# Compartmental analysis of plasma and liver n-3 essential fatty acids in alcohol-dependent men during withdrawal

**Robert J. Pawlosky,[1,*] Joseph R. Hibbeln,[†] David Herion,[§] David E. Kleiner,[**] and Norman Salem, Jr.[†]**

Laboratory of Metabolic Control,* Laboratory of Membrane Biochemistry & Biophysics,[†] Laboratory of Clinical and Translational Studies National Institutes on Alcohol Abuse & Alcoholism,[§] and Laboratory of Pathology,** National Cancer Institute NIH, Bethesda, MD

**Abstract The mechanism by which chronic ethanol consumption reduces concentrations of long chain polyunsaturated (LCP) fatty acids (FA) in tissues of humans was investigated in alcohol-dependent (AD) men during early withdrawal and to a well-matched control group by fitting the concentration-time curves of $d_5$-labeled n-3 FA from plasma and liver, which originated from an oral dose of $d_5$-linolenic acid ($d_5$-18:3n-3) ethyl ester to a compartmental model. Blood sampled over 168 h and a liver specimen obtained 96 h after isotope administration were analyzed for $d_5$-18:3n-3, $d_5$-20:5n-3, $d_5$-22:5n-3, and $d_5$-22:6n-3. Plasma 20:5n-3 and 22:5n-3 were lower in AD subjects, compared with controls (20:5n-3: -50%, 22:5n-3: -34%). Increased amounts of $d_5$-18:3n-3 were directed toward synthesis of $d_5$-20:5n-3 in AD subjects ($P < .05$). However, this effect was offset by larger amounts of 20:5n-3 lost from plasma (control: 2.0 vs. AD: 4.2 mg $d^{-1}$). In livers of AD subjects, more $d_5$-18:3n-3 and $d_5$-22:5n-3 were utilized for synthesis of $d_5$-20:5n-3 (+200%) and $d_5$-22:6n-3 (+210%), respectively, than was predicted from plasma kinetics. Although, the potential to utilize linolenic acid for synthesis of LCP FA was greater in AD subjects compared with controls, heightened disappearance rates of 20:5n-3 reduced overall plasma concentrations of several endogenous n-3 LCP FA.**—Pawlosky, R. J., J. R. Hibbeln, D. Herion, D. E. Kleiner, and N. Salem, Jr. **Compartmental analysis of plasma and liver n-3 essential fatty acids in alcohol-dependent men during withdrawal.** ***J. Lipid Res.*** **2009.** 50: **154–161.**

**Supplementary key words** ethanol • α-linolenic acid • docosahexaenoic acid • isotope tracer • compartmental modeling • $d_5$-fatty acids • eicosapentaenoic acid • cigarette smoking

One notable change associated with a prolonged alcohol insult in animals (1–10) and tobacco smoking in humans (11–13) is the reduction of long chain polyunsaturated (LCP) fatty acids (FA), such as arachidonic (20:4n-6) and docosahexaenoic acids (22:6n-3), in body tissues including the liver (1–5) and brain (9, 10). Loss of LCP FA, particularly 22:6n-3, has been related to liver pathology (2, 5) as well as reduction of visual function (10) in alcohol-consuming primates. Interestingly, higher intakes of polyunsaturated fatty acids supplementing the diet in the form of phosphatidylcholine phospholipids prevented development of fibrosis in alcohol-consuming baboons (14), suggesting that maintenance of LCP FA is important for liver function.

α-Linolenic acid, 18:3n-3, the most abundant n-3 essential fatty acid (EFA) in the North American diet, represents only a small percentage of the polyunsaturated FA intake (15). The LCP FA, eicosapentaenoic acid, (20:5n-3), docosapentaenoic acid, (22:5n-3), and 22:6n-3 are synthesized from 18:3n-3 through sequential steps of elongation and desaturation in hepatocytes (16), and stable isotope studies have indicated that men have a lower capacity to biosynthesize 22:6n-3 than women (17, 18). Observational studies in humans that have examined the effects of alcohol abuse on EFA status without controlling for tobacco smoking can be confounded by the direct effect that smoking has on dietary EFA intake and metabolism (12, 13, 19, 20).

Few animal studies have examined the direct effects of ethanol consumption on EFA metabolism using quantitative tracer techniques (6, 7) and studies, which use tissue lipid compositional data as a means of comparison, are insufficient for determining mechanistic effects of ethanol on FA metabolism.

Previously, kinetic parameters relative to LCP FA biosynthesis in humans were determined using a quantitative tracer technique together with a compartmental modeling procedure (21). Recently, the complex effects of habitual smoking on plasma n-3 EFA status and metabolism has been reported in men and women consuming a metabolically controlled diet (22). These investigations have been further extended to an examination of plasma and liver kinetics of n-3 EFA metabolism in alcohol-dependent

*Manuscript received 18 June 2008 and in revised form 25 July 2008 and in re-revised form 18 August 2008.*

*Published, JLR Papers in Press, August 22, 2008.*
*DOI 10.1194/jlr.M800322-JLR200*

Abbreviations: AD, alcohol-dependent; CRN, Clinical Research Network; LCP, long chain polyunsaturated; MAST, Michigan Alcoholism Screening Test; NASH, nonalcoholic steatohepatitis; PFB, pentafluorobenzyl; SCID, Structured Clinical Interview for Diagnosis.

[1] To whom correspondence should be addressed.
e-mail: bpawl@niaaa.nih.gov

(AD) males paired with a group of chronic smokers. Plasma kinetic parameters were determined directly from the concentration-time curves of the $d_5$-labeled FA 18:3n-3, 20:5n-3, 22:5n-3, and 22:6n-3 for each subject. This study also offered for the first time the unique opportunity to compare the plasma kinetic n-3 fatty acid profile to n-3 FA metabolism in the liver of humans directly. In vivo liver n-3-FA kinetics were extrapolated from the fatty acyl composition of specimens obtained 96 h after the $d_5$-18:3n-3 ethyl ester had been consumed.

