Effects of beef- and fish-based diets on the kinetics of n−3 fatty acid metabolism in human subjects[1,2]

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ABSTRACT

Background: The quantity and type of dietary polyunsaturated fatty acids (PUFAs) can alter essential fatty acid metabolism in humans. Diets rich in 20- and 22-carbon PUFAs may inhibit desaturase expression or activity and decrease the synthesis of long-chain unsaturated fatty acids.

Objective: It was theorized that the fat content of a fish-based diet would inhibit the kinetics of the in vivo metabolism of n−3 fatty acids compared with a beef-based diet.

Design: A compartmental model was used to determine the coefficients of the kinetic rate constants from the plasma concentration time curves of pentadeuterated (d_5) 18:3n−3, 20:5n−3, 22:5n−3, and 22:6n−3 of 10 subjects who subsisted on 3 diets with different long-chain PUFA contents. For 3 wk, subjects reported their food intake from their usual diets and then consumed a beef-based diet for 3 wk and then a fish-based diet for an additional 3 wk. Subjects consumed 1 g d_5-18:3n−3 ethyl ester at weeks 3, 6, and 9. Blood was drawn over 168 h and the plasma analyzed for fatty acids. The coefficients of the kinetic constants of n−3 fatty acid metabolism and the percentage utilization of the substrates were determined.

Results: Across all diets, < 1% of plasma 18:3n−3 was utilized for long-chain PUFA synthesis. There was a 70% reduction in the value of the rate constant coefficient that regulated transfer of the isotope from the 22:5n−3 compartment to 22:6n−3 when the fish-based diet was compared with the beef-based diet. The turnover rate of plasma d_5-22:6n−3 also decreased.

Conclusions: The primary effect of a fish-based diet on the kinetics of n−3 metabolism involves processes that inhibit the synthesis of 22:6n−3 from 22:5n−3. These processes may involve a system of feedback control mechanisms responsive to the plasma concentration of 22:6n−3 *Am J Clin Nutr* 2003;77:565–72.

KEY WORDS Fatty acid kinetics, α-linolenic acid, n−3 fatty acids, docosahexaenoic acid, compartmental model, isotope tracer, fish diet

INTRODUCTION

The American Heart Association (1) has recognized that regular consumption of long-chain (20–22 carbons) n−3 polyunsaturated fatty acids (PUFAs) found in some marine foods such as tuna, mackerel, and herring have clear positive effects in preventing cardiovascular morbidity and mortality (2–8). In addition to the multifaceted effects that long-chain n−3 PUFAs have on cardiovascular physiology, evidence shows that dietary long-chain n−3 PUFAs may improve some measures of infant neurodevelopment performance (9–12) and may also have beneficial psychiatric effects (13). Because such foods are not abundant in a typical American diet, the principle n−3 fatty acid that is consumed is α-linolenic acid (18:3n−3), which is found in limited quantities in plant seed oils (14). However, because of low utilization, the biosynthesis of long-chain PUFAs from 18:3n−3 appears to be a minor pathway in humans (15). In contrast, eicosapentaenoic acid (20:5n−3) may be well utilized for synthesis of other long-chain PUFAs such as docosahexaenoic acid (22:6n−3) (15).

Previously, we described a compartmental modeling procedure that was used to determine the coefficients of the in vivo rate constants of n−3 fatty acid metabolism in human subjects subsisting on a well-controlled beef-based diet (15). This analysis has now been extended to determine the kinetic rate constants in 10 subjects who were maintained on 3 distinct diets, each with a different fat content. The hypothesis tested was that a diet with relatively high amounts of long-chain PUFAs (fish-based diet) would lower the in vivo rate constants of n−3 fatty acid metabolism in adult humans.

The subjects were tested 3 times: initially during the self-selected dietary phase and twice while they subsisted on each of the experimental diets. During the final week of each trial period, the subjects consumed an isotope tracer of α-linolenate, and the plasma was analyzed periodically for endogenous and labeled fatty acids. The coefficients of the kinetic rate constants of n−3 fatty acid metabolism during the 3 dietary trial periods were determined from an analysis of the isotopic data by using the WinSAAM (Windows Simulation and Analysis Modeling; National Cancer Institute, Bethesda, MD) program. The endogenous concentrations of the n−3 fatty acids together with the kinetic constants were used to calculate the flux of each of the fatty acids through the plasma compartments.

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Received December 28, 2001.

Accepted for publication May 24, 2002.

TABLE 1
Fatty acid composition of the self-selected and experimental diets[1]

Fatty acid	Diet		
	Self-selected[2]	Beef-based[3]	Fish-based[3]
		g/d	
Saturates	34.4 ± 2.3	48.3 ± 4.3	45.2 ± 1.2
Monounsaturates	34.5 ± 2.0	34.6 ± 1.4	33.2 ± 0.9
n−6			
18:2	17.7 ± 1.2	5.3 ± 0.3	5.7 ± 0.2
20:3	NA	0.102 ± 0.004	0.045 ± 0.004
20:4	0.20 ± 0.02	0.24 ± 0.01	0.21 ± 0.01
n−3			
18:3	1.62 ± 0.06	0.72 ± 0.03	0.74 ± 0.02
20:5	0.05 ± 0.02	0.044 ± 0.001	0.16 ± 0.02
22:5	0.02 ± 0.01	0.092 ± 0.003	0.095 ± 0.007
22:6	0.08 ± 0.02	0.015 ± 0.001	0.40 ± 0.06

[1] $\bar{x}$ ± SD; n = 10. NA, not available from the database.

