Reduced storage of dietary eicosapentaenoic and docosahexaenoic acids in the weanling rat

R.J. Jandacek, E.J. Hollenbach, B.N. Holcombe, C.M. Kuehlthau, J.C. Peters, and J.D. Taulbee

The Procter & Gamble Company, Cincinnati, OH, USA

Studies of the utilization of fatty acids were undertaken to compare the effects of chain length and the degree of unsaturation on the accumulation of energy and of fatty acids in growing rats. Rats were fed either 17% corn oil, 17% medium chain triglyceride oil (MCT), or 17% menhaden oil in addition to 3% safflower oil. The deposition of individual fatty acids in the carcass was measured and compared with the amount of the fatty acid that was consumed during the 6 weeks of diet treatment. In all groups the percent of dietary linoleic acid in the carcass was similar with 29.2 ± 2.7, 33.0 ± 1.9, and 43.7 ± 3.8 found for the corn oil, MCT, and menhaden oil groups, respectively. In contrast, only 7.7 ± 0.9% of the dietary eicosapentaenoic acid (EPA) and 14.3 ± 1.5% of the dietary docosahexaenoic acid (DHA) were found in the carcasses of the animals fed menhaden oil. There were indications of reduced energy accumulation in the menhaden oil group, although reduced dietary consumption was also seen in this group. A paired feeding study of corn oil and menhaden oil also suggested reduced energy deposition per gram of diet in the menhaden oil group. A fat balance study comparing corn oil and fish oil showed the absorption of both fats to be essentially complete and ruled out malabsorption of the menhaden oil as a contributor to the reduced accumulation of the EPA and DHA.

Keywords: energy; linoleic; eicosapentaenoic; docosahexaenoic medium-chain

Introduction

Dietary fish oil has been the subject of numerous studies and reviews during the past several years.[1-4] Two distinct effects of dietary fish oil have been repeatedly observed. First, an increase in bleeding time resulting from decreased platelet aggregation has been generally found to accompany ingestion of fish oil.[5] Second, a reduction in plasma triglycerides has been observed in hypertriglyceridemic patients who ingested significant quantities of fish oil.[6]

The decrease in platelet aggregation tendency is generally thought to be the result of eicosapentaenoic acid (EPA) in fish oil reducing the level of pro-aggregatory prostaglandins in platelets, presumably through effects on the synthesis or activity of arachidonic acid. The reduction in plasma triglycerides, although well established, evokes questions about the transport and metabolic fate of the long chain polyunsaturated fatty acids of fish oil. The utilization of these acids may differ from that of typical long chain fatty acids as a result of events unrelated to prostaglandin metabolism.

Recently, Jones reported measurements of energy deposition in growing hamsters that were fed either fish oil or other fats.[7] In that study, body weight gain by the fish oil group was significantly (19%) less than that by the corn oil group.

Parrish and coworkers have reported recently that fish oil-fed rats deposited significantly less fat in perirenal and epididymal fat pads than lard-fed rats.[8] Since food consumption, weight gain, and fat balance were similar for the two dietary fats, these authors concluded that fish oil can limit fat cell trophic growth.

To explore the hypothesized unique metabolism of the long chain polyunsaturated fatty acids of fish oil, we have measured individual fatty acid accumulation, fat absorption, and energy storage in weanling rats fed

Address reprint requests to Dr. R.J. Jandacek at The Procter & Gamble Company, Miami Valley Laboratories, P.O. Box 398707, Cincinnati, OH 45239-8707, USA.
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fish oil containing significant quantities of EPA and docosahexaenoic acid (DHA). Consistent with Jones' hypothesis that DHA and EPA are oxidized preferentially,[7] we have found that these acids differ markedly from linoleic acid in their accumulation in body fat. Since animals do not synthesize linoleic acid, the source of this compound that is found in body tissue is strictly from diet. Similarly, only traces of EPA and docosahexaenoic acids (DHA) are found in tissues of animals fed diets low in the ω-3 acids, and adipose accumulation of DHA and EPA is of dietary origin.

Materials and methods

Fish oil was fed to weanling, male Sprague-Dawley rats (Charles River Laboratories) in three studies: (1) an ad lib feeding study measured the accumulation of total energy, fat, and individual fatty acids in rats fed either corn oil, medium chain triglycerides (MCT), or fish oil; (2) a paired feeding study compared weight gain and energy accumulation from diets containing either corn oil or fish oil; and (3) a fat balance study measured fat absorption from diets containing fish oil or corn oil. The procedures for these studies are described in detail below.