## METHODS

### Subjects

Subjects provided informed consent (protocol # 92-AA-0194) and were evaluated at the clinical research unit of the National Institute on Alcohol Abuse and Alcoholism at the Clinical Center in Bethesda, Maryland. Subjects were evaluated by physical examination and clinical laboratory testing. Tests included hepatitis A, B, and C, and electrocardiogram, chest X-ray, nurse and social worker interviews, and psychiatric diagnoses by structured interview. Recent and chronic alcohol consumption was characterized using the Michigan Alcoholism Screening Test (MAST) (23) and a structured research questionnaire (24). Age of onset of alcohol abuse was calculated by subtracting years of excessive alcohol consumption from current age. Subjects were included in the alcohol dependency group if they smoked at least 20 cigarettes $d^{-1}$ and met DSM-III-R criteria for current alcohol dependence as evaluated by the Structured Clinical Interview for Diagnosis (SCID) (25). Socioeconomic status was determined using the Hollingshead scale (26). AD subjects reported having their last drink within 7 days of admission and received benzodiazapines to treat alcohol withdrawal but were otherwise medication free during the time of the study. Control subjects (18–65 yrs) were included if they smoked at least 20 cigarettes $d^{-1}$ (range: 15–40).

Exclusion criteria for the control group included: any major medical problems, including hepatic, endocrine, and metabolic disorders, a history of head trauma, seizures, prolonged loss of consciousness, a lifetime history of a major psychiatric diagnosis, abnormal clinical laboratory findings, or the consumption of more than the equivalent of two glasses of beer/wine $d^{-1}$. AD subjects were excluded if they had major medical problems or major psychiatric disorders that were unrelated to alcoholism. AD subjects with a history of pancreatitis or conditions interfering with fat absorption were excluded. Subjects were excluded from the study if they used prescription medications within the last month or if they persistently used over-the-counter medications including aspirin, ibuprofen, acetaminophen, antihistamines, topical steroids, vitamins E and C, multivitamins, herbal and home remedies if they had unusual dietary habits, or if they donated blood within the last 3 months. Absence of illicit drug use was confirmed by random urine testing; abstinence from alcohol consumption was verified by breath testing for ethanol.

AD subjects were inpatients and received the isotope an average of 5.2 d (range: 2–14 d; SD 0.93) after their last drink. Smoking controls consumed no alcohol for at least the prior 21 d and were admitted overnight prior to receiving the isotopes. All subjects followed an ad librium diet. After training by a registered clinical nutritionist in general features of keeping accurate food intake records, subjects recorded the types and quantities of all foods consumed for two wk. Food records were used to calculate energy and macro- and micronutrient intake including an estimate of dietary EFA.

After admission for an overnight fast, subjects received 1 g of the deuterated FA ethyl ester blended into 12 oz of low-fat yogurt prior to their breakfast. The isotope used was $d_5$-17,17,18,18,18-18:3n-3 (Cambridge Isotope Labs, Woburn, MA). Blood was drawn under fasting conditions, at baseline, and at the following intervals over the following wk: 8, 24, 48, 72, 96, and 168 h and processed as previously described (22).

### Liver biopsies and grading

Nine AD subjects underwent percutaneous liver biopsy using a 19 gauge, modified Menghini biopsy needle following alcohol withdrawal (mean: 6.4 ± 0.7 d from the time subjects entered the study). Biopsy material was divided and immediately processed separately for FA analysis and histopathologic examination. Liver tissue slides were prepared in standard fashion, including staining with hematoxylin and eosin, and Masson's trichrome reagents. Biopsies were assessed and scored according to the Clinical Research Network (CRN) for nonalcoholic steatohepatitis (NASH): parenchymal inflammation (0–3), ballooning cellular injury (0–2), steatosis (0–3), and fibrosis (0–4) (27). The pathologist was blinded to patient identity and sequence of biopsies. The activity of the liver disease (NASH Activity Scores, NAS) was calculated as the sum of parenchymal inflammation, ballooning cellular injury, and steatosis (0–8) (27).

### Plasma and liver lipid analysis

Analytical procedures have been described previously (8). Briefly, plasma (0.2 ml) and liver (80–100 mg) 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. After extraction into hexane, the methyl esters were analyzed on a model HP-5890 gas chromatograph with flame ionization detection (Agilent, Wilmington, DE). Concentrations of individual FAs were calculated using the peak area counts in comparison with an internal standard.

### Mass spectral analysis

From a 0.1 ml portion of the lipid extract, chloroform was evaporated and the lipids were hydrolyzed (5% potassium hydroxide in methanol) as described (28). The FA pentafluorobenzyl (PFB) esters were made and analyzed by gas chromatography-mass spectrometry (GC-MS) according to conditions previously described (28). Data were acquired in the selected ion mode, monitoring the M-PFB anion and converted to the absolute quantity of the $d_5$-FA by reference to the concentration of an internal standard.

### Compartmental models

The hepatocyte is a main site for the biosynthesis of the 20- and 22-carbon LCP FA from 18:3n-3. Since the compartmental model for n-3 FA metabolism has been described previously (21) only a brief description follows. The model was developed from the plasma concentration-time curves of the labeled and endogenous n-3 FA using WinSAAM (Windows Simulation and Analysis Modeling) software (http://www.winsaam.com). The liver d-5 FA concentration-time curve values were extrapolated from the tissue fatty acyl concentrations of specimens obtained 96 h after isotope administration.

### Liver n-3 FA concentrations

Total liver volume was estimated as 2.5% of each subject's body weight, and liver blood volumes were estimated as 30% of the total liver volume where 70% of the total blood volume was

assumed to be of portal circulation (29). To determine amounts of $d_5$-labeled and endogenous n-3 FA within the liver proper, raw GC and GC-MS concentration data were adjusted to account for residual amounts of labeled-FA present in arterial blood volume (systemic circulation). Subsequent time point entrees for liver $d_5$-n-3 FA concentrations were estimated using a modeling paradigm appropriate for coupled multicompartmental systems that synchronizes plasma $d_5$-FA time course data to the liver compartment (30). Final $d_5$-FA concentrations were adjusted for isotope fluctuations originating from arterial blood flow. Liver $d_5$-FA rate constants were determined by fitting extrapolated values to model-derived time-course curves. Final kinetic values were determined using the program's iterative curve-fitting subroutines.