[2] The weighted mean fatty acid intakes were calculated from the subjects' food records by using Minnesota Data Systems software. The 2 experimental diets were formulated to contain either beef or fish with olive oil and butter as the major source of fats.

[3] The weighted mean values were determined from triplicate gas chromatography–flame ionization detection analysis of the fatty acid content of alternating diets providing a mean daily intake of 2700 kcal for the group.

SUBJECTS AND METHODS

Subjects

All subjects were evaluated at the Clinical Research Unit of the National Institute on Alcohol Abuse and Alcoholism (NIAAA) at the Clinical Center of the National Institutes of Health (Bethesda, MD). Selection criteria for subjects participating in the study were recently reported (15). Briefly, male (n = 5) and female (n = 5) subjects who had no major medical or psychiatric problems, did not smoke or use tobacco products within the past 2 y, and were judged to be reliable in maintaining the dietary requirements of the protocol were included. All subjects provided written informed consent, and all clinical procedures were under continuous review by the NIAAA Institutional Review Board (protocol 92-AA-0194).

Protocol

During the first 2-wk period of the study, the subjects kept accurate records of the foods and beverages they had consumed each day. The nutritional content (eg, energy, protein, and carbohydrate), including the estimated fatty acid content of the individual diets, were computed by using the Minnesota Data Systems software developed by the Nutrition Coordinating Center, University of Minnesota, MN (FOOD DATABASE version 6A and NUTRIENT DATABASE version 21). During the second 3-wk period of the study, the subjects received a beef-based diet that consisted of roast beef or tenderloin cuts of meat and hamburger patties given on alternating days. During the final 3 wk, the subjects received a fish-based diet that included salmon, tuna, and turkey breast. Dietary macronutrients were calculated by using the US Department of Agriculture nutrient data bank (Handbook no. 8, rev 11). However, the fatty acid composition of the 2 experimental diets was determined directly by using gas chromatography–flame ionization detection (GC-FID) , and is provided in **Table 1**. All meals during the experimental diet phases of the study were prepared and served in the metabolic research kitchen of the Clinical Center. Food sources were consistent throughout the study.

Oral ingestion of the deuterium-labeled fatty acids occurred at the beginning of the third, sixth, and ninth weeks after the study began, and blood samples were obtained at baseline (0 h) and at intervals (8, 24, 48, 72, 96, and 168 h) over the following week. Subjects were admitted as inpatients, fasted overnight, and then received 1 g of the labeled fatty acid ethyl ester blended into low-fat (1% fat) yogurt before consuming a standardized morning meal. The isotope used was pentadeuterated α-linolenate ethyl ester (d_5-17, 17, 18, 18, 18, 18:3n−3; Cambridge Isotope Laboratory, Andover, MA). A standardized lunch was provided 4 h later to ensure uniform absorption of the tracer. The subjects reported to the clinic for all subsequent blood drawings. Blood (40 mL) was drawn under fasting conditions (except for the 8-h sample) from the forearm into a plastic tube containing sodium citrate as an anticoagulant. The blood was placed on ice and then separated immediately into platelet-poor plasma by centrifugation at 3000 rpm (1800 × g) for 10 min at 4 °C in a clinical centrifuge. Plasma was transferred into a separate tube and frozen at −80 °C until analyzed.

Formulation and analysis of the experimental diets

Either beef or fish (the fish-based diet also included skinless turkey) provided the major source of dietary fat in the experimental diets. The only other significant sources of fat were olive oil and butter. The Harrison-Benedict equation was used to calculate each subject's energy requirements. The calculations were compared with the subject's self-selected food-record data, and, if necessary, the energy content of the metabolic diets was adjusted to satisfy energy demands. The weights of the subject were monitored during the study, and individual energy intake was adjusted to maintain a weight change of < 1 kg. During the 2 experimental dietary periods, the subjects were counseled to not eat or drink any additional foods or beverages other than those provided by the research kitchen. No alcoholic beverages or smoking were allowed.

The fatty acid contents of the alternating menus were analyzed directly. The foods from an entire day's menu were combined in a commercial 2-L blender and homogenized; aliquots were obtained and lipids were extracted by using the Folch method (16). Analysis of the fatty acid methyl esters by GC-FID is described below.

Plasma lipid extraction, preparation of fatty acid methyl esters, and analysis by GC-FID

The protocols used to extract lipids from the plasma and to prepare the fatty acid methyl esters were previously reported (15). After derivatization, the fatty acid methyl esters were extracted into hexane, and the pooled extracts were concentrated to 50 μL under nitrogen and analyzed by GC-FID. Samples were analyzed with a model HP-5890 gas chromatograph with flame ionization detector (Agilent Technologies, Wilmington, DE) according to previously published procedures (15). The concentrations of the individual fatty acids were calculated by using the peak area counts in comparison with the internal standard.