Ad lib study

Animals and diets. The animals were received immediately after weaning and then randomized into three groups of eight having equivalent mean body weights. They were caged individually and provided tap water ad lib. The diets were provided in weighed food cups, and food consumption was measured daily. Body weights were measured each week throughout the study. Diets were stored in the dark in a polyethylene bag enclosed in a second bag at 4° C. Food cups were emptied daily and refilled with fresh diet. After six weeks the animals were fasted overnight and sacrificed. The composition of the test diets and the fatty acid composition of the total diets are shown in Table 1. Linoleic acid was provided in the solution of fat soluble vitamins in safflower oil and accounted for 5% of energy. The oil sources were corn oil, ICN Biochemicals (Cleveland, OH); MCT oil, Capital City Products (Columbus, OH); fish (menhaden) oil, R.P. Scherer Company (Clearwater, FL); and safflower oil, Hollywood (Los Angeles, CA).

Necropsy. The animals were anesthetized with Nembutal, and blood was taken by cardiac puncture. The animals were sacrificed in a CO_2 chamber. Hearts, livers, brains, and epididymal fat pads were removed and frozen with liquid nitrogen. These tissue samples were stored frozen until they were analyzed. The carcasses were frozen with dry ice, cut into pieces, and passed through a Wiley mill with ground dry ice. The dry ice was allowed to evaporate, and the ground carcass was slurried with water, placed in cake pans, and frozen at −60° C in a freeze-drier (Hull Corporation) with a thermocouple in at least one pan per shelf. The drying was continued under vacuum until a constant temperature was reached. The freeze-dried carcasses were stored in glass jars closed with metal lids. Weighed portions of the dried carcass homogenate were analyzed for total energy content by bomb calorimetry at Woodson-Tenent Laboratories, Dayton, OH.

Table 1 Diet compositions

Diet composition	(Weight %)
Sucrose	47.2
Casein	25.0
Fat[a]	17.0
Salt mix USP XVII	4.0
Celluflour	3.0
Fat soluble vitamin mix[b]	3.0
Vitamin mix[c]	0.5
Choline chloride	0.3
	100.0

Fatty acid[d]	Corn oil diet	MCT diet	Fish oil diet
6:0		0.5	
8:0		51.1	
10:0		26.5	
12:0		0.5	0.1
14:0	0.1	0.3	3.3
16:0	11.4	2.5	11.6
16:1			4.4
18:0	1.8	0.9	2.5
18:1	25.8	2.9	10.0
18:2	58.9	15.0	14.2
18:3	1.0		0.9
20:4			1.0
20:5			14.9
22:6			10.7
Other			26.4

[a] Group 1, corn oil; group 2, medium chain triglyceride (MCT); group 3, fish oil.
[b] Per kg, 0.536 g retinyl palmitate, 0.067 g D_2, 2.21 g tocopheryl acetate, 0.02 g menadione, 997.2 g safflower oil.
[c] Per kg, 0.8 g thiamin, 0.6 g riboflavin, 4.0 g niacin, 20.0 g folic acid (1% in surcose), 0.04 g biotin, 18.0 g B12 (0.1% in mannitol), 1.2. g PABA, 1.2 g ascorbic acid, 1.4 g pyridoxine HCl, 1.6 g pantothenate, 36 g inositol, 915.2 g sucrose.
[d] No. of carbon atoms: no. of double bonds.

Tissue extraction. Weighed aliquots of the freeze-dried carcass or the tissue were combined with 20 volumes of 2:1 chloroform:methanol in Erlenmeyer flasks and homogenized with a polytron. The suspensions were filtered, KH_2PO_4 buffer added, and the chloroform phase was separated, dried with nitrogen, and evaporated under vacuum to obtain the lipid mass.

Fatty acid analysis. Fatty acids were analyzed by saponification of the extracted lipid with methanolic sodium hydroxide and methylation with BF_3-methanol.[9] Fatty acid methyl esters were identified by comparison with analytical standards (Supelco) using gas chromatography (30M Durabond 225 capillary; flame ionization detector, column temperature programmed from 60° to 200° C; injection port 250° C; detector,

250° C). Areas were used to quantify fatty acids with a Hewlett Packard 3390 integrator. Standards were run before and after each set of samples.

Energy and fatty acid accumulation calculations. Total carcass energy was calculated as [(dried carcass mass × energy density found by bomb combustion, kcal/g) + (organ lipid mass × 9.4 kcal/g) + (organ non-lipid mass × 5.3 kcal/g)]. The dried carcass typically contained more than 95% of the total energy. Energy accumulation was estimated by assuming 21% of the starting weight of the animal was dry weight with an energy density of 5.3 kcal/g. The initial mass of linoleic acid was assumed to be 0.7g/animal, and those of octanoic acid, decanoic acid, EPA, and DHA were assumed to be negligible. These assumed initial values were based on analysis of carcass energy and fatty acids in a separate study of weanling animals similar in age and weight to the animals at the beginning of this study. No estimation of the mass of linoleic acid converted to arachidonic acid was included.

