patient-oriented research

Compartmental analyses of plasma n-3 essential fatty acids among male and female smokers and nonsmokers

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Abstract **The effects of cigarette smoking on n-3 essential FA metabolism were studied in male and female subjects by fitting the concentration-time curves of the d_5-labeled plasma fatty acids (FAs) originating from a dose of d_5-18:3n-3 to a compartmental model of n-3 FA metabolism. For 3 weeks, female (smokers, n = 5; nonsmokers, n = 5) and male (smokers, n = 5; nonsmokers, n = 5) subjects subsisted on a beef-based diet. Beginning in the third week, subjects received a dose of d_5-18:3n-3 ethyl ester (1 g). Plasma FAs were analyzed using gas chromatography (GC) and GC-mass spectrometry, and the kinetic rate parameters were determined from the concentration-time curves for d_5-18:3n-3, d_5-20:5n-3, d_5-22:5n-3, and d_5-22:6n-3. Women smokers had a 2-fold greater percent of dose in plasma (5.8% vs. 2.9%; $P < 0.01$) and a higher fractional rate constant coefficient for formation of d_5-22:6n-3 from d_5-22:5n-3 (0.03 h^{-1} vs. 0.01 h^{-1}; $P < 0.01$), compared with nonsmokers. Male smokers had elevated total plasma n-3 FAs, more-rapid turnover of 18:3n-3 (13.3 mg/day^{-1} vs. 4.3 mg/day^{-1}; $P < 0.001$), a disappearance rate of d_5-20:5n-3 that was both delayed and slower (0.001 h^{-1} vs. 0.012 h^{-1}; $P < 0.05$), and a percentage of d_5-20:5n-3 directed into formation of d_5-22:5n-3 (99% vs. 61%; $P < 0.03$) that was greater compared with nonsmokers. Smoking increased the bioavailability of n-3 FAs from plasma, accelerated the fractional synthetic rates, and heightened the percent formation of some long-chain n-3 PUFAs in men and women.**—Pawlosky, R. J., J. R. Hibbeln, and N. Salem, Jr. **Compartmental analyses of plasma n-3 essential fatty acids among male and female smokers and nonsmokers.** ***J. Lipid Res.*** **2007.** 48: **935–943.**

Supplementary key words linolenic acid • docosahexaenoic acid • isotope tracer • mass spectrometry • humans • kinetics • controlled diet

Cigarette smoking has been shown to elevate, through a diverse set of free radical-mediated actions, lipid peroxide levels in tissues for which oxidation products of polyunsaturated fatty acids (PUFAs), as measured in the circulation, are indicators of oxidative stress (1, 2). Because PUFAs are highly susceptible to free radical oxidation, habitual smoking may decrease the amount of PUFAs in plasma (3) and red blood cells (4). Because of the constant demand for PUFAs in the replenishing of cell membranes, the making of new cells, and the counteracting of losses that occur through peroxidation, smoking tobacco may indirectly enhance the synthesis of long-chain PUFAs through its additional contribution to the system's oxidative load.

α-Linolenic acid, 18:3n-3, is the most abundant n-3 essential fatty acid (FA) in the typical North American diet. It is found in small amounts in many plant seed oils, in contrast to the more abundant n-6 essential FA, linoleic acid (5). Through a sequential series of elongation and desaturation steps, the long-chain PUFAs, eicosapentaenoic acid (20:5n-3), docosapentaenoic acid (22:5n-3), and docosahexaenoic acid (22:6n-3), are synthesized by liver hepatocytes from 18:3n-3 and are delivered to body tissues in plasma lipoproteins (6). In neural tissues, 22:6n-3 is the most abundant n-3 PUFA, and high concentrations are necessary for optimal brain development and function (7).

Observational studies that examine the effects of smoking on n-3 FA status without rigorous control for dietary intake can be confounded because of lower intake of n-3 FAs by both men and smokers (4, 8). Smoking's direct effects on n-3 FA status or the in vivo kinetics of n-3 FA metabolism have not been studied using either quantitative tracer methods or compartmental modeling procedures in subjects consuming a diet that is controlled for n-3 FA intake. Previously, we described a compartmental model of n-3 FA metabolism that was used for the determination of several rate constant parameters relative to long-chain PUFA biosynthesis (9). We now extend this analysis to examine the effects of smoking on the kinetics of n-3 FA metabolism in comparing male and female smoking (S) and nonsmoking (NS) subjects on a metabolically controlled diet. Kinetic rate parameters were

Manuscript received 17 July 2006 and in revised form 16 January 2007.

Published, JLR Papers in Press, January 17, 2007.
DOI 10.1194/jlr.M600310-JLR200

Abbreviations: ANCOVA, analysis of covariance; AUC, area under the concentration-time curve; CV, coefficient of variance; NS, nonsmoking subject; S, smoking subject.

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determined for each subject from the concentration-time curves of the experimentally determined d_5-labeled FAs 18:3n-3, 20:5n-3, 22:5n-3, and 22:6n-3 in plasma, which originated from an oral dose of d_5-18:3n-3, using compartmental modeling procedures.

METHODS

Subjects

All subjects were rigorously evaluated at the clinical research unit of the National Institute on Alcohol Abuse and Alcoholism (NIAAA) at the National Institutes of Health Clinical Center in Bethesda, Maryland. Subjects were evaluated by physical examination, extensive clinical laboratory testing, including hepatitis A, B, and C testing, HTLV3 antibody test, electro-cardiogram, chest X-ray, nurse and social worker interviews, and structured interview to evaluate psychiatric diagnoses (SCID). A psychiatrist and a social worker blind rated SCIDs to obtain medical and psychiatric diagnoses under the supervision of a senior physician. Subjects were included as healthy volunteers if they were male or female aged 18–65 years and if they were judged to be reliable in maintaining the dietary requirements of the protocol. Nonsmoking subjects did not smoke or use tobacco products within the last two years. Male and female smokers reported smoking on average 24 cigarettes d^{-1} (range: 15–40). The absence of illicit drug use was confirmed by random breath alcohol testing and urine testing throughout the protocol.

