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Differential utilization of long chain fatty acids during fasting-induced triacylglycerol depletion. III. Comparison of *n*–3 and *n*–6 fatty acids in rat plasma and liver

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Previous research has shown that arachidonic acid (20:4($n-6$)) is preferentially retained in liver triacylglycerol in fasted compared to fed rats consuming a diet containing $n-6$ fatty acids. It was hypothesized that eicosapentaenoic (20:5($n-3$)) and docosahexaneoic acids (22:6($n-3$)) would be similarly retained in liver and plasma triacylglycerol of fasted rats consuming a diet containing $n-3$ fatty acids. In comparison with fed rats, it was observed that in partially fasted rats consuming diets which contained 5% sunflower oil (78% 18:2($n-6$)) or 5% marine fish oil (30% 20:5($n-3$) and 22:6($n-3$)), both liver and plasma had significantly depleted triacylglycerol levels containing higher proportions of both arachidonic and docosahexaneoic acids but a lower proportion of eicosapentaenoic acid (marine fish oil group only). Separation of liver and plasma triacylglycerol by silicic acid column chromatography and silver nitrate TLC showed that the majority of long chain fatty acids utilized during fasting were derived from the triacylglycerol subclasses containing palmitic acid (16:0), palmitoleic acid (16:1($n-7$)) and oleic acid (18:1($n-9$)) with retention of both highly saturated and polyunsaturated subclasses. Greater utilization of eicosapentaenoic acid than either arachidonic acid or docosahexaenoic acid during fasting may be due to triacylglycerol speciation of the former with readily oxidized monounsaturated fatty acids.

Introduction

In rats consuming a diet in which the fat is mainly $n-6$ essential fatty acids (EFA), the EFA content of the triacylglycerols (TG) of liver and heart is almost exclusively of the $n-6$ type. If the total TG pool is significantly depleted, e.g., through fasting to deplete liver TG or through in vitro perfusion to deplete heart TG, it has been observed that there is differential retention of arachidonic acid (20:4($n-6$)) in particular and, to a lesser extent, stearic acid (18:0) in liver or heart TG. Whereas the total TG may be depleted as much as 90%, this is mainly a result of greater depletion of fatty acids such as palmitic acid (16:0), palmitoleic acid (16:1($n-7$)), oleic acid (18:1($n-9$)) and linoleic acid (18:2($n-6$)) [1–3] resulting in a proportional increase in 20:4($n-6$) and 18:0. In fact, even without fasting or other means of TG depletion, a significant inverse correlation exists between total TG in various organs or plasma and the proportional content of 20:4($n-6$) in the the TG pool studied [4,5].

Since the EFA composition of most TG pools reflects dietary EFA composition, it is not clear whether the retention of 20:4($n-6$) in TG during TG depletion is dependent on a unique attribute of 20:4($n-6$) itself or whether it is related to the fact that only TG enriched with $n-6$ EFA, e.g., from rats fed a dietary source of $n-6$ fatty acids, have so far been studied under these conditions. For instance, if $n-3$ EFA were fed in the diet, eicosapentaenoic acid (20:5($n-3$)) or docosahexaenoic acid (22:6($n-3$)) would appear in liver TG and might also be selectively retained in liver TG during fasting. The inverse relation between the quantitaive amount of liver TG and its proportional content of 22:6($n-3$) [3] suggests this might be the

Abbreviations: 16:0, palmitic acid; 16:1($n-7$), palmitoleic acid; 18:0, stearic acid; 18:1($n-9$), oleic acid; 18:2($n-6$), linoleic acid; 20:4($n-6$), arachidonic acid; 20:5($n-3$), eicosapentaenoic acid; 22:6($n-3$), docosahexaenoic acid; AIN, American Institute of Nutrition; EFA, essential fatty acid(s); EPA, marine fish oil diet containing 5% MaxEPA (R.P. Scherer Canada); SUN, diet containing 5% sunflower oil; TG, triacylglycerol.

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case. A comparison has therefore been made in the present study of the fatty acid composition of both liver and plasma TG of fed or fasted rats consuming dietary EFA as either 18 : 2($n-6$) (sunflower oil) or 20 : 5($n-3$) plus 22 : 6($n-3$) (marine fish oil).