Statistical analysis. All group comparisons other than tissue and carcass fatty acid compositions were by t test after treatment effects were established by analysis of variance. The statistical model used to determine differences in tissue and carcass fatty acids is given below. This method first determined whether treatment-related differences in the distribution of the fatty acids in the tissue existed. Only when there was a difference in the distributions was the method then used to determine diet treatment effects on individual fatty acids. This approach prevents erroneously claiming too many significant effects because of multiple significance testing.

For assessing the level and distribution of fatty acids in tissue, there are three sources of variability:

1. The treatment, t (type of fat consumed).
2. The fatty acid being measured, f (different fatty acids will be present at different levels).
3. Animal, a (different animals will likely have different responses).

Responses differ because of treatment, fatty acid type being measured, animal-to-animal differences, differences in distribution of fatty acid types in tissues caused by different treatments, and differences in distribution of fatty acid types in tissues caused by differences among animals on a given treatment.

This description can be translated into a statistical model for analysis as follows:

$$Y_{ijk} = m + t_i + f_j + a_k(t_i) + ft_{ij} + fa(t)_{ijk} \qquad (1)$$

where Y_{ijk} is the level for the kth animal in treatment group i for fatty acid j; m is the overall mean; t_i is the effect of treatment i, $i = 1,2,3$; f_j is the difference between the overall mean and the average level of fatty acid j, $j = 1,2,\ldots,j$ (where j is the total number of fatty acids measured); $a_k(t_i)$ is the effect (difference from the mean) due to animal k within treatment i, $k = 1,2,\ldots 8$; ft_{ij} is the effect due to interaction (synergism or antagonism) between treatment i and fatty acid type j; and $fa(t)_{ijk}$ is the effect due to interaction of fatty acid type j and animal k, within treatment i.

Typically, we express our response for each fatty acid as a percent of total fatty acid in a given tissue. For this reason, there is no variability due to animal differences (percent of fatty acid types must sum to 100% for each animal) and no variability due to treatment differences (each animal within each treatment must have the same sum of percents as well, i.e., 100%). Therefore, the factors that can influence response are fatty acid type (f_j), differences among treatments in fatty acid distribution (ft_{ij}), and differences among animals on a given treatment in fatty acid distribution ($fa(t)_{ijk}$).

These factors are put into an analysis of variance, and $fa(t)_{ijk}$ becomes the proper error term for testing whether treatment affects the distribution of fatty acids in tissue. The model uses the differences in the distributions of fatty acids from animal to animal on the same treatment to serve as the "error" or "expected variability" term to judge whether treatment differences contribute significantly to observed differences in fatty acid distribution.

Paired feeding study

This study was designed to remove the differences in food consumption of the fish oil and corn oil diets that were seen in the ad lib study. Weanling male Sprague-Dawley rats were randomized by body weight into two groups of eight. Group 1 received a diet that contained fish oil, and Group 2, a diet with corn oil. The mean food consumption for the animals in Group 1 was calculated daily, and this amount of diet was provided to each animal in Group 2 on the succeeding day. The study continued for 6 weeks with measurement of body weights, food consumption, and final carcass energy content. These values were obtained as described above for the ad lib study except that no tissues or organs were removed from the carcasses prior to freeze-drying and bomb combustion. The diets were the same as those shown in *Table 1* except that a threefold excess of the fat soluble vitamins was provided in corn oil to ensure that potentially decreased availability of these vitamins would not limit growth.

Fat balance study

The fat balance study was a paired study in which the animals received for 14 days a diet that contained either fish oil or corn oil. The corn oil group received the average amount of diet ingested by the fish oil group on the previous day. During the final 10 days of the study, feces were collected and extracted with 2:1 chloroform:methanol as described above. The diets were the same as those of the corn oil and fish oil groups shown in *Table 1* except that all vitamins were provided in an AIN-76 mix. The fish (menhaden) oil was obtained from Zapata-Haynie Corporation (Reedsville, VA), and the EPA and DHA concentra-

Table 2 Ad lib study 6-week food consumption, final body weights, weight gain, food efficiency (weight gain/food consumed)

Group	Food consumption (g)	Final body weight (g)	Weight gain (g)	Food efficiency
Corn oil	578.1 ± 20.9	340.7 ± 9.3	287.1 ± 9.7	0.497 ± 0.012
MCT	586.7 ± 16.6	333.3 ± 8.4	280.1 ± 8.1	0.478 ± 0.009
Fish oil	485.0* ± 20.0	289.6* ± 12.0	235.9* ± 11.5	0.486 ± 0.012

Note. Data are given as mean ± SEM.
* Significantly different from corn oil and MCT groups: $P < 0.01$.