Exclusion criteria included: any major medical problems, including, hepatic, endocrine, and metabolic disorders, a history of head trauma, seizures, or prolonged loss of consciousness, a lifetime history of a major psychiatric diagnosis, or abnormal clinical laboratory findings. Subjects were excluded if they consumed more than the equivalent of two glasses of beer or wine per day, used prescription medications within the last month, including birth control pills or over-the-counter medications including aspirin, ibuprofen, acetaminophen, antihistamines, and topical steroids. Subjects were excluded who had persistent use of vitamins E or C, multivitamins, lipid supplements, herbal or home remedies with unknown composition, home cures, or unusual vitamin use or other unusual dietary habits. The study required eating red meat, turkey, and yogurt, and thus excluded vegetarians; also excluded were patients with allergies to foods making up this diet, and those with lactose intolerance. Subjects were excluded who had donated blood within the previous three months or who were currently pregnant or had imminent plans to become pregnant. All subjects provided written informed consent for all clinical procedures under approval of the NIAAA Institutional Review Board under protocol #92-AA-0194.

Dietary protocol

Details of the metabolic dietary control may be found elsewhere (9). Briefly, subjects received a beef-based diet for 21 days. Based on each subject's *ad lib* food records, the metabolic diet was adjusted to maintain less than a 1 kg body weight change. Subjects were counseled not to eat or drink any foods or beverages other than those provided by the research kitchen. Food sources were consistent throughout the study. Beef, olive oil, and butter provided the major sources of dietary fat. With an average of 2,500 calories, the protein, fat, and carbohydrate of the diet were 94, 90, and 334 g/day respectively. The fatty acyl content of the diet was analyzed directly, and estimates of the mean n-3 FA intake for the groups were determined based on each subject's own energy requirements (**Table 1**). The average intake of 18:3n-3 was 0.2% of calories.

Administration of the d_5-FA ethyl ester and subsequent blood draws occurred during the final week of the study period, and details of the procedures have been reported previously (10). Briefly, subjects were given an overnight fast and then received 1 g of d_5-α-linolenate ethyl ester (d_5-17, 17, 18, 18, 18-18:3n-3; Cambridge Isotope Labs, Woburn, MA) blended into low-fat (1% fat) yogurt prior to a morning meal. Because the overall bioavailability of n-3 FAs as either an ethyl ester or a triglyceride appear to be similar, the isotope was administered as an FA ethyl ester (10). With the exception of the 8 h sample, blood (40 ml) was drawn under fasting conditions from the forearm, at baseline and at 8, 24, 48, 72, 96, and 168 h. The platelet-poor plasma was separated by centrifugation (1,800 *g* for 10 min) and frozen at −80°C until analysis.

Plasma lipid extraction and gas chromatographic analysis

Laboratory analytical procedures have been described previously (9). Briefly, plasma (0.2 ml) lipid extraction was carried out using a 1 ml solution of chloroform-methanol (2:1), and the FA methyl esters were prepared using a 14% solution of boron trifluoride in methanol. The methyl esters were extracted into hexane, concentrated to 50 μl under nitrogen, and analyzed on a model HP-5890 gas chromatograph with flame ionization detector (Agilent; Wilmington, DE). Concentrations of individual FAs were calculated using the peak area counts in comparison with the internal standard.

Sample preparation for mass spectral analysis

From a 0.1 ml portion of the lipid extract, the chloroform was evaporated under nitrogen, and the lipids were hydrolyzed in a 5% solution of potassium hydroxide in methanol as previously described (11). The FAs were reacted with 150 μl of the pentafluorobenzyl (PFB) reagent (acetonitrile-diisopropylethylamine-pentafluorobenzyl bromide; 1,000:100:1; v/v/v) at 60°C for 12 min, and cooled, and the solvent-reagent was evaporated under a steam of nitrogen and resuspended in 100 μl of hexane.

Mass spectral analysis

Gas chromatography-mass spectrometry (GC-MS) conditions were as described previously by Pawlosky, Sprecher, and Salem (11). Samples (1 μl) were analyzed on an Agilent 5989 quadrapole GC-MS using a 60 m free fatty acid polar bonded phase capillary column (0.25 mm I.D., film thickness 0.25 mm; Quadrex Corp., New Haven, CT). Data were acquired in the selected ion mode, monitoring the M-PFB anion of the FAs, and converted to the absolute quantity of the d_5-metabolite by reference to the concentration of an internal standard using an experimentally determined response factor for each FA.

TABLE 1. Estimated daily n-3 fatty acid intake for male and female subjects maintained on a beef-based diet

Fatty Acid	Male	Female
	g/day^{-1}	
18:3n-3	0.59 ± 0.02	0.47 ± 0.02
20:5n-3	0.036 ± 0.001	0.029 ± 0.001
22:5n-3	0.075 ± 0.004	0.061 ± 0.003
22:6n-3	0.012 ± 0.001	0.009 ± 0.001

Fatty acid values were determined from direct analyses of the lipid composition of the food from the control diet and intake values (in g/day^{-1}) are based on ingestion of 2,500 and 2,200 Kcal for male and female subjects, respectively.

Compartmental model

The compartmental model for n-3 FA metabolism has been validated previously in adult human subjects (9). Briefly, this model of n-3 FA metabolism was developed from the concentration-time curves of the labeled FAs and concentrations of endogenous plasma FAs using WinSAAM (Windows Simulation and Analysis Modeling) software (http://www.winsaam.com). Although the liver hepatocyte is a main site for the biosynthesis of the 20- and 22-carbon PUFAs from 18:3n-3, the rate constants determined from the model represent kinetics of d_5-FAs from their plasma pool concentrations alone and may only indirectly reflect liver metabolism.