Analysis of labeled fatty acids with GC-MS

The procedures used for the derivatization of the plasma lipids and for the analysis with quadrupole GC-mass spectrometry (MS) (HP 5989; Agilent Technologies) were previously reported (17). The pentafluorobenzyl esters of the plasma fatty acids (1 μL) were injected onto a 60-m FFAP-bonded phase capillary column (internal diameter: 0.25 mm; film thickness: 0.25 mm; Quadrex Corp, New Haven, CT)

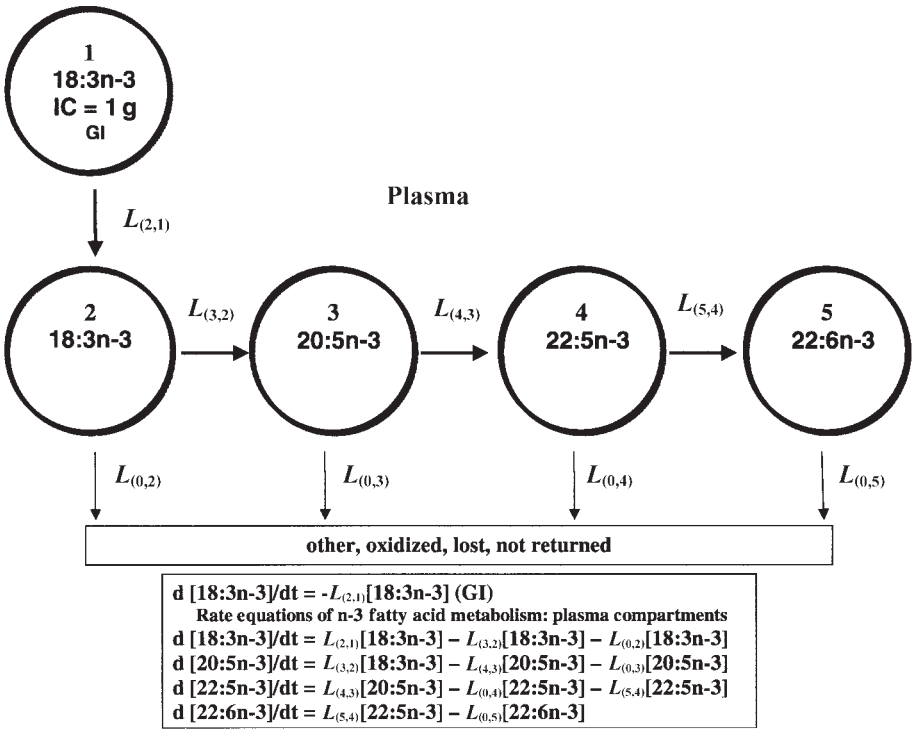

FIGURE 1. Conceptual model of α-linolenic acid metabolism. The circles represent separate n−3 fatty acid compartments in the metabolic scheme. Compartment 1 represents administration of the isotope (1 g) and intestinal absorption. Four compartments (2 through 5) represent n−3 fatty acid compartments in the plasma proceeding through successive steps of desaturation and elongation of the label. The fractional transfer rates, defined as the fraction of substrate transferred from compartment *J* to product compartment *I* [$L_{(I,J)}$], are rate parameters derived from the model-fitted experimental data. The set of differential equations used to determine the rate parameters are given in the boxed area. IC, initial concentration of the bolus; GI, gastrointestinal tract; d, differential changes; dt, differential changes in time.

into a quadrupole GC-MS. Data were acquired in the selected ion mode, monitoring the M-PFB anion of the fatty acids, and converted to the absolute quantity of the deuterium-labeled metabolite by reference to the concentration of an internal standard (17).

Compartmental analysis of n−3 fatty acid metabolism

The compartmental model used to determine the coefficients of the kinetic rate constants for n−3 fatty acid metabolism were recently described, and a diagram of the model is presented in **Figure 1** (15). The model consists of 5 compartments for which isotope data were obtained. The fractional transfer rates determined from the model represent the kinetics of labeled fatty acids from their plasma pool concentrations alone and may only be an indirect assessment of liver kinetics. The rate equations from which the kinetic parameters are derived are defined by a set of differential equations that correspond to the flux of the labeled fatty acids through their respective compartments.

Fractional transfer rates, flow rates, percentages, and turnover

The rate parameters that pertain to the metabolism of n−3 fatty acids are briefly described below. The fractional transfer rate, $L_{(I,J)}$, is defined as the fraction of substrate transferred from compartment *J* to product compartment *I*. The units are in hours. The flow rate of fatty acid, $R_{(I,J)}$, from compartment *J* to compartment *I* is obtained by multiplying the endogenous mass of the unlabeled fatty acid (M_J) in compartment *J* by $L_{(I,J)}$ and is given in μg/h. The percentage of isotope that is transferred from *J* to *I* is given as $P_{(I,J)}$ and is given as a percentage. $P_{(I,J)}$ represents the fraction of isotope that remains in the metabolic pathway as opposed to isotope that is taken up by tissues or that is irreversibly lost from the compartment. The half-life ($t_{1/2}$) of the n−3 fatty acids in the plasma is an indication of fatty acid turnover. The $t_{1/2}$ values were calculated from the summation of the fractional transfer rates for each fatty acid leaving the individual compartments: $t_{1/2} = \ln 2/\Sigma L_{(I,J)} + L_{(0,J)}$. The units are in hours and represent the disappearance rate of the fatty acid from each compartment. Mean values of these parameters were calculated for subjects consuming each of the 3 diets, and variances are reported as the group SD.

Model limits and constraints

Because the caloric intakes from the diets for each subject during the 3 trial periods were known and the dietary fatty acid composition had either been experimentally determined or calculated from each subject's food records, the daily n−3 fatty acid intake for each subject could be estimated, and upper and lower n−3 fatty acid intake limits were assigned.