Table 3 Fatty acid distribution in carcass, liver, heart, brain, and fat pad from ad lib study

	(Weight %)			Liver			Heart			Brain			Adipose (Epididymal fat pad)		
Fatty acid*	CO	MCT	FO	CO	MCT	FO	CO	MCT	FO	CO	MCT	FO	CO	MCT	FO
8:0	0.0^{1}**	1.2^{2}	0.0^{1}	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0^{1}	1.5^{2}	0.1^{1}
10:0	0.1^{1}	6.0^{2}	0.2^{1}	0.0	0.4	0.0	0.0	0.3	0.0	0.0	0.5	0.0	0.0^{1}	6.1^{2}	0.0^{1}
12:0	0.2^{1}	0.9^{2}	0.5^{1,2}	0.0	0.1	0.0	0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.7	0.2
14:0	0.9^{1}	2.6^{2}	5.7^{3}	0.2	0.6	0.8	0.6	0.5	1.3	0.0	0.2	0.0	0.8^{1}	2.4^{2}	5.9^{3}
16:0	17.3^{1}	30.9^{2}	25.6^{3}	18.8^{1}	23.4^{2}	18.4^{1}	17.2	15.2	15.4	21.1	21.4	20.8	17.0^{1}	31.3^{2}	23.5^{3}
16:1	2.4^{1}	6.4^{2}	7.5^{3}	0.6^{1}	2.4^{2}	2.2^{2}	0.9	1.1	1.7	0.4	1.0	0.8	2.7^{1}	6.7^{2}	8.1^{3}
17:0	0.1^{1}	0.2^{1}	0.9^{2}	0.2	0.1	0.4	0.4	0.2	0.5	0.1	0.0	0.0	0.1	0.1	0.2
18:0	3.3^{1}	5.4^{2}	4.7^{3}	16.8^{1}	17.2^{1}	13.6^{2}	20.0	19.7	19.6	20.9^{1,2}	19.3^{1}	21.5^{2}	2.4^{1}	4.8^{2}	3.4^{3}
18:1(t)	0.0^{1}	0.0^{1}	1.2^{2}	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0^{1}	0.0^{1}	1.0^{2}
18:1(c)	25.7^{1}	22.6^{2}	18.8^{3}	8.9^{1}	10.7^{2}	8.9^{1}	11.0^{1}	8.6^{1,2}	8.1^{2}	18.4^{1}	19.0^{1}	20.9^{2}	26.0^{1}	23.4^{2}	17.1^{3}
18:2	45.3^{1}	18.2^{2}	18.2^{2}	24.2^{1}	14.8^{2}	15.6^{3}	23.6^{1}	21.7^{1}	13.5^{2}	1.9^{1}	3.2^{1}	0.7^{2}	47.0^{1}	18.2^{2}	16.8^{3}
18:3 (*n*-3)	0.4^{1,2}	0.0^{1}	0.9^{2}	0.1	0.0	0.5	0.2	0.0	0.2	0.2	0.0	0.0	0.5^{1,2}	0.2^{1}	0.9^{2}
20:0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.0	0.0
20:1	0.3^{1}	0.1^{1}	1.5^{2}	0.0	0.0	0.4	0.1	0.1	0.6	2.1	2.0	2.1	0.2	0.2	1.5
20:2	0.4	0.2	0.3	0.5	0.4		0.5	0.4	0.2	0.0	0.0	0.0	0.4	0.2	0.0
20:3 (*n*-6)	0.1	0.0	0.2	0.3	0.4	0.8	0.3	0.6	0.5	0.0	0.0	0.0	0.1	0.1	0.0
20:4	1.4	1.2	1.0	20.9^{1}	21.0^{1}	9.8^{2}	12.7	18.3	10.3	11.2^{1}	10.2^{1}	8.8^{2}	0.8	0.3	0.6
20:5	0.0^{1}	0.0^{1}	3.6^{2}	0.0^{1}	0.0^{1}	5.4^{2}	0.0^{1}	0.0^{1}	2.4^{2}	0.0	0.0	0.0	0.0^{1}	0.0^{1}	5.8^{2}
22:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22:6	0.0^{1}	0.0^{1}	5.0^{2}	2.8^{1}	1.0^{2}	17.8^{3}	4.1^{1}	1.4^{1}	16.7^{2}	13.2^{1}	11.7^{1}	18.6^{2}	0.0^{1}	0.0^{1}	6.5^{2}
24:0	0.0	0.0	0.0				0.4	0.5	0.4	0.0	0.0	0.0	0.2	0.0	0.0

* Fatty acids are designated by the number of carbon atoms:number of double bonds; *trans* is abbreviated (t), and *cis*, (c).
** Values for each fatty acid between groups are not different if they contain no superscript or a common superscript: $P < 0.05$.

tions in the diet did not differ significantly from those in *Table 1*.