Materials and Methods

Animals

Male Wistar rats (200–250 g; Charles River Canada, St. Constant, Quebec) were given a semi-purified diet as recommended by the American Institute of Nutrition (AIN) [6] containing (g/kg): 605 corn starch, 200 casein, 100 cellulose, 50 oil, 35 AIN-76 mineral mix and 10 vitamin mix (AIN-76A). The casein, cellulose, mineral mix and vitamin mix were obtained from Teklad Test Diets (Madison, WI). The oil was either commercial grade sunflower oil (78% 18 : 2($n-6$); SUN) or marine fish oil (30% 20 : 5($n-3$) and 22 : 6($n-3$) combined; EPA) obtained courtesy of R.P. Scherer Canada (Windsor, Ontario). The diets were stored frozen and aliquots consumed by the rats were fresh daily. The rats ($n=4$ per group) consumed these two diets ad libitum for a total of 28 days with actual food intakes determined during week three. From day 23 to 27, half of the rats in each group were given 25% of their normal ad libitum intake (fasting). On day 28, all the rats were anesthetized with diethyl ether, the abdomen opened and 8–10 ml blood removed from the abdominal aorta into a heparinized syringe. The plasma was separated from the whole blood and the livers were removed from each rat; both were frozen at −2O°C until analysis.

Lipid Analyses

Total Lipids. All solvents were purchased in bulk from Fisher Scientific (Toronto) and glass-redistilled prior to use. Total lipids of plasma (2–4 ml) and liver (4–6 g) were extracted into 10 volumes of chloroform/methanol (2 : 1, v/v) containing 0.02% butylated hydroxytoluene (Sigma Chemicals, St. Louis, MO) as antioxidant. Triheptadecanoin (17 : 0) (Sigma Chemicals) was added to the total lipid extract as a quantitative external TG standard prior to the addition of 2 vol. of 0.9% saline. After centrifugation, the organic phase was quantitatively transferred to a pre-weighed tube and the lipid extract taken to dryness under nitrogen gas and weighed. An aliquot of each sample was used for separation of total TG by neutral lipid thin layer chromatography (20 × 20 cm plates pre-coated with 250 μm silica gel 60A; supplied by Chromatographic Specialities, Brockville, Ontario) using the solvent system hexane/diethyl ether/acetic acid (80 : 20 : 1).

Triglyceride Separation. The remainder of each total lipid sample was taken to dryness under nitrogen gas and dissolved in 100 μl hexane and applied to a 30 × 1 cm glass column packed with activated 100–200 mesh silicic acid (Mallenckrodt, supplied by Canlab, Toronto) for the separation of the total TG fraction [7]. Following elution of the cholesteryl esters with 100 ml of 2% ether in hexane, the TG were eluted with 300 ml 5% ether in hexane followed by 100 ml of 8% ether in hexane. The hexane eluates containing the TG were combined and concentrated to dryness on a rotary evaporator (Buchi) and stored in chloroform containing 0.02% butylated hydroxytoluene. TG recovery from the silicic columns was 98–100% as determined by weighing the TG recovered after the addition of known amounts of 17 : 0 TG to the columns.

TG subclasses were separated into major groups according to total unsaturation by silver nitrate TLC [8]. Pre-coated thin-layer plates (20 × 20 cm; 250 μm silica gel 60A) were sprayed with 500 mg silver (800 mg silver nitrate; Canlab) which had been dissolved in 25 ml methylene chloride. The total TG fraction obtained after silicic acid column chromatography was applied to the silver-impregnated thin-layer plates (10–20 mg) and the plates developed twice in the solvent system hexane/ diethyl ether/ methanol/ acetic acid (78 : 20 : 1 : 0.5). Known aliquots of 17 : 0 TG were added to the bands of TG subclasses which had been visualized under ultraviolet light after the plates had been sprayed with 0.1% dichlorofluorescein (Sigma) in methanol. Total carbon profiles of aliquots of the liver TG samples were determined as previously described [9].

Fatty acid analysis. Fatty acids in aliquots of the total liver and plasma TG obtained by TLC and in the TG subclasses obtained after silver nitrate TLC were converted to methyl esters using 14% boron trifluoride in methanol (Sigma) at 90C for 30 min. The fatty acid methyl esters were analysed by gas-liquid chromatography using a capillary column (Durabond 225; 30 m × 0.25 mm id.) coated with 25 μm cyanopropylphenyl (J&W Scientific, Folsom, CA) in a Hewlett-Packard 5890A gas-liquid chromatograph (Palo Alto, CA) with automated sample delivery and injection (H-P 7671A) and peak integration (H-P 3393). The column temperature was programmed from 150 °C to 220 °C in three stages with each run complete in 30 min.