The plasma steady state fatty acid concentrations were determined for each subject at each of the blood sampling time points (**Table 2**). During the self-selected dietary period, the mean plasma concentration of the fatty acids was used to represent the steady state mass of the endogenous substrate (M_J) available for biosynthesis. Inasmuch as the 2 standardized diets minimized the variability of dietary PUFA intake, little difference (±5%) was observed in the plasma concentrations of individual n−3 fatty acids in each subject during the experimental dietary periods. Therefore, each subject's mean concentration of the plasma

TABLE 2
Steady state mass values for the endogenous fatty acids from their plasma compartments (M_J)

Diet	Subject number									
	1	2	3	4	5	6	7	8	9	10
						μg				
Self-selected										
M_2	14494	10124	44985	13443	25574	35100	16760	9216	18620	30881
M_3	11642	9605	29848	12484	13998	17100	14078	7373	20216	15544
M_4	18770	18951	28569	19466	18844	30900	20447	16282	19950	24966
M_5	96466	47766	51808	64898	50071	45600	81454	67277	114380	86554
Beef-based										
M_2	16744	17653	27716	16071	15760	28200	23799	14438	17822	23062
M_3	19483	19470	43066	26695	16152	30600	28492	16588	21014	17385
M_4	24125	29854	32619	26695	21805	48600	35531	24268	25270	31932
M_5	96941	57372	72488	56932	51148	68100	96873	59904	106666	65159
Fish-based										
M_2	15444	17652	23452	19068	13998	22200	26816	15360	22610	23842
M_3	54648	28556	55006	64014	40111	46800	48939	56525	56126	46024
M_4	24235	31671	27503	27240	27189	34800	27486	28262	26068	36172
M_5	163231	102282	107666	138924	151021	130500	199109	159130	180348	174774

n−3 fatty acids across the 168-h period represented the steady state mass of the endogenous substrate (M_J). These values were held constant.

Model calculations and errors

The initial estimates of $L_{(I,J)}$ and $P_{(I,J)}$ of isotope transferred for this compartmental model were derived by using the WinSAAM program from the concentration-time curves that were generated from the experimental isotopic data. Values assigned to the kinetic parameters were then adjusted to compensate for individual variances in the plasma data until the model prediction gave the best fit to the experimental determinants. Final values were determined by using the program's iterative nonlinear least-squares routine. The error model for this analysis included the assumptions of independence, constant variance, and normal distribution about zero. Data points were weighted by assigning a fractional SD of 0.1 to each measurement, which is consistent with the error associated with these analyses.

TABLE 3
Fatty acid composition of the total lipid extract of plasma from subjects consuming the self-selected and 2 experimental diets[1]

Fatty acid	Diet		
	Self-selected	Beef-based	Fish-based
		$\mu g/mL$	
16:0	314 ± 24	356 ± 21	335 ± 14
18:0	117 ± 7	130 ± 7	123 ± 5
18:1n−9	271 ± 19	340 ± 22	296 ± 13
n−6			
18:2	505 ± 30	477 ± 32	490 ± 20
20:3	24 ± 2	33 ± 2	24 ± 2[2]
20:4	133 ± 9	150 ± 12	137 ± 8
22:4	4.8 ± 0.4	5.3 ± 0.4	3.7 ± 0.2[3]
22:5	4.3 ± 0.3	4.4 ± 0.3	3.3 ± 0.2[2]
n−3			
18:3	7.4 ± 2.0	6.8 ± 0.8	7.2 ± 0.5
20:5	5.7 ± 1.1	8.8 ± 1.1	19.4 ± 1.5[3]
22:5	7.7 ± 0.8	10.2 ± 0.7	10.1 ± 0.4
22:6	26.8 ± 3.7	26.8 ± 2.4	55.1 ± 2.5[3]

[1] $\bar{x}$ ± SD; n = 10. The values were determined by gas chromatography–flame ionization detection analysis of the fatty acid methyl esters in the plasma of subjects during the third week of the dietary period. Data were analyzed as paired observations from subjects during the 2 experimental dietary periods.

[2,3] Significantly different from the beef-based diet (Student's t test): [2] $P < 0.05$, [3] $P < 0.001$.

Statistical analysis

For comparison of the effects of the 2 experimental diets on n−3 fatty acid metabolism, individual kinetic rate constants and other in vivo parameters were analyzed as paired observations from individual subjects. Differences were determined by using Student's t test. For all kinetic parameters, a P value ≤ 0.05 was considered significant. Mean (± SD) values of the kinetic parameters are also reported.

RESULTS

Subject characteristics, dietary intake, and plasma composition

All 10 of the subjects completed the 9 wk of the study. The subjects had a mean age of 26 y (range: 22–37 y), height of 170 cm (range: 116–187 cm), body weight of 70.4 kg (range: 53–83 kg), and body mass index (in kg/m^2) of 23 (range: 19–25). The mean energy content of the experimental diet was 2700 kcal/d and the protein, fat, and carbohydrate contents of the diet were 102 g/d (16% of energy), 98 g/d (33% of energy), and 364 g/d (51% of energy), respectively. The mean plasma fatty acid concentrations of the subjects at the end of each dietary period are presented in **Table 3**. Significant differences in several individual fatty acids, which resulted from changes in PUFA intakes during the different dietary treatment periods, were observed. Consistent with the changes in dietary PUFAs, the primary changes in plasma fatty acids were elevations in the concentrations of 20:5n−3 and 22:6n−3 during the fish-based diet compared with the beef-based diet. Small but significant decreases in the plasma concentrations of both 22:4n−6 and 22:5n−6 after the fish-based diet were also observed.