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

The fractional rate constant coefficient, $\mathbf{L_{(I,J)}}$, represents the fraction of substrate, which is transferred from substrate-compartment **J** to product-compartment **I**. The units are in $h^{-1}$. 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 fractional synthetic rate (**fsr**) (i.e., the fraction of isotope remaining within the metabolic pathway as opposed to isotope taken up by tissues or irreversibly lost from the compartment). Initial **fsr** estimates were derived from the concentration-time curves generated from the experimental data. Values assigned to kinetic parameters were then adjusted to compensate for each subject's individual variances in the data (e.g., variances in isotope dilution due to differences in body mass) 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. 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 (21). 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 or coefficient of variance (**cv**) where appropriate.

### Model and rate equations

Both liver and plasma models consisted of five compartments for which isotope data was obtained (**Fig. 1**). Compartment 1 represents the $d_5$-18:3n-3 dosage and absorption through the gastrointestinal tract. Compartments 2 through 5 denote individual pools of the n-3 FA (18:3n-3, 20:5n-3, 22:5n-3, and 22:6n-3). Rate equations corresponding to the flux of the $d_5$-FA substrates through their respective compartments are defined by a set of differential equations.

### Limits, constraints, and statistics

Daily energy, macronutrient, and EFA intake for each subject were estimated using values determined from an analysis of each subject's responses to the food frequency questionnaire. Upper and lower n-3 FA intake limits were assigned to each subject, which constrained 18:3n-3 daily intake values to within known limits.

Throughout the168 h, variances of all the endogenous plasma FA concentrations were less than 15% (range: $9 \pm 2\%$ to $14 \pm 2\%$). Therefore, mean values of the plasma n-3 FA concentrations over the sampling period were used to approximate the steady state mass of the endogenous substrate $\mathbf{M_{(J)}}$ available for biosynthesis and these values were held constant (Table 4).

To determine differences in plasma FA concentrations and model-derived rate parameters between groups, an unpaired *t*-test analysis was performed. A *P* value of .05 or lower was considered significant.

## RESULTS

### Subject characterizations

Seven control and 12 AD subjects were recruited into the protocol. Of these, five control and nine AD subjects completed the study. Smokers were well matched to AD subjects in age, body mass index, cigarette smoking, and socioeconomic status (**Table 1**). Medium age of smoking subjects was 36.5 yr ($\pm$ 3.1) and that of AD subjects was 41.0 yr ($\pm$ 2.3). Mean body weight for smoking subjects

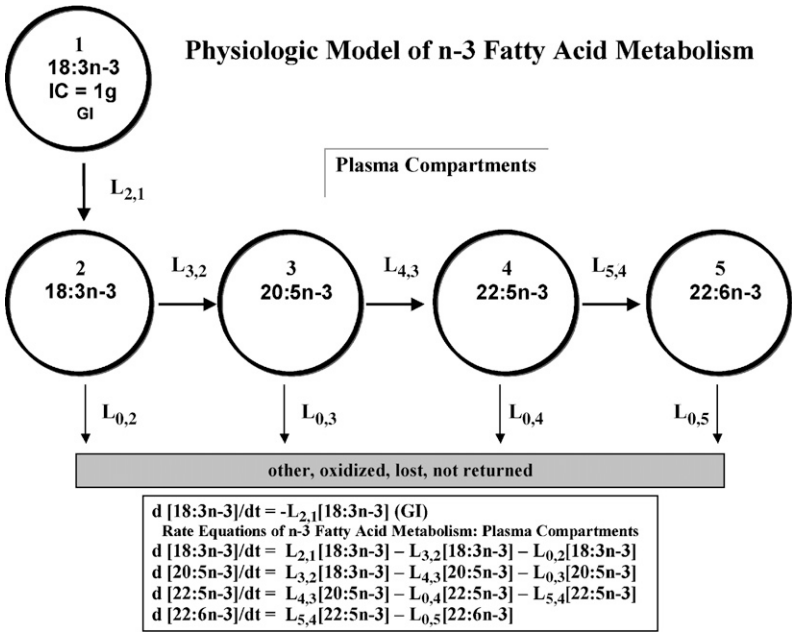


**Fig. 1.** Conceptual model of n-3 fatty acid metabolism. The open circles represent separate fatty acid compartments. Compartment 1 represents administration of the isotope (1 g) and absorption through the gastrointestinal tract. Four compartments (2 through 5) represent plasma fatty acid compartments following along successive steps of desaturation and elongation of the tracer. Arrows connecting the five compartments indicate flow along the path and losses of isotope from the system are indicated by arrows drawn perpendicular to the path The fractional transfer rates, $L_{(I,J)}$ are rate parameters derived from the model-fitted experimental data. The set of differential equations used in determining individual rate parameters are given in the boxed area.

TABLE 1. Subject characteristics

| | Smokers | Alcohol-dependent |
|---|---|---|
| | n = 5 | n = 9 |
| Age (yr) | 36.5 ± 3.1 | 41 ± 2.3 |
| Height (cm) | 175.1 ± 1.2 | 171.0 ± 2.2 |
| Weight (kg) | 84.4 ± 3.1 | 77.9 ± 5.3 |
| BMI ($kg/m^2$) | 27.5 ± 1.5 | 26.5 ± 2.1 |
| Socioeconomic status | 45 ± 2 | 37 ± 3 |
| Cigarettes (n $d^{-1}$) | 21.1 ± 1.3 | 26.5 ± 4.1 |
| Alcohol frequency (d/last 180) | 14.1 ± 8.7 | 172.5 ± 9.5[a] |
| Alcohol quantity (g $d^{-1}$) | 30.6 ± 6.6 | 206.0 ± 50.4[a] |
| Lifetime alcohol intake (kg) | 31.2 ± 12.1 | 512.3 ± 121.1[a] |
| MAST | 0.8 ± 0.3 | 42.1 ± 5.0[a] |
| CAGE[b] | 0.3 ± 0.1 | 3.2 ± 0.6[a] |
| Energy intake (kcal $d^{-1}$) | 2614 ± 270 | 3043 ± 233 |
| Protein (g $d^{-1}$) | 104 ± 17 | 85 ± 17 |
| Carbohydrate (g $d^{-1}$) | 342 ± 33 | 240 ± 36 |
| Fat (g $d^{-1}$) | 96 ± 12 | 102 ± 22 |
| Saturated (g $d^{-1}$) | 36 ± 5 | 36 ± 8 |
| Monounsaturated (g $d^{-1}$) | 33 ± 4 | 37 ± 8 |
| 18:2n-6 (g $d^{-1}$) | 17 ± 3 | 18 ± 8 |
| 18:3n-3 (g $d^{-1}$) | 1.3 ± 0.2 | 1.1 ± 0.3 |

Values are expressed as mean ± standard deviation.