Fractional transfer rates, flow rates, percents, turnover, and errors

The fractional rate constant coefficient, $\mathbf{L_{(I,J)}}$ represents the fraction of substrate that is transferred from the substrate compartment, **J**, to the product compartment, **I**. The units are in h^{-1}. Here, $\mathbf{L_{(I,J)}}$ represents an assemblage of several independent enzymatic processes, each having a separate rate constant, for which no intermediates were isolated. The rate of flow, $\mathbf{R_{(I,J)}}$, from **J** to **I** is obtained by multiplying the mass $\mathbf{(M_J)}$ of unlabeled FA in compartment **J** by $\mathbf{L_{(I,J)}}$ and is given in $\mu g\ h^{-1}$. The percentage of isotope transferred from **J** to **I** is given as $\mathbf{P_{(I,J)}}$ and is the fraction of isotope that stays in the metabolic pathway, as opposed to isotope taken up by tissues or irreversibly lost from the compartment. The half-life ($\mathbf{t_{1/2}}$) of the n-3 FA in the plasma was calculated from the sum of the fractional transfer rates leaving the compartment: $\mathbf{t_{1/2} = ln2 / \Sigma L_{(I,J)} + L_{(0,J)}}$. Initial estimates of the fractional transfer rates and percentages of isotope transfer were derived using the model from the concentration-time curves generated from the experimental data. Data points were weighted by assigning a fractional standard deviation of 0.1 to each measurement, which is consistent with the precision of the methods for the determination of labeled and unlabeled FAs in the plasma. Values assigned to 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 using the program's iterative nonlinear least-squares routine. The error model included the assumptions of independence, constant variance, and normal distribution about zero. Variances for the determined parameters are reported as standard deviation (SD), or coefficient of variance (CV) where appropriate.

Model illustration and rate equations

The model consisted of five compartments for which isotope data were obtained (**Fig. 1**). Compartment 1 represents the dose of d_5-18:3n-3 and its absorption through the gastrointestinal (GI) tract. Compartments 2 through 5 denote plasma pools of the n-3 FA (18:3n-3, 20:5n-3, 22:5n-3, and 22:6n-3). Arrows connecting the five compartments indicate flow along the path. Losses of isotope from the system are indicated by arrows drawn from each compartment toward the bottom of the figure. The rate equations are defined by a set of differential equations corresponding to the flux of the d_5-FA substrates through their respective compartments.

Limits, constraints, and statistical analysis

Because the FA composition of the diet had been experimentally determined and the caloric intake of the diet had been adjusted to the energy requirements of each subject, the daily n-3 FA intake for each subject could be estimated so that upper and lower n-3 FA limits were assigned.

Plasma steady-state FA concentrations were determined for each subject at each blood sampling time point, and because the

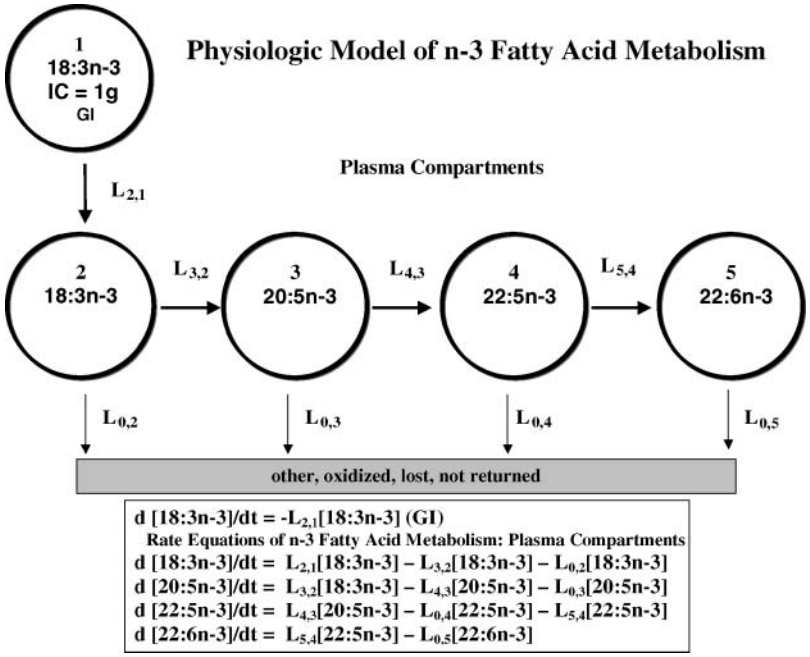

Fig. 1. Conceptual model of n-3 fatty acid (FA) metabolism. The circles represent separate FA compartments. Compartment 1 represents administration of the isotope (1 g) and absorption through the gastrointestinal tract. Four compartments (2 through 5) represent plasma FA compartments following along successive steps of desaturation and elongation of the tracer. The fractional transfer rates, $L_{(I,J)}$, are rate parameters derived from the model-fitted experimental data. The differential equations used in determining individual rate parameters are given in the boxed area.

standardized diet minimized the variability of n-3 FA intake, there was little difference (± 5%) in the concentrations of the individual n-3 FAs within the plasma of a given subject over this period. Therefore, the mean concentration of each plasma n-3 FA from each subject was used to approximate the steady-state mass of the endogenous substrate **(M_J)** available for biosynthesis. These values were held constant.

For statistical analyses, data were imported into StatView 5 (SAS Institute; Cary, NC). Initially, plasma FA concentrations and model-derived rate parameters from male and female subjects were pooled and analyzed for the effects of smoking using unpaired *t*-tests. A *P* value of 0.05 or lower was considered significant. The data were also analyzed with respect to gender in unpaired *t*-tests. A *P* value of 0.05 or lower was considered significant. Where differences were observed in either plasma FA concentrations or kinetic parameters, the findings were further analyzed in an analysis of covariance (ANCOVA) model for possible confounding influences of age or body mass. Additionally, these data sets were analyzed in multiple linear regression models to assess interactions of age and body mass on plasma FAs or kinetic parameters. A *P* value of 0.05 or lower was considered significant.

