.4 |
| 22:6(n-3) | 100% | 9.6 ± 1.3[a] | 4.7 ± 1.4[b] | 6.8 ± 1.6[ab] |
| | 85% | 4.9 ± 2.6 | 4.7 ± 3.3 | 4.5 ± 1.0 |
| | 70% | 6.6 ± 1.9 | 5.6 ± 2.1 | 4.0 ± 1.1 |
| ΣSaturated | 100% | 76.6 ± 2.4 | 70.0 ± 8.8 | 66.9 ± 5.3 |
| | 85% | 84.7 ± 3.3 | 86.5 ± 3.9 | 72.9 ± 4.5 |
| | 70% | 82.9 ± 3.1 | 67.4 ± 8.3 | 74.7 ± 4.3 |
| ΣMonounsaturated | 100% | 4.5 ± 1.3[ab] | 4.8 ± 1.6[ab] | 9.4 ± 2.2[b] |
| | 85% | 3.3 ± 0.9 | 3.7 ± 1.3 | 7.4 ± 1.7 |
| | 70% | 3.1 ± 0.8 | 4.4 ± 0.5 | 6.0 ± 0.7 |
| Σ(n-6) | 100% | 4.3 ± 0.8[a] | 19.9 ± 7.9[b] | 13.3 ± 4.3[ab] |
| | 85% | 1.7 ± 0.02[a] | 11.2 ± 3.3[b] | 13.3 ± 2.5[b] |
| | 70% | 3.1 ± 1.7[a] | 20.7 ± 7.3[b] | 12.6 ± 2.4[ab] |
| P/S ratio | 100% | 0.3 ± 0.04 | 0.5 ± 0.2 | 0.4 ± 0.1 |
| | 85% | 0.2 ± 0.1 | 0.1 ± 0.1 | 0.3 ± 0.1 |
| | 70% | 0.2 ± 0.04 | 0.5 ± 0.2 | 0.3 ± 0.1 |

NOTE. Values are the mean ± SEM (n = 3-7). Means with different letter superscript in the same row are significantly different ($P < .05$, Fisher's protected LSD).

**Table 8. SPH FA Composition in Liver Cell Membrane of Rats Fed Diets Varying in Fat Source and Energy Intake Level (% wt/wt)**

| FA | Energy Intake Group | Dietary Fat: Fish Oil | Safflower Oil | Beef Tallow |
|---|---|---|---|---|
| 18:2(n-6) | 100% | 2.1 ± 0.5[a] | 8.8 ± 1.0[b] | 8.4 ± 1.1[b] |
| | 85% | 2.1 ± 0.6[a] | 8.0 ± 1.7[b] | 3.9 ± 0.7[a]* |
| | 70% | 2.8 ± 0.2[a] | 5.9 ± 1.3[b] | 3.3 ± 0.6[ab]* |
| 20:4(n-6) | 100% | 8.4 ± 1.8[a] | 26.2 ± 5.3[b] | 14.2 ± 3.2[a] |
| | 85% | 7.2 ± 1.5[a] | 19.9 ± 5.4[b] | 12.0 ± 2.9[ab] |
| | 70% | 7.9 ± 1.1 | 17.2 ± 5.1 | 12.8 ± 5.3 |
| 22:6(n-3) | 100% | 8.7 ± 2.9[a] | 3.6 ± 0.9[b] | 4.6 ± 1.1[b] |
| | 85% | 8.3 ± 1.9[a] | 2.8 ± 0.9[b] | 2.9 ± 0.8[b] |
| | 70% | 4.9 ± 1.2 | 3.4 ± 1.3 | 4.7 ± 1.8 |
| ΣSaturated | 100% | 66.5 ± 6.5 | 52.0 ± 7.3 | 56.5 ± 5.6 |
| | 85% | 64.2 ± 4.5 | 61.9 ± 7.8 | 64.4 ± 4.9 |
| | 70% | 68.7 ± 3.4 | 62.9 ± 6.2 | 58.1 ± 7.5 |
| ΣMonounsaturated | 100% | 4.6 ± 0.6[a] | 2.9 ± 0.4[a] | 11.0 ± 1.6[b] |
| | 85% | 8.2 ± 3.1[ab] | 3.5 ± 0.6[a] | 12.1 ± 2.8[b] |
| | 70% | 5.9 ± 1.5[a] | 3.4 ± 0.4[a] | 14.9 ± 3.6[b] |
| Σ(n-6) | 100% | 11.5 ± 2.9[a] | 39.2 ± 6.9[b] | 23.2 ± 6.0[ab] |
| | 85% | 11.0 ± 2.8[a] | 29.5 ± 7.2[b] | 17.1 ± 4.1[ab] |
| | 70% | 14.1 ± 1.9 | 26.4 ± 7.0 | 19.2 ± 6.7 |
| P/S ratio | 100% | 0.5 ± 0.2 | 1.1 ± 0.3 | 0.7 ± 0.2 |
| | 85% | 0.4 ± 0.1 | 0.8 ± 0.3 | 0.4 ± 0.1 |
| | 70% | 0.4 ± 0.1 | 0.6 ± 0.2 | 0.7 ± 0.3 |

NOTE. Values are the mean ± SEM (n = 6-8). Means with different letter superscript in the same row are significantly different ($P < .05$, Fisher's protected LSD). For each FA in the same column, *significantly different *v* 100% group.