[a] Value is significantly different from smoking control group at $P < .05$ using paired Student's *t* test.

[b] CAGE values are derived from the first four questions from the Michigan Alcohol Screening Test (MAST).

TABLE 2. Clinical chemistries of smokers and alcohol dependent (AD) subjects

| | Smokers | Alcohol dependent |
|---|---|---|
| | n = 5 | n = 9 |
| Hepatic profile | | |
| Ammonia | 26 ± 3 | 32 ± 4 |
| Direct bilirubin | 0.08 ± 0 | 0.12 ± 0[a] |
| Alkaline phosphatase | 80 ± 12 | 112 ± 17 |
| Lactate dehydrogenase | 153 ± 11 | 255 ± 4[a] |
| ALT/GPT | 24 ± 7 | 142 ± 93 |
| AST/GOT | 20 ± 3.5 | 166 ± 122 |
| GGTP | 28 ± 5 | 167 ± 62[a] |
| Aldolase | 4 ± 0.3 | 11 ± 6 |
| Amylase | 50 ± 6 | 41 ± 5 |
| Clinical chemistries | | |
| Cholesterol, mg $dl^{-1}$ | 190 ± 6 | 208 ± 24 |
| Triglyceride, mg $dl^{-1}$ | 112 ± 18 | 185 ± 63 |
| Carotene, g $dl^{-1}$ | 141 ± 4 | 92 ± 28 |
| Chloride | 102 ± 0.7 | 100 ± 1.4 |
| Urea | 15.2 ± 2.2 | 11.4 ± 2.0[a] |
| Creatinine | 1.1 ± 0.1 | 0.8 ± 0.0[a] |
| Vitamin C, g $dl^{-1}$ | 0.5 ± 0.1 | 0.6 ± 0.1 |
| Vitamin A, g $dl^{-1}$ | 101 ± 13 | 76 ± 10 |
| Immunoglobulin G | 1257 ± 107 | 913 ± 85 |
| Iron | 98 ± 12 | 132 ± 21 |
| Transferin | 290 ± 17 | 247 ± 22 |
| Haptoglobin | 154 ± 22 | 147 ± 23 |
| Hematology | | |
| Erythrocytes | 4.1 ± 0.1 | 4.4 ± 0.1[a] |
| Mean corpustular volume | 88.7 ± 2.3 | 98.8 ± 3.2[a] |
| Reticulocyte count | 1.1 ± 0.3 | 1.6 ± 0.2 |
| Platelet count | 297 ± 17 | 197 ± 34 |
| Prothrombin time | 28 ± 1.5 | 26 ± 0.7[a] |

Values are expressed as mean ± standard deviation. All clinical chemistries were from blood samples.

[a] Value is significantly different from smoking control group at $P < .05$ using Student's paired test.

was 84.4 kg (± 3.1) and that of AD subjects was 77.9 (± 5.9). AD subjects consumed an average of 206 g $d^{-1}$ of alcohol and had a mean time to last drink of 5.2 days (min, 2 d; max, 14 d) measured up until the time of isotope administration. AD subjects who had consumed alcohol on an average of 173 d during the previous 180 d, had a mean lifetime consumption of 512 ± 121 kg of absolute ethanol, and significantly elevated MAST and CAGE scores (Table 1). AD subjects exhibited marked increases in serum bilirubin, lactate dehydrogenase, and GGTP relative to the smoking group, but decreases in urea and creatinine (**Table 2**). Although, AD subjects had elevated plasma ALT/GPT and AST/GOT levels, values were widely distributed and differences between groups had not reached significance (*P* values: .09 and .07 for ALT/GPT and AST/GOT, respectively) (Table 2). No differences were observed in the following serum measures: albumin, amylase, aldolase, ferritin, electrolytes, glucose, calcium, magnesium, zinc, phosphorus, uric acid, folate, vitamin E, vitamin B12, T3, T4, and thyroid stimulating hormone between the groups. AD subjects had a higher erythrocyte count and corpuscular volume but a decrease in prothrombin time compared with smoking controls (Table 2).

### Histopathology

Fat was present in eight subjects' biopsies (88%; CRN steatosis score: 1.2 ± 0.8, range: 0–3), parenchymal inflammation was observed in seven (78%; CRN score 1.0 ± 0.9, range: 0–3) and ballooning injury was present in two (22%; CRN ballooning injury score 0.2 ± 0.4, range 0–1). Three subjects (33%) had fibrosis, including one who had cirrhosis (CRN fibrosis score 0.8 ± 1.4, range 0–3). Four subjects (44%) had steatohepatitis by CRN criteria (NAS: 2.4 ± 1.5, range 0–5). Three subjects (33%) had evidence of mitochondrial injury (mega mitochondria), and none had Mallory's hyaline.

### Nutrient intake

Nutrient compositions were estimated for each subject based on their responses to the food frequency questionnaire using values obtained from the Minnesota Food and Nutrient Database (Table 1). Calculated caloric intake for control and AD subjects were 2,614 ± 270 and 3,084 ± 233 kcal $d^{-1}$, respectively. There were no differences in protein, fat, and carbohydrate intake between groups (smokers: 104, 96, and 342; AD subjects: 85, 102, and 240 g $d^{-1}$) and no differences in estimated 18:2n-6 and 18:3n-3 FA intake values. None of the subjects reported eating fish during the 2 week study period, and intake of LC n-3 FA from other food sources was not significant.

### Plasma FA and $d_5$-18:3n-3 AUC

Mean concentrations of plasma FAs obtained from blood samples taken over 168 h, and concentrations of liver FA from AD subjects are given in **Table 3**. AD subjects had significantly lower plasma concentrations of 20:5n-3 and 22:5n-3 and tended to have lower concentrations of 22:6n-3 ($P < .1$) compared with smokers. There were no differences in saturated, monounsaturated or other polyunsaturated FA between groups.