Results

Ad lib study

Food consumption, weight gain, and food efficiency. *Table 2* displays the food consumption, weight gain, and food efficiency (weight gain/diet consumed). The food consumption and body weight of the animals that received fish oil were significantly less than the values obtained with the corn oil and MCT diets. There were no differences in the food efficiency values among the three groups.

Tissue fatty acids. The fatty acid distributions of the carcass and tissues are given in *Table 3*. In the MCT-fed animals, fat storage depots contained approximately 1% octanoic acid and 6% decanoic acid. The linoleic acid found in the carcasses was similar for all groups when expressed as percent of that fed—29.2 ± 2.7 (SEM), 33.0 ± 1.1, and 34.5% ± 3.0 for the corn, MCT, and fish oil groups, respectively (*Figure 1*).

The fatty acid deposition in the carcass for those acids of dietary origin for the MCT and fish oil groups in shown in *Figure 2*. In the MCT group, the octanoic and decanoic acids were deposited in a manner quite different from linoleic acid. These data suggest that only small amounts of decanoic acid and essentially no octanoic acid are deposited directly into the adipose tissue. In the fish oil group, EPA and DHA in the carcass accounted for 7.7 ± 0.9 and 14.3 ± 1.5%, respectively, of that in the diet. The ratio of DHA to EPA in the carcass was 1.4, twice that of the dietary fat.

Carcass energy. *Table 4* gives bomb calorimetric measurements of the energy density of the freeze-dried carcasses and the percentage of the carcass that was calculated to be fat by organic solvent extraction. The energy density of the fish oil-fed animals was signifi-

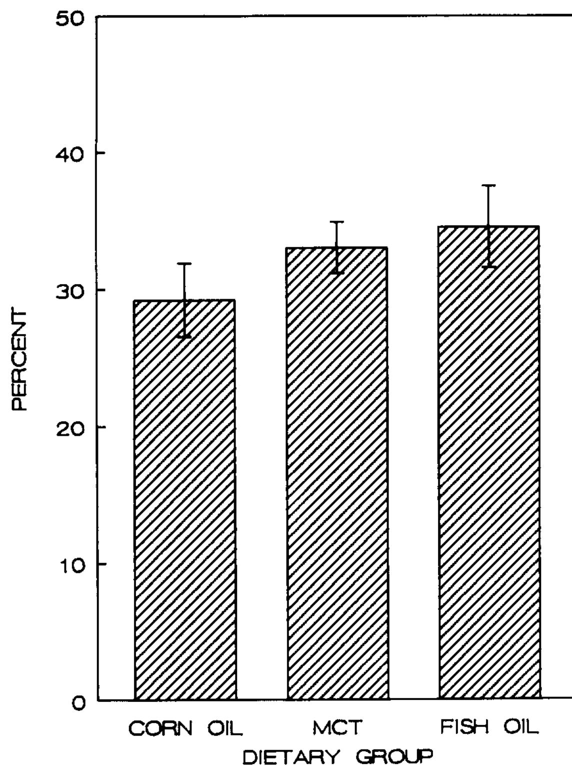


Figure 1 Linoleic acid recovered from the carcasses of rats fed diets based on corn oil, MCT oil, or fish oil. Values are expressed as percent of linoleic acid ingested during the 42-day feeding; mean with ± 1 SEM bars. There were no significant differences between groups.

cantly less than that observed for the corn oil-fed animals.

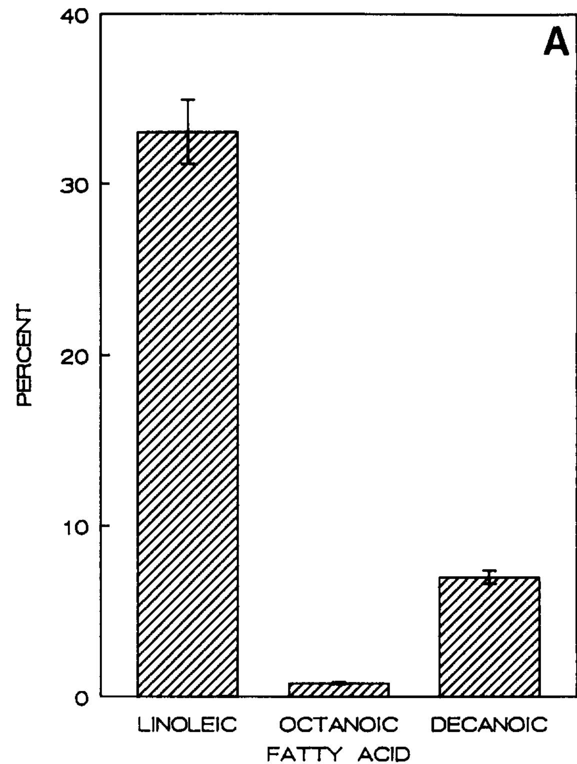
The energy gain was calculated by subtracting the carcass energy at the beginning of the study (see Methods). The energy gain and energy efficiency (kcal gained per gram of diet) are shown in *Table 5*. Energy gain by the fish oil-fed animals was less than that of the other groups.