PC, PE, PS, SPH, and total PL. Similar to the findings in many individual FAs, such differences were abolished in total PL and most of the PL species (PC, PS, and SPH) when energy intake was restricted to 70% of the ad libitum intake. However, the content of n-3 FA in PE was not influenced by food restriction. In PI, the proportion of n-3 FA was higher ($P < .02$) in fish oil–fed animals only when the energy supply was reduced to 70% of the ad libitum intake.

## DISCUSSION

The present study was designed to investigate whether changes in membrane PL FA composition induced by dietary fat type remain constant when energy balance is altered. Our results demonstrate PL-specific modifications in liver cell structural lipid composition induced by the qualitative dietary fat intake and level of energy intake. Notably, energy restriction abolished the finding of an increased proportion of n-6 FA in membrane SPH and n-3 FA in PC, PS, and SPH occurring in response to the consumption of safflower oil and fish oil, respectively.

The results of the present study support existing evidence suggesting that membrane FA composition undergoes modification by qualitative dietary FA consumption. Rats fed a diet containing 20% fat as fish oil for 10 weeks showed an increased proportion of palmitic and docosahexaenoic acids and a decreased proportion of linoleic and arachidonic acids in hepatic mitochondrial PL.[19] Similar results were reported in plasma membrane PL of the heart and skeletal muscle in swine fed a high–n-3 FA diet.[20] Again, feeding rats a high–P/S ratio fat diet

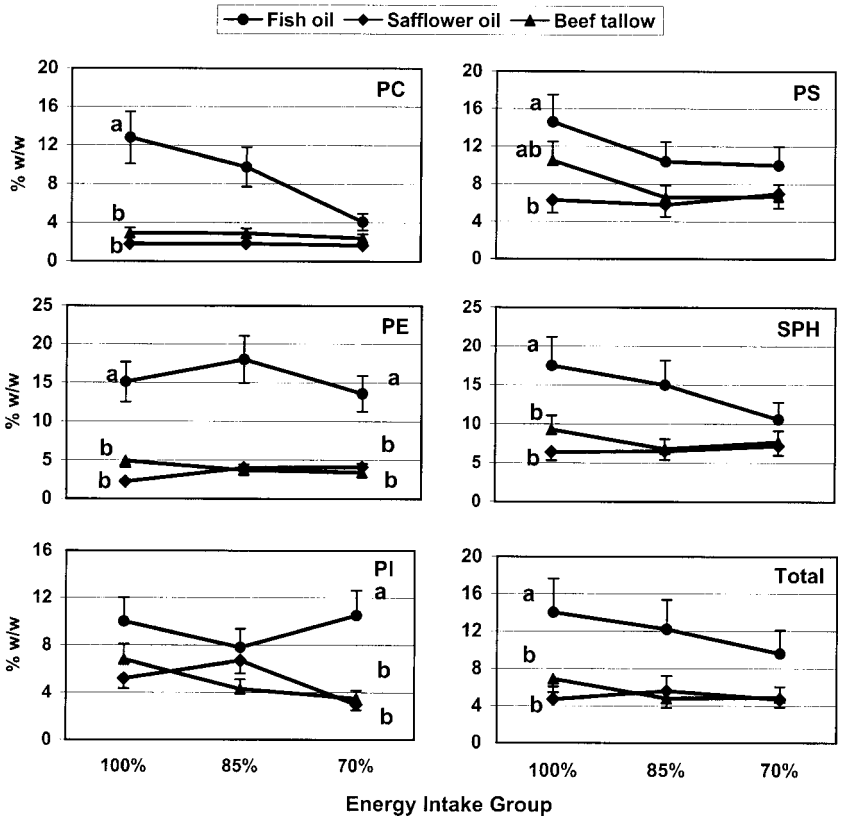


**Fig 1. Proportion of n-3 FAs in liver cell membrane PC, PE, PI, PS, SPH, and total PL in rats fed diets varying in fat source and energy intake level. Values are the mean ± SEM. Values within each PL species with a different letter at the same energy intake level are significantly different ($P < .05$, Fisher's protected LSD).**

for 6 weeks increased the PUFA content of adipocyte membrane PL.[6] In the present study, despite increased PL levels of n-3 FA in the fish oil–fed rats and n-6 FA in the safflower oil–fed rats, neither the P/S FA ratio nor the total PUFA content differed across groups. This could be due to the competition between n-3 and n-6 FA for incorporation into structural PL. The outcome of such competition is to maintain a constant membrane PUFA content.