TABLE 3. Plasma and liver fatty acyl composition and area under the concentration-time curves (AUC) for smokers and alcohol-dependent subjects

| | Smoker's plasma | Alcohol-dependent plasma | Alcohol-dependent liver |
|---|---|---|---|
| | n = 5 | n = 9 | n = 9 |
| Fatty acids[a] | | | |
| Saturated | 341 ± 49 | 585 ± 26 | 11,322 ± 2,360 |
| Monounsaturated | 718 ± 35 | 443 ± 56 | 12,283 ± 3,910 |
| n-6 | | | |
| 18:2 | 600 ± 31 | 594 ± 45 | 1,270 ± 209 |
| 20:2 | 4.4 ± 0.2 | 5.1 ± 0.8 | 147 ± 34 |
| 20:3 | 45 ± 2 | 34 ± 4 | 393 ± 60 |
| 20:4 | 198 ± 12 | 166 ± 10 | 874 ± 139 |
| 22:4 | 8.0 ± 0.8 | 6.3 ± 0.4 | 121 ± 16 |
| 22:5 | 5.5 ± 0.4 | 8.6 ± 0.6 | 148 ± 18 |
| n-3 | | | |
| 18:3 | 13 ± 1 | 11 ± 2 | 161 ± 48 |
| 20:5 | 17 ± 2 | 10 ± 1[b] | 79 ± 11 |
| 22:5 | 18 ± 2 | 11 ± 1[b] | 132 ± 20 |
| 22:6 | 40 ± 4 | 29 ± 2 | 424 ± 54 |
| $d_5$-n-3 | | | |
| 18:3 | 0.093 ± 0.011 | 0.067 ± 0.009 | 0.78 ± 0.12 |
| 20:5 | 0.062 ± 0.008 | 0.039 ± 0.006 | 0.32 ± 0.06 |
| 22:5 | 0.018 ± 0.005 | 0.024 ± 0.003 | 0.31 ± 0.05 |
| 22:6 | 0.012 ± 0.001 | 0.011 ± 0.002 | 0.38 ± 0.10 |
| AUC | | | |
| $d_5$-18:3 | 58.3 ± 17.8 | 50.6 ± 11.6 | |
| $d_5$-20:5 | 6.53 ± 1.17 | 8.78 ± 1.68 | |
| $d_5$-22:5 | 2.06 ± 0.37 | 3.59 ± 0.60 | |
| $d_5$-22:6 | 0.67 ± 0.16 | 1.44 ± 0.33 | |

Values are given in $\mu g\ ml^{-1}$ plasma (mean values over 168 h) or $g^{-1}$ of liver tissue corrected for blood volume at 96 h ± the standard deviation. Labeled fatty acid values from plasma are at 96 h. Values for AUC are given in $\mu g\ ml^{-1}$ h.

[a] "Saturated" and monounsaturated" indicates the sum of all plasma saturated and monounsaturated fatty acids, respectively.

[b] Plasma value is significantly different from smoking control group at $P < .05$ using Student's *t* test.

The model carried the assumption that fat absorption was essentially complete for all subjects (98%). Therefore, variances in the amount of labeled-precursor appearing in plasma represent differences in bioavailability among subjects. Bioavailability of $d_5$-18:3n-3 from plasma was calculated as percent of dose (parameter, $\mathbf{P_{(2,1)}}$, **Table 4**) using isotope values obtained from compartments 1 and 2 (Fig. 1). There were no differences in the percent of $d_5$-18:3n-3 appearing in plasma between smokers (4.7%) and AD subjects (4.2%) and hence no apparent differences in bioavailability of the precursor. Plasma $d_5$-18:3n-3 values may also be expressed in terms of area under the concentration-time curve (AUC) over the 168 h period (expressed here as $ug\ ml^{-1} \cdot h \pm SEM$). There were no differences in AUC values for $d_5$-18:3n-3 between these groups (smokers were: 51 ± 17; AD subjects: 58 ± 16) (Table 3). Data in Table 3 may be used to calculate a tracer/trace ratio by dividing AUC by the appropriate unlabeled fatty acid.

### n-3 FA concentration-time curves

**Figure 2** illustrates composite time course curves for $d_5$-20:5n-3 $d_5$-22:5n-3, $d_5$-22:6n-3 among smokers and AD subjects. Smokers had both a delayed (mean value, 48 h) and more prolonged rate of disappearance of $d_5$-20:5n-3 from plasma compared with AD subjects (mean value, 26 h). Disappearance rates of $d_5$-20:5n-3 in plasma, quantified as fractional rate constant coefficients, $\mathbf{L_{(0,3)}}$, are given in Table 4 (smokers: 0.0014 $h^{-1}$; AD subjects: 0.0061 $h^{-1}$, $P < .05$). The 5-fold difference in the rate of disappearance of 20:5n-3 from the plasma in AD subjects emphasizes a greatly accelerated turnover rate and consequently a shorter plasma half-life of this FA. A somewhat lower turnover rate of 22:6n-3, $\mathbf{L_{(0,5)}}$, in AD subjects compared with smokers was another difference noted between groups (Table 4).

Among AD subjects the fractional synthetic rate (**fsr**) for $d_5$-20:5n-3, $\mathbf{P_{(3,2)}}$, was twice that of smokers (smokers: 0.32; AD subjects: 0.72, Table 4). A larger **fsr** indicates that a higher percentage of 18:3n-3 is utilized for synthesis of 20:5n-3. As a consequence of the spike in the 20:5n-3 **fsr** occurring at this stage in the biosynthetic sequence, then both $d_5$-22:5n-3 and $d_5$-22:6n-3 also carried higher amounts of the deuterium label in the plasma of AD subjects relative to the controls (Fig. 2). However, there were no differences in the **fsr** for formation of either 22:5n-3 or 22:6n-3 ($\mathbf{P_{(4,3)}}$ and $\mathbf{P_{(5,4)}}$) indicating that chronic alcohol consumption had not negatively influenced the synthesis of either of these FA (Table 4).

The daily plasma turnover rate of 20:5n-3 was 2.5 times greater in AD subjects than in smokers (smokers, 1.95; AD subjects, 4.24 mg $d^{-1}$) (Table 4). However, AD subjects also tended to have a lower plasma turnover of 22:6n-3 (103 mg $d^{-1}$) compared with smokers (169 mg $d^{-1}$; $P < .1$).