TABLE 4
Individual kinetic constant coefficients ($L_{I,J}$) describing the in vivo metabolism of n−3 fatty acids in 10 subjects sustained with a self-selected or 2 experimental diets[1]

	Subject number									
Diet	1	2	3	4	5	6	7	8	9	10
Self-selected										
$L_{(2,1)}$	0.0190	0.0292	0.0398	0.0409	0.0470	0.0478	0.0325	0.0216	0.0314	0.0701
	(0.0583)	(0.0420)	(0.0455)	(0.0662)	(0.0397)	(0.0426)	(0.0478)	(0.0235)	(0.0421)	(0.0196)
$L_{(0,2)}$	11.99	11.24	33.23	7.79	12.88	20.00	18.12	9.09	26.69	3.49
	(1.015)	(0.025)	(0.04)	(0.05)	(0.04)	(0.03)	(0.027)	(0.05)	(0.02)	(0.12)
$L_{(3,2)}$	0.0013	0.0019	0.0032	0.0068	0.0018	0.0011	0.0023	0.0009	0.0027	0.0013
	(0.9600)	(0.0495)	(0.0653)	(0.0816)	(0.0630)	(0.1027)	(0.0399)	(0.0984)	(0.0331)	(0.0356)
$L_{(0,3)}$	0.0112	0.0000	0.0000	0.0305	0.0088	0.0155	0.0087	0.0112	0.0162	0.0112
	(0.0842)	(0.0000)	(0.0000)	(0.0913)	(0.1103)	(0.0768)	(0.1886)	(0.1460)	(0.1008)	(0.1076)
$L_{(4,3)}$	0.0136	0.0170	0.0122	0.0141	0.0152	0.0066	0.0162	0.0092	0.0138	0.0136
	(0.1336)	(0.0526)	(0.0712)	(0.0632)	(0.0467)	(0.1077)	(0.0712)	(0.1258)	(0.0838)	(0.0725)
$L_{(0,4)}$	0.0000	0.0360	0.0015	0.0541	0.0158	0.0355	0.0428	0.0000	0.0281	0.0000
	(0.0000)	(0.1434)	(7.6550)	(0.0586)	(0.1727)	(0.0768)	(0.1046)	(0.0000)	(0.1595)	(0.0000)
$L_{(5,4)}$	0.0449	0.0091	0.0382	0.0018	0.0116	0.0004	0.0127	0.0320	0.0117	0.0449
	(0.3091)	(0.1751)	(0.1645)	(0.0650)	(0.1820)	(5.4850)	(0.0562)	(0.0222)	(0.0609)	(0.9885)
$L_{(0,5)}$	0.0488	0.0464	0.1616	0.0119	0.0535	0.0463	0.0295	0.6000	0.0368	0.0488
	(0.2799)	(0.2252)	(0.1768)	(0.0700)	(0.2110)	(0.2438)	(0.1024)	(0.0050)	(0.0821)	(0.3500)
Beef-based										
$L_{(2,1)}$	0.0289	0.0260	0.0185	0.0795	0.0285	0.0271	0.0298	0.0274	0.0229	0.0313
	(0.0241)	(0.0164)	(0.0362)	(0.0306)	(0.0200)	(0.0196)	(0.0173)	(0.0167)	(0.0289)	(0.0330)
$L_{(0,2)}$	7.82	5.34	22.60	7.05	8.79	11.97	19.00	7.05	20.00	4.61
	(0.04)	(0.09)	(0.02)	(0.02)	(0.02)	(0.01)	(0.01)	(0.02)	(0.018)	(0.12)
$L_{(3,2)}$	0.0025	0.0012	0.0023	0.0033	0.0017	0.0016	0.0011	0.0012	0.0011	0.0006
	(0.0562)	(0.0493)	(0.0267)	(0.0397)	(0.0793)	(0.0830)	(0.1189)	(0.1067)	(2.3975)	(0.1050)
$L_{(0,3)}$	0.0000	0.0000	0.0000	0.0198	0.0063	0.0000	0.0128	0.0097	0.0119	0.0106
	(0.0000)	(0.0000)	(0.0000)	(0.0761)	(0.2374)	(0.0000)	(0.1174)	(0.1549)	(0.0002)	(0.0974)
$L_{(4,3)}$	0.0216	0.0206	0.0126	0.0167	0.0124	0.0127	0.0096	0.0065	0.0100	0.0147
	(0.1096)	(0.0197)	(0.0321)	(0.0767)	(0.1033)	(0.1007)	(0.1335)	(0.1984)	(0.1827)	(0.0276)
$L_{(0,4)}$	0.0157	0.0000	0.0045	0.0475	0.0289	0.0529	0.0062	0.0000	0.0000	0.0236
	(1.1300)	(0.0000)	(0.7955)	(0.0914)	(0.1500)	(0.0820)	(0.6990)	(0.0000)	(0.0000)	(0.1527)
$L_{(5,4)}$	0.0470	0.0632	0.0355	0.0060	0.0169	0.0013	0.0249	0.0294	0.0314	0.0106
	(0.3032)	(0.0478)	(0.0851)	(0.0684)	(0.0243)	(0.3164)	(0.0164)	(0.0139)	(0.3065)	(0.2848)
$L_{(0,5)}$	0.1900	0.1313	0.0531	0.0257	0.0693	0.0227	0.0519	0.2808	0.0683	0.0416
	(0.2588)	(0.0399)	(0.0985)	(0.0824)	(0.0305)	(0.0930)	(0.0407)	(0.0075)	(0.6325)	(0.1257)
Fish-based										
$L_{(2,1)}$	0.0248	0.0255	0.0239	0.0505	0.0347	0.0279	0.0319	0.0300	0.0305	0.0266
	(0.0146)	(0.0096)	(0.0250)	(0.0073)	(0.0101)	(0.0203)	(0.0016)	(0.0105)	(0.0172)	(0.0270)
$L_{(0,2)}$	7.04	10.00	45.72	32.16	9.98	19.66	17.32	7.78	19.02	5.36
	(0.04)	(0.03)	(0.002)	(0.004)	(0.01)	(0.006)	(0.030)	(0.07)	(0.03)	(0.02)
$L_{(3,2)}$	0.0006	0.0010	0.0047	0.0010	0.0012	0.0011	0.0010	0.0012	0.0014	0.0009
	(0.0896)	(0.0658)	(0.0059)	(0.0282)	(0.0228)	(0.0267)	(0.0594)	(0.0506)	(0.0433)	(0.0320)
$L_{(0,3)}$	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0152	0.0157	0.0062	0.0030
	(0.0000)	(0.0000)	(0.0000)	(0.0000)	(0.0000)	(0.0000)	(0.0676)	(0.0656)	(0.1646)	(0.2629)
$L_{(4,3)}$	0.0234	0.0196	0.0157	0.0100	0.0172	0.0140	0.0046	0.0040	0.0104	0.0091
	(0.1683)	(0.1286)	(0.0295)	(0.0462)	(0.0269)	(0.0331)	(0.0881)	(0.1009)	(0.0388)	(0.0509)
$L_{(0,4)}$	0.0000	0.0675	0.0535	0.0501	0.0000	0.0541	0.0048	0.0182	0.0381	0.0234
	(0.0000)	(0.1602)	(0.0408)	(0.0436)	(0.0000)	(0.0404)	(0.7480)	(0.1980)	(0.0947)	(0.0934)
$L_{(5,4)}$	0.0084	0.0075	0.0128	0.0150	0.0053	0.0055	0.0102	0.0083	0.0098	0.0037
	(0.3426)	(0.1803)	(0.0816)	(0.0696)	(0.1958)	(0.1905)	(0.2966)	(0.3638)	(0.3086)	(0.2789)
$L_{(0,5)}$	0.1695	0.0138	0.0150	0.0090	0.0109	0.0540	0.0170	0.0310	0.0276	0.0101
	(0.3099)	(0.1950)	(0.4017)	(0.6730)	(0.5535)	(0.1119)	(0.3078)	(0.1687)	(0.1900)	(0.5985)