Although diet-induced changes in membrane FA composition may occur within a relatively short period, the evidence suggests that insensitivity to dietary fat develops over the longer term. Mice fed a PUFA-containing diet for 4 weeks showed an increased proportion of unsaturated FA in the hepatocyte plasma membrane; however, the unsaturated FA content decreased with continued feeding of the PUFA-enriched diet.[21] It was suggested that a homeostatic mechanism may operate in the biological membranes to buffer the membranes against changes in the nature of the dietary lipid intake.[22] The biochemical explanation for this mechanism is not known. It has been shown that tissue lipoprotein lipase activity[23] and body energy expenditure[24] are stimulated to a greater extent by a high-PUFA diet. It can be assumed that longer-term PUFA consumption may result in an increased partitioning of PUFA for oxidation with decreased retention. Decreased PUFA retention may diminish the inhibition of FA synthetase activity by PUFA, thus enhancing tissue accretion of the newly synthesized saturated FA. In addition, it has been shown that the presence of linoleic acid can increase Δ 6-desaturase activity.[25] Greater oxidation and thus less retention of linoleic acid may decrease the activity of this enzyme. Taken together, more saturated FAs become available for the synthesis of structural lipids, and therefore the P/S ratio in membrane PL remains unchanged despite the higher PUFA content in the fish and safflower oil diets.

Membrane FA composition has been shown to be modulated by food restriction. After lifelong food restriction, spleen cell membrane PL in food-restricted corn oil–fed rats had a higher linoleic acid content and lower arachidonic and docosatetraeoic acid content than spleen cell PL in ad libitum–fed rats.[26] Similar findings were also reported in liver mitochondria membranes.[12] In the present study, under conditions of constant fat intake, mild energy restriction decreased the proportion of linoleic acid in PC and SPH of the beef tallow–fed rats, while the concentration of this FA remained unchanged in food-restricted animals fed the fish oil diet, despite comparable levels of linoleic acid in the fish oil as compared with the beef tallow diet. It has been demonstrated that the metabolic fate of dietary FA is influenced by the overall FA profile of the diet.[27] The discrepancy between the present findings and previous reports could be due to the apparent difference in the type of fat consumed. Our results suggest that the relationship between the accretion of linoleic acid in membrane lipids and energy restriction depends on the FA composition of the diet. Less linoleic acid will be incorporated into structural lipids in the face of food restriction when the dietary PUFA content is low.

Our results demonstrate further that energy restriction abolished the proportional change of a number of key FAs in membrane PC, PS, and SPH produced by varying dietary FA consumption. For example, the differences in the content of arachidonic and docosahexaenoic acids observed in SPH of the animals fed different fats ad libitum or food-restricted to 85% of

the ad libitum intake no longer existed when energy intake was further restricted. Membrane SPH may play a specific role as a messenger molecule and may be a potent inhibitor of protein kinase C, which is an enzyme involved in the regulation of cellular metabolism.[28] Arachidonic acid in membrane PL is the major substrate pool for the synthesis of the biologically active compounds, eicosanoids, which are the potent mediators of many physiological processes, including certain biochemical reactions within the cell. Docosahexaenoic acid is also an important structural component in the brain and retina.[29] Increased membrane permeability following incorporation of docosahexaenoic acid has already been reported.[30] The diminished differences regarding the amount of these FAs in membrane SPH may also abolish the different biological functions associated with the varied dietary fat consumption. We previously reported that the variation in the concentration of total FA in the liver of rats consuming different types of dietary fat was abolished when energy intake was restricted.[13] The present findings might be offered as an explanation.

In summary, the results of the present study demonstrate that membrane FA composition, particularly n-3 and n-6 FAs, is modified by the FA composition in the diet. Mild energy restriction had limited effects on structural lipid FA composition. However, these changes in PL FA composition following different dietary FA consumption were influenced by body energy status. Energy deficiency tended to dilute the effect of different types of fat intake on the membrane FA composition. Thus, the reciprocal argument follows that the influence of dietary fat type on cellular structure and perhaps function becomes increasingly important with progressively positive energy balance. This contention can be supported by the evidence that the varied composition of FAs in the diets influenced the FA composition of tissue PLs more substantially in obese rats as compared with their lean littermates.[31] The present results thus emphasize the importance of considering whole-body energy status when selecting the dietary fat source in relation to the maintenance of optimal health.

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