RESULTS

Subject characteristics

Twenty subjects (10 male and 10 female) completed the study. The medium age of male subjects was 25.2 years (NS) (range, 20–44; CV, 0.33) and 33.3 years (S) (range, 27–60; CV, 0.38), and that of female subjects was: 27.6 years (NS) (range, 22–37; CV, 0.10) and 38.4 years (S) (range, 32–43; CV, 0.25; $P < 0.01$). The mean body mass index (BMI) for men was 24 (NS) (range, 19–26; CV, 0.11) and 27 (S) (range, 21–31; CV, 0.11; $P < 0.01$). The mean BMI for women was 27 (NS) (range, 22–41; CV, 44) and 24 (S) (range, 20–27; CV, 11). The rotational menu restricted dietary fat intake; however, the caloric content of each subject's diet was adjusted to maintain his or her body weight. The average caloric intake for men consuming the control diet was 2,664 Kcal (NS) (range, 1,960–3,340) and 2,536 Kcal (S) (range, 1,823–3,323) and that for women was 2,172 (NS) (range, 1,876–2,400) and 2,056 Kcal (S) (range, 1,480–2,370).

The n-3 FA intake was estimated using values obtained from a direct determination of the lipid content of the food. These values are reported in amounts that take into consideration energy density differences of the diets for male and female subjects (Table 1). The mean concentrations of plasma n-3 FAs obtained from blood samples drawn over the third week of the dietary period are given in **Table 2**. As a group, both male and female smokers had higher concentrations of plasma n-3 FAs compared with nonsmokers. These values, however, were strongly influenced by the higher plasma FA concentrations observed in male smokers as compared with female subjects.

Appearance of d_5-18:3n-3 in plasma and area-under-the-curve values

Two 18:3n-3 compartments were included in the model, one for isotope administration and absorption through the GI tract and the second for the appearance of the FA in the plasma. To perform proper kinetic analysis, blood collection began 8 h from the time of dosing to assess accurate concentrations of labeled 18:3n-3 entering the plasma as well as quantification of the subsequent labeled FAs appearing there (12). Bioavailability of the precursor in plasma was calculated as percent of dose using isotope values obtained from these two compartments. The model carried with it an assumption that fat absorption was essentially complete for all subjects (98%) and that therefore, variances in the amount of labeled precursor appearing in plasma represent differences in bioavailability among subjects. The $P_{(2,1)}$ parameter (**Table 3**) returns the value for percent of dose of d_5-18:3n-3 appearing in plasma over 168 h from the model estimations. Among women smokers, this was significantly greater (5.8%) compared with nonsmokers (2.9%), an outcome that is reflected in the composite time course curves for d_5-18:3n-3 (**Fig. 2A**). Plasma deuterated FA values may also be expressed in terms of area under the concentration-time curve (AUC) over the time course (here expressed in total amount of label, in mg/hour ± SD of d_5-18:3n-3). The mean AUC values for all groups were: 85 ± 17; 89 ± 8; 101 ± 21; and 118 ± 19 mg/hour for male S and NS and female S and NS subjects, respectively. There were no differences in the AUC values; however, the AUC does not inform on either the rate of appearance or disappearance of the isotope plasma, nor is it descriptive of the contour of the concentration-time curve.

n-3 FA plasma concentration-time curves

Figure 2 and **Figure 3** give illustrations of the composite time course curves of labeled FAs in plasma of both female and male subjects. Figure 3B illustrates the time course curve for d_5-20:5n-3 in male smokers and nonsmokers.

TABLE 2. Mean plasma n-3 fatty acid concentrations for male and female smoking and nonsmoking subjects

	Male and Female			Male			Female		
Fatty Acid	Nonsmokers (n = 10)	Smokers (n = 10)	*P*	Nonsmokers (n = 5)	Smokers (n = 5)	*P*	Nonsmokers (n = 5)	Smokers (n = 5)	*P*
	μg/ml			*μg/ml*			*μg/ml*		
18:3n-3	7.3 (2.8)[a]	11.3 (3.7)	0.01	6.9 (2.7)	13.1 (4.5)	0.01	7.9 (2.9)	9.4 (1.2)	ns[b]
20:5n-3	9.1 (3.8)	13.9 (6.2)	0.03	8.4 (1.1)	16.5 (7.9)	0.03	9.9 (5.3)	11.3 (2.6)	ns
22:5n-3	10.9 (2.6)	14.5 (5.2)	0.04	10.2 (1.4)	16.8 (6.6)	0.02	11.9 (3.3)	12.1 (1.7)	ns
22:6n-3	27.1 (8.5)	37.9 (13.2)	0.05	24.8 (7.4)	40.8 (8.0)	0.04	29.9 (9.6)	34.6 (9.4)	ns

The mean values (in μg/ml) are presented for blood samples obtained over the final week of the study period.
[a] Standard deviations of the mean.
[b] Not significant.

TABLE 3. Mean P-parameter values (percent transfer values) for male and female smoking and nonsmoking subjects

	Male and Female			Male			Female		
P(I,J)	Nonsmokers (n = 10)	Smokers (n = 10)	*P*	Nonsmokers (n = 5)	Smokers (n = 5)	*P*	Nonsmokers (n = 5)	Smokers (n = 5)	*P*
	% (cv)			*% (cv)*			*% (cv)*		
P(2,1)	3.5 (0.14)[a]	5.3 (0.20)	0.004	4.0 (0.13)	4.9 (0.16)	ns	2.9 (0.09)	5.8 (0.18)	0.009
P(3,2)	0.22 (0.37)	0.26 (0.39)	ns	0.28 (0.27)	0.32 (0.31)	ns	0.17 (0.13)	0.20 (0.42)	ns
P(4,3)	67.4 (0.24)	99.7 (0.12)	0.01	61.7 (0.28)	97.5 (0.20)	0.03	79.5 (0.28)	100 (1.7)	0.14
P(5,4)	45.0 (0.43)	53.8 (0.26)	ns	28.0 (0.54)	45.5 (0.47)	ns	66.1 (0.27)	62.1 (0.27)	ns

[a] Values for each group are expressed as the percent of the total flux of each tracer transferred between compartments. Numbers in parentheses in the left column refer to specific compartments. For example, in P(2,1) the 1 represents isotope dosage (d5-18:3n-3) transferred through the gastrointestinal tract and the 2 is the d5-18:3n-3 that appeared in the plasma over the time course. cv, coefficient of variance; ns, not significant.