Results

Liver Total Triacylglycerols

Fasting decreased total liver TG by 73% in the SUN group and by 49% in the EPA group. Absolute amounts of individual long-chain fatty acids in the liver TG were therefore markedly decreased with the following exceptions: 20 : 4($n-6$) in the SUN group decreased 13% and 18 : 2($n-6$), 20 : 4($n-6$) and 22 : 6($n-3$) in the EPA group changed < 20% (Table I). The quantitative depletion of individual fatty acids from liver TG could therefore be calculated. In the SUN group, fasting

TABLE I

Fatty acid composition (mg/g wet weight) of liver total triacylglycerols of fed or fasted rats consuming diets containing 5% sunflower oil (SUN) or 5% marine fish oil (EPA)

Percent depletion (%) is fasted/fed×100. Each value is the mean of data from two rats.

	SUN		EPA	
	fed	fasted (%)	fed	fasted (%)
16:0	4.98	0.81 (−84)	2.45	1.00 (−59)
16:1($n-7$)	1.27	0.06 (−95)	0.73	0.11 (−85)
18:0	0.21	0.08 (−62)	0.21	0.15 (−29)
18:1($n-9$)	4.08	0.52 (−87)	2.45	1.29 (−47)
18:2($n-6$)	5.41	2.47 (−54)	0.40	0.39 (−2)
20:4($n-6$)	0.52	0.43 (−17)	0.15	0.16 (+6)
20:5($n-3$)	n.d.	n.d.	0.69	0.17 (−75)
22:6($n-3$)	0.02	0.01 (−50)	1.03	0.85 (−17)
Total	16.5	4.4 (−73)	8.1	4.2 (−49)

n.d., not determined.

depleted > 80% of 16:0, 16:1($n-7$) and 18:1($n-9$); 18:0 and 18:2($n-6$) were reduced 62 and 54%, respectively, but 20:4($n-6$) was depleted only 17%. In the EPA group, only 16:1($n-7$) was depleted > 80%. 20:5($n-3$) was unique among the EFA in being depleted by 75%. 18:2($n-6$) and 20:4($n-6$) were not quantitatively depleted and 22:6($n-3$) was decreased only 17% from liver TG in the fasted EPA group (Table I).

Liver Triacylglycerol Subclasses

Silver nitrate TLC resulted in eight bands being observed in each total liver TG sample which had been separated from the other lipid classes by silicic acid column chromatography. These bands corresponded to significant differences in total unsaturation of liver TG subclasses (in the figures, highly saturated TG subclasses have low numbers and highly unsaturated TG subclasses have high numbers). The actual amount of TG in each of the eight subclasses in each group is shown in Fig. 1.

The absolute amount (mg/g liver, wet weight) of the two most saturated TG subclasses (1 and 2) and the three most unsaturated TG subclasses (6, 7 and 8) were very similar in both the SUN and EPA groups (unfasted). The major difference in TG between the unfasted SUN and EPA groups was in the intermediate subclasses (3, 4 and 5) which were each above 3 mg/g in the SUN group but below 1 mg/g in the EPA group. In the SUN group, fasting dramatically reduced subclasses 2–5 while leaving subclasses 6–8 virtually unchanged. In contrast, fasting in the EPA group caused a decrease in subclasses 1, 2 and 6, leaving subclasses 3, 4, 5, 7 and 8 unchanged (Fig. 1). In the SUN group, the marked depletion of subclasses 3, 4 and 5 after fasting corresponded to a decrease in the proportion of TG containing 48 and 50 carbons and an increase in TG containing 54, 56 and 58 carbons (data not shown). As a result of these quantitative changes in TG subclasses, the proportional distribution of residual liver TG after fasting was altered such that, in the SUN group, the more unsaturated TG subclasses (6, 7 and 8) were increased from 14.7 to 41.6% of the total liver TG. In contrast, in the EPA group, fasting proportionally altered subclass 2 (−54%) and subclass 8 (+67%).