[1] L values represent model-determined kinetic constants for the transfer of fatty acids from compartment J to I (precursor to product). The fractional SDs are given in parentheses. The fractional SD is equal to the variance divided by the value; it is a measure of goodness of fit of the function curve to the experimental data.

Compartmental model

The compartmental model shown in Figure 1 is a simplification of the physiologic reality in that each rate constant $L(_{I,J})$ reflects several steps of metabolism that occur within the liver hepatocyte and also incorporates a transport step of fatty acids from the liver to the plasma. Two 18:3n−3 compartments are included in the model, one for the isotope administration and gastrointestinal tract and the second for the appearance of the fatty acid in the plasma. The individual model-determined kinetic parameter estimates for $L(_{I,J})$ in **Table 4** are the in vivo

TABLE 5
Summary of kinetic parameters of labeled n−3 fatty acid metabolism determined by compartmental analysis of the 3 diets[1]

	Diet		
	Self-selected	Beef-based	Fish-based
Mean fractional transfer rate, $L_{(I,J)}$			
$L_{(3,2)}$	0.0023 ± 0.0008	0.0017 ± 0.0004	0.0014 ± 0.0005
$L_{(4,3)}$	0.0131 ± 0.0016	0.0137 ± 0.0024	0.0128 ± 0.003
$L_{(5,4)}$	0.0207 ± 0.0081	0.0266 ± 0.008	0.0086 ± 0.0012[2]
$L_{(x,1)}$ $\Sigma L_{(I,J)}$ leaving 1	0.0379 ± 0.0078	0.0320 ± 0.008	0.0306 ± 0.004
$L_{(x,2)}$ $\Sigma L_{(I,J)}$ leaving 2	15.45 ± 4.56	11.42 ± 3.31	17.40 ± 6.43
$L_{(x,3)}$ $\Sigma L_{(I,J)}$ leaving 3	0.0113 ± 0.004	0.0071 ± 0.003	0.004 ± 0.003
$L_{(x,4)}$ $\Sigma L_{(I,J)}$ leaving 4	0.0214 ± 0.0104	0.0179 ± 0.009	0.0310 ± 0.012
$L_{(x,5)}$ $\Sigma L_{(I,J)}$ leaving 5	0.1084 ± 0.0441	0.0935 ± 0.008	0.0358 ± 0.025[2]
Mean flow rate, $R_{(I,J)}$ (μg/h)			
$R_{(3,2)}$	49.5 ± 19.9	32.6 ± 8.3	29.7 ± 14.6
$R_{(4,3)}$	197 ± 42	325 ± 68	613 ± 154[3]
$R_{(5,4)}$	450 ± 211	751 ± 277	244 ± 43[3]
$R_{(0,2)}$	$3.91 \times 10^5 \pm 2.18 \times 10^5$	$2.44 \times 10^5 \pm 9.34 \times 10^4$	$3.69 \times 10^5 \pm 1.53 \times 10^5$[2]
$R_{(0,3)}$	161 ± 64	159 ± 90	212 ± 169
$R_{(0,4)}$	461 ± 225	597 ± 403	934 ± 387
$R_{(0,5)}$	$7.2 \times 10^3 \pm 5.9 \times 10^3$	$6.8 \times 10^3 \pm 3.0 \times 10^3$	$3.1 \times 10^3 \pm 9.9 \times 10^2$
Fatty acids transferred from *J* to *I* (%)			
$P_{(3,2)}$	0.20 ± 0.01	0.19 ± 0.01	0.10 ± 0.02
$P_{(4,3)}$	56 ± 14	71 ± 13	73 ± 16
$P_{(5,4)}$	51 ± 28	56 ± 20	26 ± 15[2]
Half-lives (h)			
18:3n−3	0.70 ± 0.03	0.86 ± 0.02	0.60 ± 0.02
20:5n−3	31 ± 6	36 ± 5	44 ± 6
22:5n−3	17 ± 2	17 ± 3	41 ± 12
22:6n−3	18 ± 8	13 ± 4	42 ± 12[2]