Paired feeding study

The weight gain, food consumption, and food efficiency calculations for the animals in the paired feeding experiment are shown in *Table 6*. The final body weights and weight gain of the fish oil group were significantly less ($P < 0.05$) than those of the corn oil group. The food efficiency calculations, both as weight gain/food consumed and as energy gained/food consumed, also resulted in significantly lower values for the fish oil group.

Fat balance study

The results of the fat balance study are shown in *Table 7*. The absorption of the fish oil and of the corn oil was essentially complete with extractable fecal fat from both groups accounting for less than 3% of the total fat consumed during the 10 days of fat balance measurements. Food efficiency calculated for the 14 days of feeding showed a trend toward a lower value



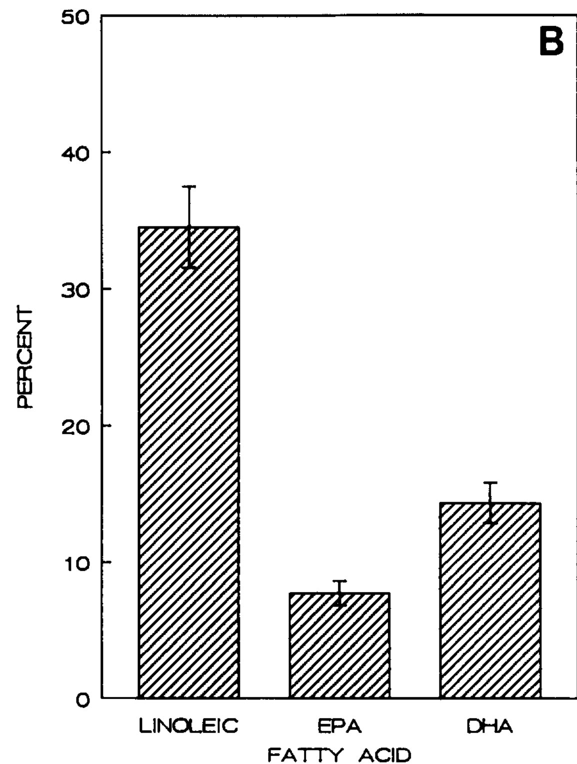


Figure 2 Comparison of the carcass accumulation of linoleic acid with other fatty acids of dietary origin. Expressed as percent of fatty acid ingested during the 42 days of feeding; mean with ± 1 SEM bars. (A) Linoleic, octanoic and decanoic acids from the MCT group. Linoleic acid accumulation was significantly greater than that of octanoic or decanoic acid. Decanoic acid accumulation was greater than that of octanoic acid ($P < 0.05$). (B) Linoleic acid, EPA and DHA from the fish oil group. Linoleic acid accumulation was greater than that of EPA and DHA. DHA accumulation was greater than that of EPA ($P < 0.05$).

Table 4 Ad lib study results

Group	Energy (kcal/g)	% Fat
Corn oil	6.5 ± 0.3	40.2 ± 3.0
MCT	5.9 ± 0.2	36.5 ± 2.2
Fish oil	5.6 ± 0.1*	35.2 ± 1.8

Note. Carcass energy density is measured by kcal/gram of dried tissue. The weight percentage of dry carcass as fat by extraction is given as mean ± SEM.
* Significantly different from corn oil: $P < 0.01$.

Table 5 Ad lib study results: cumulative energy gain and energy gain/food consumed

Group	Carcass energy gain (kcal)	Energy gained/Food consumed (kcal/g)
Corn oil	717 ± 101	1.21 ± 0.12
MCT	585 ± 40	0.99 ± 0.05
Fish oil	467 ± 40*	0.95 ± 0.05

* Different from corn oil group: $P < 0.05$.

for the fish oil group as seen in the 6-week paired study.