Fatty Acids in Liver Triacylglycerol Subclasses

Saturated and monounsaturated fatty acids (16:0, 18:0, 16:1($n-7$), 18:1($n-9$)) were distributed among all liver TG subclasses but were primarily in subclasses 1–5. In general, in both the SUN and EPA groups, fasting decreased the proportion of 16:0, 16:1($n-7$) and 18:1($n-9$) in subclasses 1–5 but had much less effect on their proportions in subclasses 6–8 (the most unsaturated TG subclasses) (Fig. 2). Fasting increased the proportion of 18:0 in most TG subclasses in both groups. Polyunsaturated fatty acids (18:2($n-6$), 20:4($n-6$), 20:5($n-3$) and 22:6($n-3$)) were distributed mainly in TG subclasses 5–8 (except 18:2($n-6$) which was found as high as subclass 2). In both the SUN and EPA groups, fasting increased the proportion of the polyunsaturated fatty acids in subclasses 5–8 or moved the distribution curve to the right (towards greater unsaturation, i.e., 18:2($n-6$) in the fasted EPA group). 20:5($n-3$) was an exception; in the fasted EPA group, the proportion of 20:5($n-3$) in subclasses

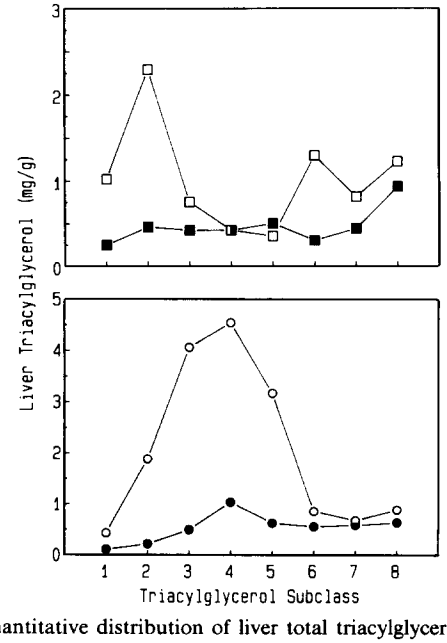

Fig. 1. Quantitative distribution of liver total triacylglycerols according to unsaturation in rats consuming 5% sunflower oil (SUN: ○, fed; ●, fasted) or 5% marine fish oil (EPA: □, fed; ■, fasted). The triacylglycerol species have been separated by silver nitrate thin-layer chromatography with the numerical designation: 1 = highly saturated (top of the plate); 8 = highly unsaturated (origin).

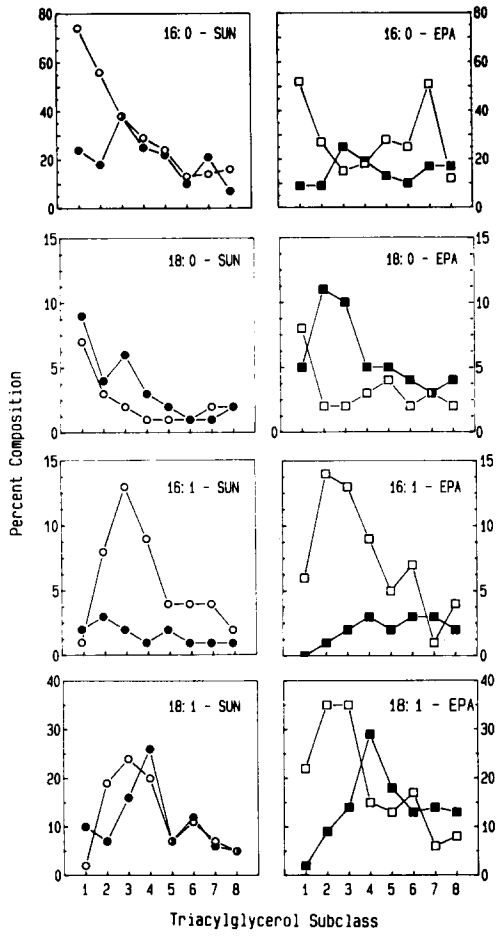

Fig. 2. Proportional distribution of saturated fatty acids (palmitic, 16:0; stearic, 18:0) and monounsaturated fatty acids (palmitoleic acid, 16:1($n-7$); oleic acid, 18:1($n-9$)) in liver triacylglycerol species separated according to unsaturation in rats consuming 5% sunflower oil (SUN: ○, fed; ●, fasted) or 5% marine fish oil (EPA: □, fed; ■, fasted). 1 = highly saturated (top of the plate); 8 = highly unsaturated (origin).