[1] $\bar{x}$ ± SD; $n = 10$. The kinetic rate constants and other parameters were analyzed as paired observations of data from subjects during the 2 experimental dietary periods.

[2,3] Significantly different from the beef-based diet: [2] $P < 0.05$, [3] $P < 0.01$.

constants of n−3 fatty acid metabolism for each subject during each of the 3 dietary periods. In some cases, a residual amount of isotope was present in the n−3 fatty acid compartment at the 0-h time point. These residues were the result of a prior dose of the label that the subjects had ingested. When present, the isotopes were integrated into the model so that the initial conditions reflected the availability of the labeled substrates at early time points.

A summary of the values for the parameters $L_{(I,J)}$, $P_{(I,J)}$, and $R_{(I,J)}$ and the $t_{1/2}$ values of the n−3 fatty acids across all diets are shown in **Table 5**. Turnover of n−3 fatty acids in these plasma compartments were calculated from the summation of the $L_{(x,J)}$ for each subject and are represented by the mean intervals for $t_{1/2}$. Mean flow rates, designated as $R_{(I,J)}$, pertain to the transfer of mass from compartment *J* to compartment *I* and are expressed in μg/h.

In general, the concordant values observed in several of the mean kinetic constants $L_{(I,J)}$ suggest that the experimental procedures were well performed and that the metabolism of the labeled fatty acids was consistent across the 3 trials (Table 5). However, differences in 2 of the mean rate constant coefficients was observed. The values for $L_{(5,4)}$ and $L_{(0,5)}$ were both lower while subjects subsisted on the fish-based diet. A lower value for $L_{(5,4)}$ (beef-based diet: 0.0266 ± 0.008 h; fish-based diet: 0.0086 ± 0.012 h) reflects a reduction in the quantity of the isotope that was transferred between the 22:5n−3 and 22:6n−3 compartments. Also, a lower value for $L_{(0,5)}$ (beef-based diet: 0.0935 ± 0.008 h; fish-based diet: 0.0358 ± 0.025 h) indicates a reduced turnover rate of 22:6n−3, as evidenced by an increase in the $t_{1/2}$ of 22:6n−3 in the plasma (beef-based diet: 13 ± 5 h; fish-based diet: 41 ± 12 h).

As shown in Table 5, a difference in the flow rate of a fatty acid $R_{(I,J)}$ results from an interaction of the individual kinetic constant (Table 4) and changes in the steady state concentration (Table 2), which are responsive to variations in dietary PUFAs (Table 1). During the period when subjects were maintained on the fish-based diet, a greater amount of 18:3n−3 exited the biosynthetic pathway $R_{(0,2)}$ (beef-based diet: $2.45 \times 10^5 \pm 9.37 \times 10^5$ μg/h; fish-based diet: $3.69 \times 105 \pm 1.54 \times 10^5$ μg/h; $P < 0.05$) compared with the beef-based dietary period. Similarly, a greater amount of mass of 20:5n−3 was transferred to 22:5n−3 $R_{(4,3)}$ (beef-based diet: 325 ± 68 μg/h; fish-based diet: 613 ± 154 μg/h; $P < 0.01$). This was primarily due to a greater dietary intake of 20:5n−3 during the fish-based diet (Table 1), which gave rise to a greater steady state concentration of 20:5n−3 (Table 2). However, during the same dietary period, there was a sharp reduction in the amount of mass of 22:5n−3 transferred to 22:6n−3 $R_{(5,4)}$ (beef-based diet: 751 ± 277 μg/h; fish-based diet: 244 ± 43 μg/h).

The mean values for the proportion of the n−3 fatty acid substrates directed toward biosynthesis $P_{(I,J)}$ are given in Table 5. The percentage of 18:3n−3 destined for synthesis of 20:5n−3, $P_{(3,2)}$, appeared to be lower during the fish-based dietary period than during the beef-based dietary period; however, this difference was not significant (self-selected diet: 0.2%; beef-based diet: 0.2%; fish-based diet: 0.1%). While the subjects were sustained on the fish-based diet, the percentage of 22:5n−3 that was utilized for synthesis of 22:6n−3, $P_{(5,4)}$, was lower than

that synthesized during the beef diet, which was consistent with changes in the kinetic constants (beef-based diet: 56%; fish-based diet: 26%).