Discussion

The low recovery of EPA and DHA and the reduced carcass energy accumulation in animals fed fish oil are evidence that these acids are utilized in a manner that is different from fatty acids of more typical fats such as corn oil. Consistent with Jones' finding in growing hamsters,[7] dietary energy provided as fish oil was less efficiently utilized for storage and growth compared to dietary energy provided as corn oil. The ad lib fed animals consumed less of the diets containing fish oil than diets with corn oil or MCT. However, based on body weight gain and on body energy accumulation per gram of diet consumed, the food efficiency of the fish oil and MCT animals was still lower than that of corn oil, suggesting different utilization of these dietary fats compared with corn oil. The energy accumulation observed in the pair-fed study supports this hypothesis.

Since there was the possibility that the reduced energy efficiency and low levels of tissue EPA and DHA could have resulted from malabsorption of fish oil, we carried out a fat balance study to compare the absorption of fish oil and corn oil using a paired feeding study design. However, the fat balance study eliminated the possibility that malabsorption of fish oil was responsible for the low levels of EPA and DHA in the carcass.

The relative inefficiency of the contribution of MCT oil to fat storage in rats has been reported by several investigators.[10,11] The reason for this difference in storage efficiency of MCT involves differences both in transport and metabolism of MCT-derived medium chain fatty acids compared to long chain fatty acids. Unlike long chain fatty acids, newly absorbed medium chain fatty acids are transported via the portal vein directly to the liver where they undergo rapid oxidation to acetyl-CoA. The acetyl-CoA thus formed can undergo further oxidation to CO_2, or it can serve as a substrate for synthesis of ketone bodies or of long chain fatty acids. Both ketogenesis and de novo lipogenesis from acetyl-CoA are energy requiring processes. The obligatory cost of lipogenesis from medium chain fatty acid-derived acetyl-CoA can account for the reduced energy accumulation in rats fed MCT compared with long chain triglycerides.

The postabsorption metabolism of fish oil long chain fatty acids differs from other long chain fatty acids in that a significant portion of fish oil fatty acids is oxidized in peroxisomes.[12] Peroxisomes are thought to be important in the oxidation of both saturated and unsaturated fatty acids having 20 or more carbons.

Table 6 Body weights, food consumption, food efficiency, and liver weights from 6-week paired feeding study

Group	Initial body wt. (g)	Weight gain (g)	Food consumption (g)	Energy gain/ Food consumed (kcal/g)	Weight gain/ Food consumed	Final dry weight (g)	Carcass energy kcal/g final dry weight
Fish oil	47.2 ± 1.4	195.5* ± 6.0	453.9 ± 11.6	0.91* ± 0.03	0.43* ± 0.007	73.6* ± 3.1	6.31 ± 0.07
Corn oil	47.1 ± 1.4	215.2 ± 2.1	445.9 ± 0.6	0.99 ± 0.02	0.48 ± 0.004	78.9 ± 1.2	6.26 ± 0.08

Note. Data are given as mean ± SEM.
* $P < 0.05$, fish oil differs from corn oil.

Table 7 The results of the fat balance study comparing corn oil and fish oil fed animals

Group	Initial wt. (g)	Food consumed (g)	Food efficiency, 14-Day (Weight gain/Food consumed)	10-Day balance		Dietary fat excreted (%)
				Fat consumed (g)	Fat excreted (g)	
Fish oil	91.5 ± 2.4	189.5 ± 5.6	0.46 ± 0.014	26.6 ± 0.9	0.57 ± 0.04	2.1 ± 0.1
Corn oil	91.9 ± 2.3	184.2 ± 0.7	0.49 ± 0.008	26.4 ± 0.04	0.60 ± 0.05	2.6 ± 0.2

Fatty acid oxidation in peroxisomes occurs by a mechanism that is not coupled to ATP production. Thus, peroxisomal oxidation of very long chain fatty acids would not contribute useful energy to the maintenance or growth of the animal. Our observations of reduced adipose tissue deposition of EPA and DHA along with the reduced feed efficiency of rats fed fish oil are consistent with extensive oxidation of these acids and inefficient utilization of the derived energy.

Anderson and Connor[13] compared the fate of intravenously injected [1-^{14}C]-labeled fatty acids and found a three- to fourfold increase in water-soluble oxidation products of DHA compared with those from linoleic acid in the serum of hepatectomized and sham-operated rats. This increase is consistent with the substantial oxidation of DHA that we have inferred from its low recovery in the carcass. It suggests, however, that the liver may not be the only site capable of oxidizing these substrates.

Lin and Connor have reported recently measurements of the deposition of dietary EPA and DHA in rabbit adipose tissue.[14] They concluded that the deposition of these acids seemed low, but since linoleic deposition was not measured, a direct comparison with linoleic acid was not possible. Our data have reproduced their observation that the DHA to EPA ratio in the adipose tissue was greater than that in the diet. They suggested conversion of EPA to DHA or preferential oxidation of EPA as possible explanations for this phenomenon.