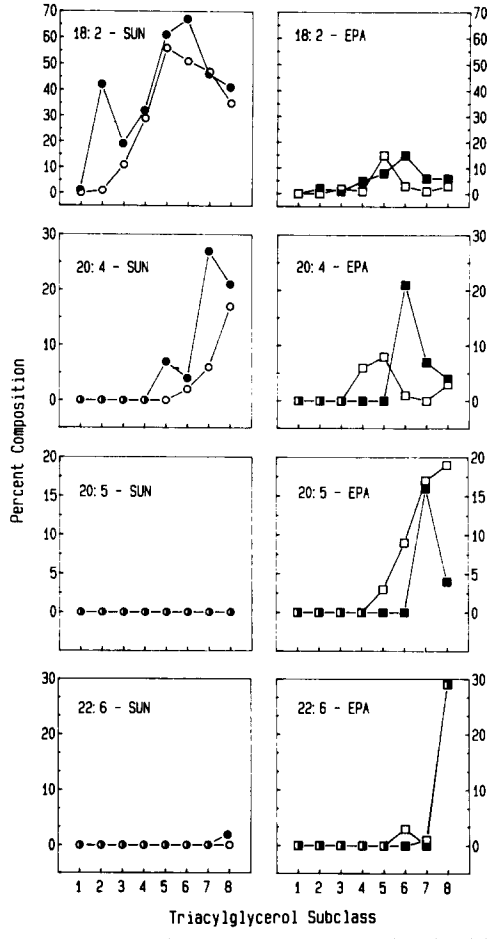

Fig. 3. Proportional distribution of $n-6$ fatty acids (linoleic, 18:2($n-6$); arachidonic, 20:4($n-6$)) and $n-3$ fatty acids (eicosapentaenoic, 20:5($n-3$); docosahexaenoic, 22:6($n-3$)) in liver triacylglycerol species separated according to unsaturation in rats consuming 5% sunflower oil (SUN: ○, fed; ●, fasted) or 5% marine fish oil (EPA: ○, fed; ■, fasted). 1 = highly saturated (top of the plate), 8 = highly unsaturated (origin).

6 and 8 decreased from 7% to 0% and from 17 to 4%, respectively, while remaining at 16–17% in subclass 7 (Fig. 3).

Plasma Total Triacylglycerols

Fasting decreased plasma total TG by 90% in the SUN group and 89% (1.0 to 0.1 mg/ml) in the EPA group. As a result, all fatty acids in plasma TG of both the SUN and EPA groups were quantitatively decreased by fasting. However, the percent depletion of individual fatty acids varied as it did in liver. In plasma of both groups, only 20:4($n-6$) was depleted < 70% by fasting; all other fatty acids were depleted > 80% (except 22:6($n-3$) in the fasted SUN group, −62% depletion) (Table II).

Plasma Triacylglycerol Subclasses

In plasma, nine TG subclasses could be distinguished except in the fasted rats consuming the EPA diet (eight

TABLE II

Fatty acid composition (μg/ml) of plasma total triacylglycerols from fed or fasted rats consuming diets containing 5% sunflower oil (SUN) or 5% marine fish oil (EPA)

Percent depletion (%) is fasted/fed×100. Each value is the mean of data from two rats.

	SUN		EPA	
	fed	fasted (%)	fed	fasted (%)
16:0	643	31 (−95)	211	14 (−93)
16:1($n-7$)	4	<1 (−>75)	18	3 (−83)
18:0	64	10 (−84)	26	9 (−65)
18:1($n-9$)	618	98 (−84)	297	29 (−90)
18:2($n-6$)	1216	86 (−93)	58	8 (−86)
20:4($n-6$)	141	46 (−67)	9	3 (−67)
20:5($n-3$)	n.d.	n.d.	161	9 (−94)
22:6($n-3$)	16	6 (−62)	206	32 (−84)
Total	2702	277 (−90)	986	107 (−89)

n.d., not determined.