DISCUSSION

This study was undertaken to determine the effects of dietary treatments with different fat contents on the kinetics of the in vivo biosynthesis of long-chain n−3 PUFAs in human subjects. Rate equations, which were fitted to the flux of the isotope tracer through the various plasma compartments, were used to determine the coefficients of the in vivo kinetic constants. Individual rate constants were calculated for each subject, and mean values were determined for the cohort during each of the dietary treatment periods. The steady state plasma fatty acid concentrations for each subject were used to estimate the amount of mass of the n−3 fatty acids transferred through the biosynthetic pathway. The data in Table 5 can be used to calculate the mean synthesis rates of n−3 fatty metabolism in these subjects during the different dietary trials. While subjects were sustained on the beef-based diet, the mean synthesis rate of 22:6n−3 from 22:5n−3 was ≈18 mg/d compared with ≈5 mg/d when they were consuming the fish-based diet. However, there was a higher rate of synthesis of 22:5n−3 from 20:5n−3 during consumption of the fish-based diet (beef-based diet: 7.8 mg/d; fish-based diet: 14.7 mg/d). The higher synthesis rate of 22:5n−3 was primarily due to a greater availability of substrate (20:5n−3) entering the plasma pool from the diet, which indicates that the homeostasis of plasma n−3 fatty acids is determined by the steady state concentrations of their precursors and the kinetic parameters.

Confirming our previous observation, we found that a high flow of 18:3n−3 exiting the biosynthetic pathway restricted the rate of long-chain PUFA biosynthesis across all 3 diets (15). A high rate of oxidation of 18:3n−3 in humans and a substantial transfer to the skin in rodents was reported by other investigators, which may account for the loss of isotope from the system (18–20). Also consistent with this observation are several reports indicating that 18:3n−3 does not support neural concentrations of 22:6n−3 as well as a preformed source of 22:6n−3 (21–24). The $t_{1/2}$ values of n−3 fatty acids in the plasma also indicated that 18:3n−3 was more rapidly removed from the plasma than were the other n−3 fatty acids, which is consistent with its rapid catabolism.

The individual in vivo rate constants calculated from data obtained during the self-selected and the beef-based dietary periods were not significantly different (Table 4). This finding indicates that these diets probably produce similar effects on n−3 fatty acid metabolism and suggests that the fatty acid composition of the beef-based and self-selected diets were similar. This was also noted for many parameters while subjects were maintained on the fish-based diet, with the exception of 2 rate constants. There was a 70% reduction in the value of the rate constant coefficient that regulated transfer of the isotope from the 22:5n−3 compartment to 22:6n−3 when the fish-based diet was compared with the beef-based diet. The fish-based diet had the effect of reducing the amount of mass of 22:5n−3 utilized for synthesis of 22:6n−3 by 68%.

The percentage of isotope that was transferred through the n−3 fatty acid compartments along the pathway was calculated for each intermediate, and these values were used to determine the efficiency of the biosynthetic processes (Table 5). Although a somewhat lower percentage of 18:3n−3 was used for the biosynthesis of 20:5n−3 during the fish-based diet than during the beef-based diet, the major effect of this diet was that the percentage of 22:5n−3 utilized for synthesis of 22:6n−3 (−46%) was much lower during the fish-based diet than during the beef-based diet.

While the subjects were subsisting on the fish-based diet, a significant reduction was observed in the turnover rate of 22:6n−3, as deduced from its $t_{1/2}$ value in the plasma compared with that during the beef-based diet (Table 5). Although the fish-based diet appeared to have a similar effect on the $t_{1/2}$ values of 22:5n−3, this difference was not significant in this group of subjects. In contrast, the turnover rate of 20:5n−3 was similar across all 3 diets. This contrast is interesting because the fish-based diet elevated the steady state concentrations of both 20:5n−3 and 22:6n−3, and the dilution of these tracers in their respective pools was similar. The concomitant increase in the concentrations of these fatty acids while the subjects remained on the fish-based diet might be expected to inhibit the rate of synthesis from their precursors. However, this only occurred for the synthesis rate of 22:6n−3 from 22:5n−3. This suggests that plasma 22:6n−3 concentrations may exhibit feedback inhibition, whereas plasma 20:5n−3 concentrations do not.

In the current study, it was shown that the plasma concentrations of n−3 fatty acids were responsive to dietary changes in 10 healthy subjects. The plasma concentrations of both 20:5n−3 and 22:6n−3 were significantly greater during the fish-based diet than during the beef-based diet. The effects of the fish-based diet on the kinetics of n−3 fatty acid metabolism appear to be centered on processes that inhibit the synthesis of 22:6n−3 from 22:5n−3. A feedback control mechanism responsive to the plasma concentration of 22:6n−3 may effect processes that regulate its own synthesis, thereby maintaining 22:6n−3 homeostasis during dietary changes.

We thank the clinical and technical support staff of the Laboratory of Clinical Studies, National Institute on Alcohol Abuse and Alcoholism, for their participation in this study. We appreciate the work of the staff in the metabolic kitchen at the Clinical Center of the National Institutes of Health, especially the dietitians Patti Riggs and Nancy Sebring. We especially recognize the excellent work of Brent Wegher in analyzing and reporting the isotope data and managing the database.

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