Male smokers had both a delayed and less rapid rate of disappearance of the labeled FA in plasma compared with nonsmokers. Plasma disappearance rates of d_5-20:5n-3 were quantified as the fractional rate constant coefficient, $L_{(0,3)}$, given in **Table 4** for nonsmokers (0.012 h^{-1}) and smokers (0.0014 h^{-1}; $P < 0.05$). By contrast, the rate constant coefficient for disappearance of d_5-18:3n-3, $L_{(0,2)}$, from plasma was greater among male smokers (12.7 h^{-1}) compared with nonsmokers (8.8 h^{-1}; $P < 0.04$) (see Fig. 3A, Table 4). The dissimilarity resulted in an accelerated turnover rate of 18:3n-3 among smokers [$R_{(0,1)}$ 1,340 mg/day^{-1}] compared with nonsmokers [$R_{(0,1)}$ 429 mg/day^{-1}] (**Tables 5 and 6**). The 3-fold greater throughput of 18:3n-3 among smokers strongly suggests that smoking acts to stimulate n-3 FA entrance into the plasma, thereby increasing its bioavailability to tissues. We note that this value was well above estimated dietary intake amounts for 18:3n-3 for male subjects (Table 1) but is consistent with the

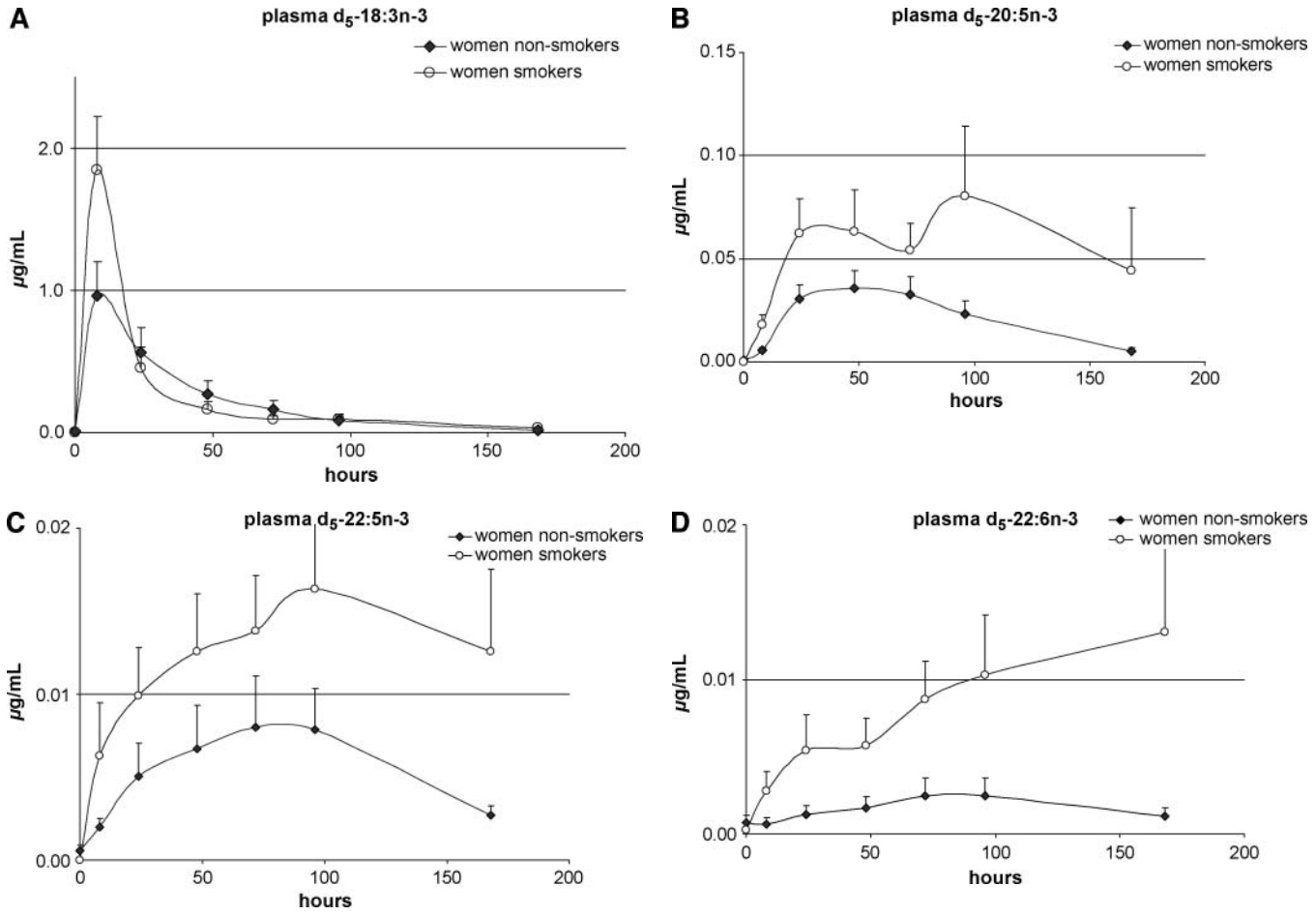

Fig. 2. Graphical comparison of mean values of the plasma concentration (μg/ml^{-1}) time curves for d_5-labeled FAs in women smokers (n = 5) and nonsmokers (n = 5) following a 1 g oral dose of d_5-18:3n-3 ethyl ester. A: d_5-18:3n-3. In women smokers, significantly greater amounts of the label appeared in the plasma (as determined by the area under the curve calculation), representing a higher percentage of available dose compared with nonsmokers (see text for group values). B: d_5-20:5n-3. C: d_5-22:5n-3. D: d_5-22:6n-3. There is a sustained increase of plasma d5-22:6n-3 in women smokers over the time course compared with nonsmokers. Error bars represent SD of mean values.