### Liver FA and rate constants

The livers of AD subjects contained greater amounts of n-3 FA compared with plasma whether expressed in units of concentration ($\mu g\ g^{-1}$, Table 3) or in absolute amounts ($\mathbf{M_{(j)}}$, Table 4). Also, the livers contained greater amounts of $d_5$-n-3 FA compared with the plasma at 96 h (Table 3). The mean **fsr** for formation of 20:5n-3, $\mathbf{P_{(3,2)}}$, in the liver was 1.5 (± 1.9) indicating a greater capacity for utilizing 18:3n-3 for synthesis in the liver than that which was predicted from the plasma kinetics (0.72 ± 0.2) (Table 4). Also, the **fsr** for 22:6n-3 formation, $\mathbf{P_{(5,4)}}$, in the liver was greater (85 ± 8) than that predicted from subjects' plasma kinetic profiles (41 ± 9). The higher **fsr** values together with the greater endogenous mass of n-3 FA indicates a potentially greater synthetic output of LCP n-3 FA from liver than was predicted from the plasma kinetic profile for subjects (Table 4). The mean daily liver production of 20:5n-3, 22:5n-3, and 22:6n-3 were estimated as 17 (± 7), 71 (± 17), and 104 (± 15) mg, respectively, and these production rates were 3-, 5-, and 5-fold, respectively, above those predicted from the plasma kinetic profiles.

## DISCUSSION

To our knowledge, this is the first detailed study examining the effects of chronic alcohol dependency on plasma and liver kinetics of essential n-3 FA metabolism in a group of habitual smokers during early alcohol withdrawal. A compartmental model, previously validated (21), was used to compare plasma kinetic rate profiles from AD subjects

TABLE 4. Kinetic constants, fractional transfer rates, fatty acids, and synthetic rates from plasma and liver compartments in smokers and alcohol-dependent subjects

| | | Smoker's (n = 5) plasma | Alcohol-dependent (n = 9) Plasma | Alcohol-dependent (n = 9) liver |
|---|---|---|---|---|
| | n-3 FA | $k^{-1}$ | $k^{-1}$ | $k^{-1}$ |
| $L_{(3,2)}$ | 18:3 -> 20:5 | 0.004 ± 0.002 | 0.006 ± 0.002 | 0.008 ± 0.002 |
| $L_{(0,2)}$ | 18:3 -> out | 12.7 ± 1.6 | 8.7 ± 2.42 | 4.5 ± 1.3 |
| $L_{(4,3)}$ | 20:5 -> 22:5 | 0.015 ± 0.003 | 0.019 ± 0.002 | 0.021 ± 0.003 |
| $L_{(0,3)}$ | 20:5 -> out | 0.0014 ± 0.0001 | 0.0061 ± 0.0002 | 0.002 ± 0.001[a] |
| $L_{(5,4)}$ | 22:5 -> 22:6 | 0.020 ± 0.007 | 0.022 ± 0.006 | 0.021 ± 0.003 |
| $L_{(0,4)}$ | 22:5 -> out | 0.032 ± 0.014 | 0.029 ± 0.007 | 0.006 ± 0.002 |
| $L_{(0,5)}$ | 22:6 -> out | 0.048 ± 0.011 | 0.031 ± 0.004 | 0.033 ± 0.011[a] |
| | | fsr | fsr | fsr |
| $P_{(2,1)}$ | 18:3 -> in | 4.7 ± 1.6 | 4.2 ± 0.7 | 5.1 ± 1.9 |
| $P_{(3,2)}$ | 18:3 -> 20:5 | 0.32 ± 0.11 | 0.72 ± 0.15 | 1.5 ± 0.1[a,b] |
| $P_{(4,3)}$ | 20:5 -> 22:5 | 98 ± 13 | 91 ± 7 | 94 ± 4 |
| $P_{(5,4)}$ | 22:5 -> 22:6 | 45 ± 21 | 41 ± 9 | 85 ± 8[b] |
| | | mg | mg | mg |
| $M_{(2)}$ | 18:3 | 45 ± 8 | 41 ± 17 | 350 ± 24 |
| $M_{(3)}$ | 20:5 | 58 ± 11 | 29 ± 4 | 121 ± 2[a] |
| $M_{(4)}$ | 22:5 | 62 ± 9 | 39 ± 6 | 230 ± 3[a] |
| $M_{(5)}$ | 22:6 | 145 ± 25 | 98 ± 14 | 657 ± 14 |
| | | mg $d^{-1}$ | mg $d^{-1}$ | mg $d^{-1}$ |
| $R_{(0,1)}$ | 18:3 -> in | 668 ± 97 | 430 ± 201 | 1,330 ± 618 |
| $R_{(3,2)}$ | 18:3 -> 20:5 | 4.4 ± 1.9 | 4.8 ± 2.2 | 17 ± 7 |
| $R_{(0,2)}$ | 18:3 -> out | 656 ± 82 | 421 ± 198 | 505 ± 88 |
| $R_{(4,3)}$ | 20:5 -> 22:5 | 12.1 ± 3.5 | 14.3 ± 1.5 | 71 ± 17 |
| $R_{(0,3)}$ | 20:5 -> out | 1.95 ± 0.45 | 4.24 ± 0.21 | 4.9 ± 2.4[a] |
| $R_{(5,4)}$ | 22:5 -> 22:6 | 34 ± 11 | 20 ± 4 | 104 ± 15 |
| $R_{(0,4)}$ | 22:5 -> out | 35 ± 17 | 30 ± 9 | 33 ± 14 |
| $R_{(0,5)}$ | 22:6 -> out | 169 ± 106 | 103 ± 39 | 463 ± 211 |

Values are determined from $d_5$-labeled FA concentration-time curves and the mass of endogenous fatty acids from plasma and liver compartments from alcohol-dependent subjects. L(i,j) values are the kinetic rate constant coefficients; P(i,j) values are fractional synthetic rate values; M(i) values represent total mass of fatty acid within the compartment; and R(i,j) values are the FA turnover and synthetic amounts within a 24 h period. Data are expressed as the mean ± standard deviation.

[a] Plasma value is significantly different from smoker at $P < .05$ using a paired $t$'test.