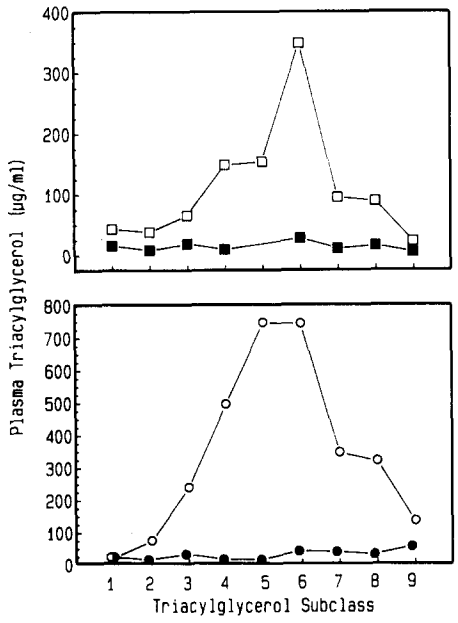

Fig. 4. Distribution of plasma total triacylglycerols according to unsaturation in rats consuming 5% sunflower oil (SUN: ○, fed; ●, fasted) or 5% marine fish oil (EPA: □, fed; ■, fasted). 1 = highly saturated (top of the plate), 8 = highly unsaturated (origin).

subclasses). The missing subclass in the fasted EPA-fed rats was in the area of subclass 4–5. All TG subclasses in both groups (except subclass 1 in the SUN group) were quantitatively decreased by fasting with proportionally greater depletion of subclasses 4 to 6, e.g., TG of mixed unsaturation (Fig. 4). No clear trends in the proportional distribution of plasma TG subclasses occurred in the fasted groups.

Fatty Acids in Plasma Triacylglycerol Subclasses

Amongst the saturated and monounsaturated fatty acids in plasma TG, there was no clear effect of fasting except that 18 : 0 was found in a higher proportion in most TG subclasses of both the SUN and EPA groups and 16:1($n-7$) was proportionally increased across TG subclasses in the fasted EPA group (Fig. 5). Amongst the polyunsaturated fatty acids, 18 : 2($n-6$) was increased in most subclasses in the fasted EPA group but in the fasted SUN group was decreased except in the highly unsaturated TG subclasses. 20 : 4($n-6$) was increased after fasting in most TG subclasses in both groups. Except for 22 : 6($n-3$) in the SUN group, fasting decreased 20 : 5($n-3$) and 22 : 6($n-3$) (Fig. 6).

Discussion

The present results support the previously demonstrated proportional retention of 20 : 4($n-6$) and 18 : 0 in liver TG after TG depletion by fasting in rats consuming a diet containing primarily ($n-6$) EFA [2]. The principle of selective utilization of long-chain fatty acids from TG during TG depletion in liver and heart [1,2] has been extended in this study to include plasma which was > 90% depleted of TG during fasting. The plasma fatty acid data obtained after fasting also gave a clear indication that 20 : 4($n-6$) was not unique amongst EFA in being selectively retained during TG depletion; even in rats consuming the SUN diet (< 0.5% $n-3$ fatty acids in the diet), 38% of 22 : 6($n-3$) was retained in plasma TG after fasting (similar to the 33% retention of 20 : 4($n-6$)). In comparison, all other fatty acids were retained to a maximum of 16%, e.g., > 84% depletion (Table II).

An $n-3$ fatty acid-enriched diet (EPA) was fed to increase the amount of $n-3$ fatty acids in liver and plasma TG so that effects of fasting on $n-3$ fatty acid levels in TG could be more completely studied and compared with 20 : 4($n-6$) retention during TG depletion. The most interesting and consistent result obtained from total TG values in both liver and plasma