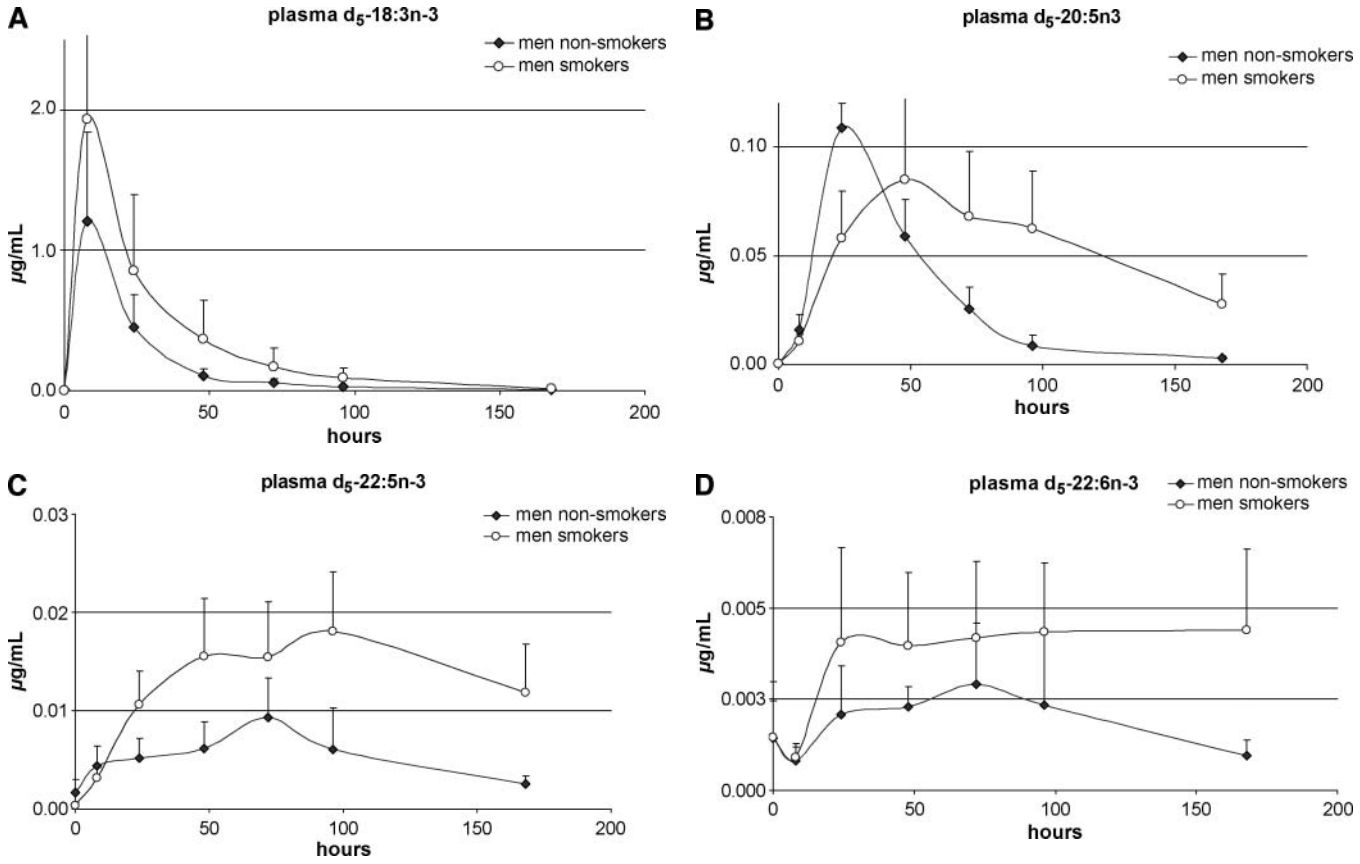

Fig. 3. Graphical comparison of mean values of the plasma concentration ($\mu g/ml^{-1}$) time curves for d_5-labeled FAs in men smokers (n = 5) and nonsmokers (n = 5) following a 1 g oral dose of d_5-18:3n-3 ethyl ester. A: d_5-18:3n-3. Men smokers had a disappearance rate of the label from the plasma, $L_{(0,2)}$, representing a greater percentage of turnover of the label compared with nonsmokers. B: d_5-20:5n-3. Smokers had both a delayed and prolonged disappearance rate of the label from the plasma compared with nonsmoking subjects. C: d_5-22:5n-3. D: d_5-22:6n-3. Error bars represent SD of mean values.

accelerated turnover rate. The proposition of accelerated throughput of 18:3n-3 is supported by higher plasma concentrations of n-3 FA among both male and female smokers compared with nonsmokers (Table 2) as well as the 2-fold greater percentage of d_5-18:3n-3 that appeared in the plasma of female smokers, as discussed above (Fig. 2A).

Among both male and female smokers, the fractional rate constant coefficient for transfer of d_5-22:5n-3 to d_5-22:6n-3, $L_{(5,4)}$, was greater compared with nonsmokers (Table 4). Among women who smoked, the rate constant coefficient was 3-fold greater compared with nonsmokers (0.028 vs. 0.009; $P < 0.03$) eliciting a 3-fold greater rate of synthesis of 22:6n-3 from 22:5n-3 (20 vs. 6.2 mg/day^{-1}; $P < 0.04$) (Table 6). The difference in magnitude between these coefficients is reflected in the positive incline of the curves in the composite concentration-time curves for d_5-22:6n-3 (Fig. 2D). Notably, among female smokers, the appearance rate of d_5-22:6n-3 continued to increase throughout the 168 h (Fig. 2D) compared with that of male smokers (Fig. 3D).