[b] Liver value is significantly different from plasma value in alcohol-dependent subjects at $P < .05$ using a paired $t$'test.

going through withdrawal to those of a group of habitual smokers. We extended this analysis to an evaluation of liver n-3 FA kinetics in AD subjects using tissue fatty acyl profiles obtained from biopsied specimens. Compartmental models based on total plasma values are often limited as they do not account for kinetics within individual lipoprotein fractions and a more detailed model of lipoprotein kinetics is desirable.

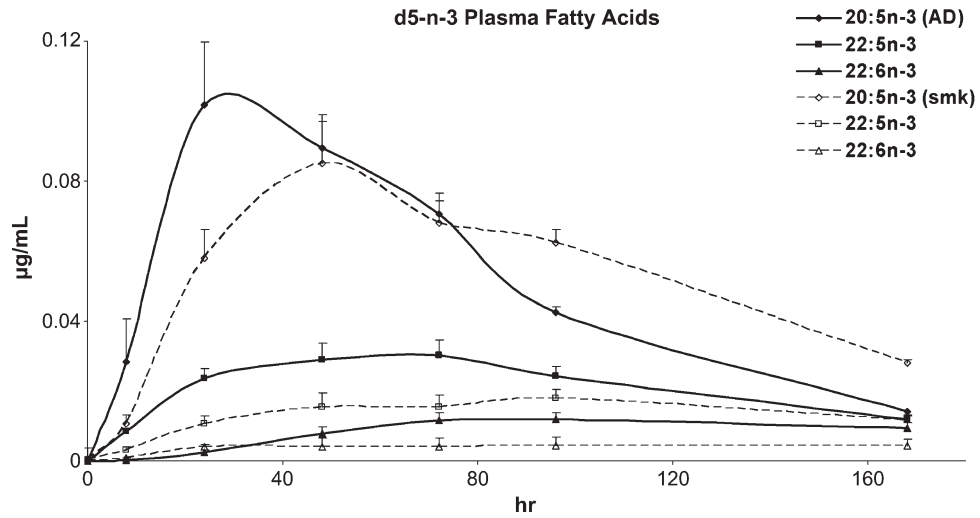


**Fig. 2.** Graphical comparison of mean values of plasma concentration (µg $ml^{-1}$) time curves for $d_5$ -20:5n-3, -22:5n-3, and -22:6n-3 in male smokers (n = 5) and alcohol dependent subjects (n = 9) following a 1 g oral dose of $d_5$-18:3n-3 ethyl ester over 168 h. Error bars represent SEM of mean value.

All but one AD subject presented with some degree of liver steatosis and inflammation, and one third of these subjects had initial stage fibrosis accompanied with other alcohol-related liver injury. There was no correlation in the amount of liver fat accumulation with any kinetic parameter of n-3 FA metabolism. Liver synthetic output was determined using mean values of the daily dietary intake of linolenic acid (averaged over 2 wk) for each subject as the principle n-3 FA for the biosynthesis of all other LCP FA. The predicted liver synthetic output of 20:5n-3, 22:5n-3, and 22:6n-3 from the livers of AD subjects was potentially greater than that determined from the plasma kinetic profile (Table 4). A greater synthetic output of LCP n-3 FA is consistent with the larger amounts of liver n-3 FA available for biosynthesis relative to the plasma and possibly the higher turnover rate of 22:6n-3 in the liver (Table 4). The **fsr** for formation of 20:5n-3 and 22:6n-3 were also greater in the liver compared with the values from the total plasma profile, suggesting that perhaps an analysis of specific plasma lipoproteins may give a more accurate reflection of liver metabolism (Table 4).

Smoking (31, 32) and ethanol metabolism (33–37) are well known to increase formation of oxygenated LCP FA. Since highly unsaturated FA are more likely to be oxidized, this may lead to decreases in the percentage and/or total amounts of the LCP FA in tissues. Also, 20:5n-3, an analog of 20:4n-6 may also be recruited as a potential cycoloxygenase substrate in cytokine-mediated inflammatory response processes. AD subjects who smoked had significantly lower plasma amounts of 20:5n-3 and 22:5n-3 compared with a well-matched group of habitual smokers having similar availability to dietary n-3FA.

A persistent question has been what effect ethanol has on LCP FA formation, desaturation, and catabolism. The desaturases (Δ-9, Δ-6, and Δ-5) dehydrogenate FA between specific carbon positions and in vitro studies across different species have suggested that an ethanol-induced decrease in liver LCP FA may result from an inhibition of either Δ-5 or Δ-6 desaturase (38–40). Others have found little or no change in desaturase activity (41), and findings from a cell culture study reported a positive effect of ethanol on desaturase activity across a wide range of concentrations (42). In vivo investigations of EFA metabolism in alcohol-consuming monkeys found no difference in the plasma uptake of labeled-18:2n-6 or -18:3n-3 and AUC for labeled-20:4n-6 and -22:6n-3 resulting from the metabolism of these precursors tended to be greater in the alcohol group than in the controls (7). A study in felines (6) also suggested that ethanol consumption may have had a positive influence on the production of labeled LCP n-3 FA based on AUC determinations.

Evidence obtained from the fatty acyl composition of livers suggested that desaturation was not affected in humans with alcoholic liver disease since concentrations of LCP n-6 FA, 20:4n-6, 22:4n-6, and 22:5n-6 (products of Δ-5 and Δ-6 desaturation) were no different than normal livers (43). However, lower amounts of LCP n-3 FA in these livers may have been the result of a low dietary supply of n-3 FA or perhaps greater catabolic rates. These findings were consistent with evidence from alcohol studies in micropigs (4), felines (9), and rhesus monkeys (7).

An n-3 FA compartmental model was used to determine the efficiency of individual biosynthetic steps by estimating the **fsr** for LCP FA formation along the synthetic pathway. A greater percentage of 18:3n-3 was used for formation of 20:5n-3 in AD subjects who smoked compared with controls. Previously, we reported that men who smoked had a higher **fsr** for formation of 22:5n-3 compared with nonsmokers (smokers: 97; nonsmokers: 62), and in the present study both smokers and AD subjects had similarly high 22:5n-3 **fsr** values (smokers: 98; AD subjects: 91) (26). Male smokers subsisting on a low 18:3n-3 diet (0.59 g $d^{-1}$) were also shown to have higher plasma turnover rates of 18:3n-3 (1330 mg $d^{-1}$) compared with nonsmokers (432 mg $d^{-1}$). In the present study turnover rates for 18:3n-3 in subjects on ad-librium diets were more moderate (smokers: 656 mg $d^{-1}$; AD subjects: 421 mg $d^{-1}$) reflecting perhaps the higher 18:3n-3 content of their diet (~1.1 g $d^{-1}$).