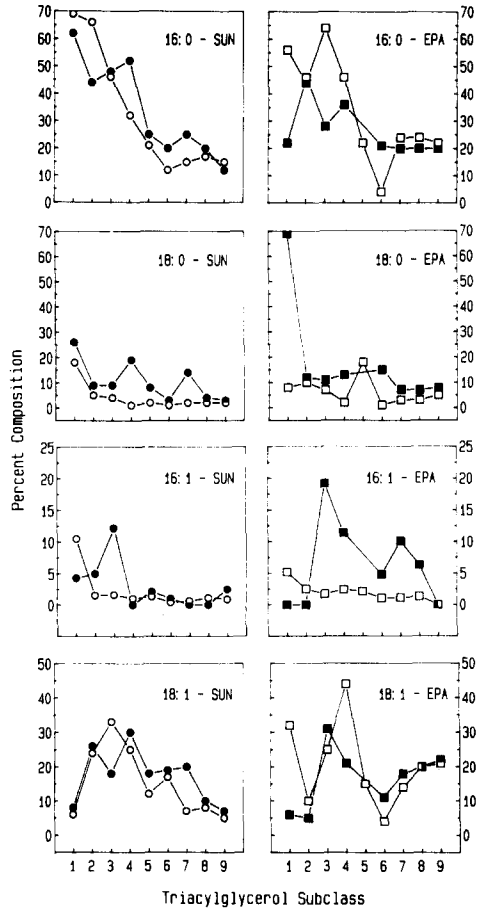

Fig. 5. Proportional distribution of saturated fatty acids (palmitic, 16 : 0; stearic, 18 : 0) and monounsaturated fatty acids (palmitoleic acid, 16 : 1($n-7$); oleic acid, 18 : 1($n-9$)) in plasma triacylglycerol species separated according to unsaturation in rats consuming 5% sunflower oil (SUN: ○, fed; ●, fasted) or 5% marine fish oil (EPA: ○, fed; ■, fasted). 1 = highly saturated (top of the plate), 8 = highly unsaturated (origin).

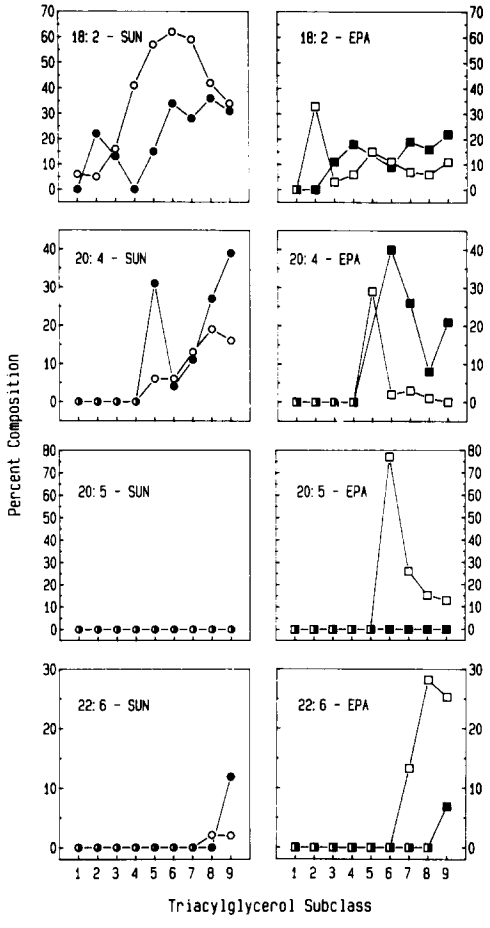

Fig. 6. Proportional distribution of ($n-6$) fatty acids (linoleic, 18 : 2($n-6$); arachidonic, 20 : 4($n-6$)) and $n-3$ fatty acids (eicosapentaenoic, 20 : 5($n-3$); docosahexaenoic, 22 : 6($n-3$)) in plasma triacylglycerol species separated according to unsaturation in rats consuming 5% sunflower oil (SUN: ○, fed; ●, fasted) or 5% marine fish oil (EPA: □, fed; ■, fasted). 1 = highly saturated (top of the plate), 8 = highly unsaturated (origin).

was that 20 : 5($n-3$) was depleted from TG whereas 22 : 6($n-3$) was selectively retained in TG during fasting (Tables I and II). These data suggest that some dietary 20 : 5($n-3$) and 22 : 6($n-3$) may be acylated into the same TG subclass but due to the greater depletion of 20 : 5($n-3$) than 22 : 6($n-3$) during fasting, at least 50% of the 20 : 5($n-3$) must be in TG that is readily utilized during fasting. It appears that the TG fatty acid combination which contains much of the 20 : 4($n-6$) and/or 22 : 6($n-3$) and/or 18 : 0 in plasma and liver TG and which is selectively retained during fasting is unlikely to contain very much 20 : 5($n-3$). Hence, selective retention of long chain fatty acids in plasma or liver TG during TG depletion is not a function of fatty acid class ($n-3$ or $n-6$) or structure alone but is probably a function of the TG subclass into which the fatty acid is acylated.