TABLE 4. Mean fractional rate constant coefficients for male and female smoking and nonsmoking subjects

	Male and Female			Male			Female		
l(I,J)	Nonsmokers	Smokers	*P*	Nonsmokers	Smokers	*P*	Nonsmokers	Smokers	*P*
	h^{-1} (*cv*)			h^{-1} (*cv*)			h^{-1} (*cv*)		
L(0,2)	9.8 (0.25)	11.6 (0.15)	ns	8.8 (0.15)	12.7 (0.21)	0.04	10.7 (0.31)	10.5 (0.21)	ns
L(0,3)	0.008 (0.62)	0.001 (0.47)	0.02	0.012 (0.49)	0.0014 (0.17)	0.05	0.004 (0.76)	0.0003 (1.1)	ns
L(0,4)	0.24 (0.41)	0.28 (0.37)	ns	0.28 (0.33)	0.32 (0.45)	ns	0.02 (0.54)	0.021 (0.58)	ns
L(0,5)	0.048 (0.48)	0.042 (0.27)	ns	0.028 (0.25)	0.048 (0.22)	0.09	0.069 (0.44)	0.036 (0.47)	ns
L(3,2)	0.002 (0.41)	0.003 (0.31)	ns	0.002 (0.40)	0.004 (0.26)	ns	0.001 (0.14)	0.002 (0.32)	ns
L(4,3)	0.013 (0.22)	0.013 (0.21)	ns	0.012 (0.23)	0.015 (0.18)	ns	0.014 (0.23)	0.0012 (0.24)	ns
L(5,4)	0.019 (0.25)	0.024 (0.15)	0.01	0.009 (0.33)	0.02 (0.33)	0.10	0.009 (0.16)	0.028 (0.31)	0.03

Numbers in parentheses in left column identify particular plasma compartments for which mean values of the coefficients are listed (see Fig. 1 for compartment numerical assignments). Values having a zero in parentheses indicate loss of isotope from the system through compartment J.

TABLE 5. Mean plasma n-3 fatty acid total amounts for male and female smoking and nonsmoking subjects

Fatty Acid	Male and Female		P	Male		P	Female		P
	Nonsmokers	Smokers		Nonsmokers	Smokers		Nonsmokers	Smokers	
	μg (cv)			μg (cv)			μg (cv)		
$M_{(2)}$ 18:3n-3	20,387 (0.14)	36,173 (0.22)	0.01	21,960 (0.09)	45,357 (0.18)	0.02	18,813 (0.16)	26,988 (0.16)	0.1
$M_{(3)}$ 20:5n-3	26,555 (0.23)	43,142 (0.23)	0.05	23,454 (0.18)	58,132 (0.20)	0.01	29,657 (0.19)	28,153 (0.12)	ns
$M_{(4)}$ 22:5n-3	28,897 (0.08)	44,181 (0.23)	0.03	30,181 (0.12)	59,273 (0.16)	0.01	27,613 (0.08)	29,089 (0.05)	ns
$M_{(5)}$ 22:6n-3	81,274 (0.12)	126,235 (0.18)	0.01	74,945 (0.11)	147,236 (0.17)	0.02	87,602 (0.12)	105234 (0.13)	ns

Values (μg) represent mean values of total plasma amounts of each fatty acid from groups of subjects determined over the final week of the study period. For purposes of compartmental analyses, total amounts of individual fatty acids from each subject were used as representative substrate masses available for synthesis.

Male smokers had a 30% increase in the percent conversion of d_5-20:5n-3 to d_5-22:5n-3 compared with nonsmokers (Table 3), which was reflected in the greater rate of synthesis of 22:5n-3 from 20:5n-3 among smokers (6.2 vs. 19.9 mg/day^{-1}; $P < 0.03$) (Table 6). Among females, there were no differences in the values of the fractional synthetic rate for conversion of 20:5n-3 to 22:5n-3; however, there was a tendency for an increase in the percent conversion of 20:5n-3 to 22:5n-3. There were no substantial differences noted in the half-lives of n-3 FAs between the groups other than a trend toward a shorter half-life of 18:3n-3 among male smokers and a trend toward a longer half-life of 20:5n-3 among female smokers compared with their NS controls (**Table 7**).

DISCUSSION

To our knowledge, no detailed studies examining the effects of smoking on the kinetics of essential FA metabolism in human subjects subsisting on a controlled diet have been reported. We undertook an examination of the effects of cigarette smoking on the in vivo kinetics of n-3 essential FA metabolism among male and female subjects maintained on a rigorously controlled diet that was designed to enhance biosynthesis of long-chain PUFAs using a quantitative isotopic tracer technique in conjunction with a compartmental modeling procedure.

Although in this study female smokers were older, they also tended to be leaner than nonsmokers, whereas male smokers had greater adiposity than nonsmokers. To assess whether age and/or adiposity had possible confounding influences on plasma FA concentrations or kinetic parameters, positive findings were subjected to further statistical analyses using an ANCOVA and multiple linear regression models. No direct effects of age or adiposity on plasma FA concentrations or kinetic parameter outcomes were detected. Neither age nor adiposity was found to correlate with those effects of smoking in this group of subjects, and there were no apparent significant interactions between these parameters and cigarette smoking. We did observe that smoking possibly affected an increase in the bioavailability of n-3 essential FAs from plasma and altered several rate parameters that appeared to both accelerate and heighten the synthesis of some long-chain n-3 PUFAs in men and women.

The compartmental model of n-3 FA metabolism was previously validated in subjects subsisting on various diets (13). Inasmuch as the model makes use of FA data from the plasma alone, the derived kinetic parameters may only indirectly reflect liver synthesis of long-chain PUFAs. In vivo rate constant coefficients for successive steps of long-chain PUFA biosynthesis were determined for each subject using the concentration-time curves of the d_5-labeled FAs, and plasma steady-state FA amounts were used as estimations of total mass of n-3 FA substrate available for transfer through the pathway. Because the number of subjects in this study was small, several perceived differences in rate parameters approached but did not reach significance.