Another recent paper suggests that the post-absorptive transport of EPA and DHA may contribute to the unique metabolism of fish oil by the liver. The appearance of fish oil fatty acids in the lymph of rats 24 hours after gavage dosing was only 45–57% of that found for the fatty acids of corn oil.[15] Our fat balance data, however, show that absorption of fish oil is complete. Although there is a possibility that the appearance of a portion of the fish oil fatty acids in lymph might have been delayed past the 24 hours of collection, another explanation for the incomplete lymphatic recovery is possible. It has been reported that in the rat the portal vein transport of fatty acids increased with increasing number of double bonds.[16] This trend would predict that a portion of EPA and DHA could follow the portal vein route, and this prediction is consistent with complete absorption but incomplete lymphatic transport of the fish oil acids. As in the case of MCT fatty acids, the portal vein transport of the fish oil acids directly to the liver may contribute to the rapid oxidation and decreased carcass deposition of these polyunsaturated lipids.

Although our data support the idea that the reduced efficiency of energy utilization by rats fed fish oil is due to its unique metabolism, we cannot be certain that the net result was not due to an effect on some other component of the energy balance equation. For example, rats fed the fish oil diet may have been more active than animals fed the corn oil diet, and thus would have expended more energy and deposited less than the controls. Jones's study of hamsters, however, showed no difference in oxygen consumption during fish oil consumption.[7]

A discussion of the data presented above would not be complete without consideration of the artifacts that would result, had significant oxidation of EPA and DHA occurred either in the diet or in the tissue analyses. The rapid loss of EPA and DHA in animal diet cups has been reported[17] and is a potential source of error in all studies of the biological effects of EPA and DHA. The storage of diet in double bags without headspace at 4° C and the daily complete replacement of diet minimized the loss of EPA and DHA in our diet studies. Analysis of the diet for fatty acids after completion of the study showed no change in the linoleic:EPA:DHA ratio compared with the ratio based on the original values of the component oils.

The extraction procedures were also selected to minimize oxidation. Tissues were rapidly frozen and air was excluded by nitrogen or vacuum whenever possible. If technique had been responsible for the loss of EPA and DHA, we would have expected greater losses of DHA because of its greater unsaturation. The loss of EPA was two- or threefold times that of the DHA. Moreover, the levels of anachidonic acid, EPA, and DHA found in the brains and livers are similar to those of other studies.[13,18] We, therefore, conclude that the low recovery of EPA and DHA represents metabolic oxidation rather than artifacts of diet decomposition or analysis.

In summary, our data showing reduced energy deposition, reduced EPA and DHA accumulation, and complete absorption of fat by fish oil fed animals combine with earlier reports on incomplete lymphatic transport and peroxisomal oxidation of fish oils to suggest a unqiue process by which a portion of fish oil may be metabolized. In the liver, EPA and DHA from chylomicrons, the portal vein, and lipoprotein triglycerides can be oxidized (at least partially) in peroxisomes without the production of biologically useful energy. As a result of this overall metabolic scheme, the oxidation of EPA and DHA may be increased relative to the fatty acids of corn oil and their deposition in adipose tissue is diminished.

Although not evident in the carcass fatty acid distribution measurements, it is nevertheless possible that EPA and DHA were shortened to lower molecular weight fatty acids or degraded to acetyl CoA that was used in fatty acid synthesis. The data in this paper cannot exclude these explanations for reduced EPA and DHA recovery.

One implication of this view of fish oil metabolism is the interpretation of studies indicating that fish oil is not a promoter of tumor growth or incidence in rats challenged with carcinogens.[19,20] Studies by Pariza[21] and Kritchevsky[22] have shown body size and stored energy to be a determinant of a diet's effect on tumor promotion in animals. Thus, tumor promotion studies with fish oil in which decreased food consumption was observed should be considered in terms of its effect on total caloric intake. Moreover, even if animals fed fish oil should ingest the same amount of diet as the

control groups, the utilizable energy or stored carcass energy should be considered when comparing different dietary fats.

Finally, our data are consistent with the well-documented effect of the reduction of plasma triglycerides by fish oil in hyperlipidemic patients, which may result in part from preferential oxidation of EPA and DHA. The reduced availability of these acids would provide less substrate for triglyceride-rich lipoprotein production. A link between the hepatic peroxisome proliferation observed in fish oil fed animals and the triglyceride lowering effect is suggested by the peroxisome proliferation produced by hypolipidemic drugs including clofibrate.[23]

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