TG speciation is a function of both dietary levels and synthesis of fatty acids. One reason why 20 : 5($n-3$) may have been depleted from plasma and liver TG during fasting in amounts greater than 20 : 4($n-6$) is that, unlike 20 : 4($n-6$), 20 : 5($n-3$) was already present in TG in the diet. As a result most of the 20 : 5($n-3$) in plasma and liver TG of the EPA group was probably derived from dietary $n-3$ fatty acids. The location of 20 : 5($n-3$) in TG of marine fish origin consumed in the diet predetermines to some extent the liver TG species in which the 20 : 5($n-3$) becomes located [10]. Hence, if the amount of dietary 20 : 5($n-3$) exceeded the capacity to insert all the 20:5($n-3$) into its preferred TG species in liver and plasma, then some 20 : 5($n-3$) may have been inserted into alternate TG species. On the basis of the present results, such alternate TG species probably contain 16 : 1($n-7$) and 18 : 1($n-9$), which appear to be more readily available during fasting. However, 18 : 2($n-6$) and 22 : 6($n-3$) were both present in the diet and certainly no 18 : 2($n-6$) was synthesized. 22 : 6($n-3$) was retained in liver and plasma and 18 : 2($n-6$) was retained in liver only during fasting-induced TG depletion (Tables I and II). This suggests that the presence of 20 : 5($n-3$) in the diet was not as significant a determinant of its utilization from liver or plasma TG during fasting as its natural TG speciation or position within a given TG species. In contrast, 20 : 4($n-6$) in liver and plasma TG was only derived from synthesis; no 20 : 4($n-6$) was present in the diet. Furthermore, 4% by weight 18 : 2($n-6$) in the diet would not have resulted in excess synthesis of 20 : 4($n-6$). Hence, the TG species into which 20 : 4($n-6$) was inserted would only be those which normally contain 20 : 4($n-6$) and which the present results as well as prior evidence [2] suggests are avidly retained during TG depletion.

The positional distribution of fatty acids of individual TG subclasses was not determined in this study. Exact differences in liver and plasma TG subclasses which would account for differential retention of 20 : 4($n-6$) and 22 : 6($n-3$) but utilization of 20 : 5($n-3$) are therefore unknown at this stage. From the proportional distribution of fatty acids according to unsaturation of liver TG subclasses, 20 : 5($n-3$) depletion occurred in TG subclasses which also were depleted of 16 : 0, 16 : 1($n-7$) and 18 : 1($n-9$) (Figs. 2 and 3). This suggests that the greater utilization of 20 : 5($n-3$) in comparison to 20 : 4($n-6$) and 22 : 6($n-3$) may have been due to a high proportion of 20 : 5($n-3$) being acylated in TG containing 16 : 0, 16 : 1($n-7$) and 18 : 1($n-9$). In contrast, TG subclasses with higher proportions of 20 : 4($n-6$) after fasting also had higher amounts of 18 : 0 and 18 : 2($n-6$) (Figs. 2 and 3) suggesting these three fatty acids probably make up a large proportion of at least one TG subclass which is not readily hydrolysed during fasting.

The effect of manipulation of the size of other lipid classes besides TG on their relative composition of long

chain fatty acids has also been noted. The percent composition of $20:4(n-6)$ in plasma cholesteryl esters has been reported to be inversely proportional to the total plasma cholesteryl ester pool size [4]. With the exception of $22:6(n-3)$, the proportional composition of $n-6$ and $n-3$ fatty acids in phospholipids is unrelated to the total phospholipid pool size in either liver or plasma. The percent composition of $22:6(n-3)$ is significantly positively correlated with the quantitative amount of liver total phospholipid and significantly negatively correlated with the amount of plasma total phospholipids [3]. A more direct comparison with the present data has shown that short-term fasting reduces the plasma total phospholipid pool size by 50–70%. Under these conditions, the proportion of $20:4(n-6)$ was shown to increase and that of $20:5(n-3)$ decrease but only in rats given dietary $20:5(n-3)$ and $22:6(n-3)$. There was no such effect in rats fed $18:2(n-6)$ without dietary $n-3$ fatty acids [4]. In the same study, short-term fasting did not affect liver total phospholipid content irrespective of dietary fat type. However, as in plasma phospholipids, fasted rats had proportionally higher $20:4(n-6)$ and proportionally lower $20:5(n-3)$ in liver phospholipids only if they were fed $20:5(n-3)$ and $22:6(n-3)$. Hence, in comparison with the plasma or liver TG pool(s), both the pool size and the essential fatty acid composition of plasma and liver phospholipids appears less responsive to the effects of fasting. Presumably, this is related to the different metabolic and structural roles that essential fatty acids have in TG compared to phospholipids.

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