Gender-specific differences in the metabolism of n-3 FAs have been previously reported (14, 15), where it has been noted that a greater percent of 22:5n-3 was utilized

TABLE 6. Mean values for plasma synthesis and disappearance rates for male and female smoking and nonsmoking subjects

R(I,J)	Male and Female		P	Male		P	Female		P
	Nonsmokers	Smokers		Nonsmokers	Smokers		Nonsmokers	Smokers	
	mg d^{-1} (cv)			mg d^{-1} (cv)			mg d^{-1} (cv)		
R(0,1)	445 (0.27)	1030 (0.26)	0.01	428 (0.16)	1,340 (0.15)	0.001	462 (0.43)	719 (0.36)	0.44
R(0,3)	4.5 (0.83)	0.19 (1.11)	0.09	8.5 (0.60)	0.28 (1.14)	0.07	1.5 (0.96)	0.14 (1.18)	0.24
R(0,4)	14 (0.48)	27 (0.68)	0.14	16.4 (0.54)	37.5 (0.51)	0.24	11.9 (0.58)	16 (0.58)	0.72
R(0,5)	114 (0.42)	136 (0.61)	0.71	56 (0.59)	180 (0.56)	0.07	173 (0.26)	92 (0.75)	0.36
R(3,2)	1.3 (0.24)	2.8 (0.56)	0.06	1.3 (0.27)	4.4 (0.46)	0.13	0.50 (0.20)	1.23 (0.32)	0.09
R(4,3)	7.8 (0.43)	14.0 (0.31)	0.21	6.2 (0.22)	19.9 (0.30)	0.03	9.5 (0.41)	8.1 (0.33)	0.66
R(5,4)	7.3 (0.47)	15.5 (0.47)	0.24	8.4 (0.63)	29 (0.51)	0.12	6.2 (0.26)	20 (0.49)	0.04

Numbers in parentheses in the left column identify specific compartments described in Fig. 1. Zero-numbered compartments such as R (0,1) indicate the rate of disappearance of the fatty acid from plasma. Here, R (0,1) represents turnover rate for 18:3n-3 through the system.

TABLE 7. Half-lives of n-3 fatty acids in plasma for male and female smoking and nonsmoking subjects

	Male and Female			Male			Female		
Fatty acid	Nonsmokers	Smokers	*P*	Nonsmokers	Smokers	*P*	Nonsmokers	Smokers	*P*
	hours (cv)			*hours (cv)*			*hours (cv)*		
$t_{1/2}$ 18:3n-3	0.9 (0.27)	0.75 (0.19)	ns	0.9 (0.19)	0.72 (0.00)	0.090	0.86 (0.42)	0.79 (0.35)	ns
$t_{1/2}$ 20:5n-3	39 (0.23)	62 (0.25)	ns	38 (0.33)	54 (0.25)	ns	41 (0.09)	70 (0.23)	0.06
$t_{1/2}$ 22:5n-3	27 (0.24)	18 (0.40)	ns	22 (0.24)	19 (0.40)	ns	36 (0.38)	17 (0.26)	ns
$t_{1/2}$ 22:6n-3	30 (0.41)	17 (0.33)	ns	39 (0.48)	17 (0.23)	ns	20 (0.40)	19 (0.46)	ns

$t_{1/2}$, half-life.

for synthesis of 22:6n-3 among women compared with men (14). In this study, as well, we observed that women smokers and nonsmokers tended to have greater percent conversions of 22:5n-3 to 22:6n-3 compared with male subjects (Table 3; Figs. 2C, D and 3C, D).

Cigarette smoking is well known to increase formation of oxygenated PUFA (1, 2) and because smoking has also been associated with lower amounts of serum PUFA phospholipids, it has been suggested that cigarette smoking may influence processes related to the absorption, synthesis, or metabolism of PUFAs (3). Among men and women subsisting on the control diet, cigarette smoking did not appear to have a negative impact on PUFA absorption, because there were greater concentrations of n-3 FAs in the plasma of male smokers compared with nonsmokers and nonsignificant elevations of plasma n-3 FAs among female smokers compared with nonsmokers. This suggested that cigarette smoking may act to increase transport of PUFA into the plasma, a supposition that was supported by observations among women smokers, where there was a greater influx of the dosed d_5-18:3n-3 into the plasma compared with nonsmokers. The difference in plasma influx of d_5-18:3n-3 in male smokers was not as great as that in female smokers when compared with their NS controls (Table 3). However, to sustain plasma 18:3n-3 concentrations with their rapid turnover rate in male smokers ($L_{(0,2)}$; Table 4), it was estimated that the 18:3n-3 influx in smokers would need to be three times that of nonsmokers (Table 6).

The compartmental model was used to determine the efficiency of individual steps in the biosynthetic processes by estimating the percentage of isotope transferred between the n-3 FA compartments along the pathway. Cigarette smoking was not found to increase the percent utilization of 18:3n-3 for formation of 20:5n-3 ($P_{(3,2)}$; Table 3) in either men or women. However, among both male and female smokers, a higher percentage of 20:5n-3 was utilized for 22:5n-3 synthesis ($P_{(4,3)}$; Table 3), and this difference tended to be greater in men than in women.

This is the first detailed study in which the effects of cigarette smoking on n-3 essential FA metabolism were examined in human subjects on a controlled diet. Results from the kinetic analyses (isotope tracer determinations) and steady-state measurements (plasma FA concentrations) in male and female subjects subsisting on the beef-based diet indicated that cigarette smoking increased the amount of n-3 FAs transfered to the plasma. As a consequence, bioavailability of PUFAs to tissues may be enhanced in persons who smoke compared with nonsmokers, in part counteracting losses that occur through peroxidation. Also, among male and female smokers, the percent conversion of 20:5n-3 to 22:5n-3 was enhanced, and the fractional synthetic rate for formation of 22:6n-3 among women smokers was triple that of nonsmokers. The increased synthetic rates and the greater percent transfer of isotope between compartments among smokers indicate that smoking also had a positive effect on the production of some long-chain PUFAs in vivo, possibly as another means to compensate for losses resulting from lipid oxidation (1, 2). Notably, the chronic effects of smoking on the enhancement of long-chain PUFA biosynthesis observed here in humans is in contrast to results obtained from cell culture, where an acute dose of cigarette smoke appeared to inhibit long-chain PUFA formation (16).

The authors wish to thank the clinical and technical support staff of the Laboratory of Clinical Studies, National Institutes on Alcohol Abuse and Alcoholism, National Institutes of Health for their participation in this study. The authors appreciate the work of the staff in the metabolic kitchen at the Clinical Center of the National Institutes of Health, especially that of the dieticians, Ms. Patti Riggs, RD and Ms. Nancy Sebring, RD. The authors especially wish to recognize the excellent work of Mr. Brent Wegher in analyzing and reporting the isotope data and managing the database.

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