AD subjects had both a greater disappearance and turnover rate of plasma 20:5n-3 compared with smokers ($\mathbf{L}_{(0,3)}$ and $\mathbf{R}_{(0,3)}$, Table 4). An alcohol-induced utilization or catabolism of 20:5n-3, causing a deficit of this fatty acid, may have had an indirect effect on increasing Δ-6 desaturase activity to compensate for losses of 20:5n-3 (thereby also extending isotope accumulation to both 22:5n-3 and 22:6n-3) (44). However, the **fsr** values for formation of 22:5n-3 and 22:6n-3 were no different in AD subjects compared with controls (Table 4), suggesting that enzyme systems leading to their production may be saturated or are perhaps more refractory to the ethanol effects. The FA kinetic parameters in AD subjects were consistent with observations of lower plasma concentrations of 20:5n-3, 22:5n-3, and 22:6n-3 compared with a group of smoking subjects and support the view that chronic alcohol consumption enhances specific anabolic processes in the metabolism of EFA while also enhancing the catabolism of 20:5n-3 in the plasma. These findings also appear to be consistent with observations of lower concentrations of n-3 FA in livers of humans with alcoholic liver disease (43). This study provided no evidence to support the view that chronic ethanol consumption inhibits desaturation of LCP FA in vivo, because production rates of 22:5n-3 and 22:6n-3 were not diminished and production of 20:5n-3 was greater in AD subjects during early stages of alcohol withdrawal.

The authors wish to thank the clinical and technical support staff of the Laboratory of Clinical Studies, NIAAA for their participation in this study, especially the dieticians, Ms. Patti Riggs, RD and Ms. Nancy Sebring, RD. We wish to recognize the excellent work of Mr. Brent Wegher in analyzing and reporting the isotope data and managing the database. There were no known conflicts of interest between any author who participated in this study and any commercial company. R.J.P. was responsible for the design of the compartmental model, database management, and preparation of the manuscript. J.R.H. was responsible in part for the design of the human protocol and had major responsibility for screening subjects and admission of patients into the protocol. N.S.was responsible for the study design

and in editing the manuscript. D.H. performed the liver biopsies and contributed to portions of the manuscript. D.E.K. was the pathologist responsible for grading the micrographs from prepared liver biopsies.

## REFERENCES


1. Corbett, R., H. H. Floch, J-F. Menez, and B. E. Leonard. 1991. The effects of chronic ethanol administration on rat liver and erythrocyte lipid composition: modulatory role of evening primrose oil. *Alcohol Alcohol.* **26:** 459–464.
2. Cunningham, C. C., G. Bottenus, R. E. Spach, and L. L. Rudel. 1983. Ethanol-related changes in liver microsomes and mitochondria from the monkey *Macaca fascicularis*. *Alcohol. Clin. Exp. Res.* **7:** 424–433.
3. Cunnane, S. C., M. S. Manku, and D. F. Horrobin. 1985. Effect of ethanol on liver triglycerides and fatty acid composition in the golden Syrian hamster. *Ann. Nutr. Metab.* **31:** 246–252.
4. Villanueva, J., C. J. Chandler, N. Shimasaki, A. B. Tang, M. Nakamura, S. D. Phinney, and C. H. Halsted. 1994. Effects of ethanol feeding on liver, kidney and jejunal membranes of micropigs. *Hepatology.* **19:** 1229–1240.
5. Pawlosky, R. J., B. M. Flynn, and N. Salem, Jr. 1997. The effects of a low essential fatty acid diet on alcohol-induced liver fibrosis in rhesus monkeys. *Hepatology.* **26:** 1387–1392.
6. Pawlosky, R. J., S. Gupta, and N. Salem, Jr. 1998. Essential fatty acids and eicosanoid: alcohol consumption in felines increases lipid peroxidation in the brain and stimulates polyunsaturated fatty acid metabolism. *In* Invited Papers from the 4th International Congress. R. A. Reimersma, R. Armstrong, R. W. Kelly, and R. Wilson, editors. AOCS Press, Champaign IL. 312–320.
7. Pawlosky, R. J., and N. Salem, Jr. 1999. Alcohol consumption in rhesus monkeys depletes tissues of polyunsaturated fatty acids and alters essential fatty acid metabolism. *Alcohol. Clin. Exp. Res.* **23:** 311–317.
8. Nakamura, M., A. B. Tang, J. Villanueva, C. H. Halsted, and S. D. Phinney. 1992. Reduced tissue arachidonic acid concentration with chronic ethanol feeding in miniature pigs. *Am. J. Clin. Nutr.* **56:** 467–474.
9. Pawlosky, R. J., and N. Salem, Jr. 1995. Ethanol exposure causes a decrease in docosahexaenoic acid and an increase in docosapentaenoic acid in feline brains and retinas. *Am. J. Clin. Nutr.* **61:** 1284–1289.
10. Pawlosky, R. J., J. Bacher, and N. Salem, Jr. 2001. Ethanol consumption alters electroretinograms and depletes neural tissues of docosahexaenoic acid in rhesus monkeys: Nutritional Consequences of a Low n-3 Fatty Acid Diet. *Alcohol. Clin. Exp. Res.* **25:** 1758–1765.
11. Simon, J. A., J. Fing, J. T. Bernert, Jr., and W. S. Browner. 1996. Relation of smoking and alcohol consumption to serum fatty acids. *Am. J. Epidemiol.* **144:** 325–334.
12. Hibbeln, J. R., K. K. Makino, C. E. Martin, F. Dickerson, J. Boronow, and W. S. Fenton. 2003. Smoking, gender, and dietary influences on erythrocyte essential fatty acid composition among patients with schizophrenia or schizoaffective disorder. *Biol. Psychiatry.* **53:** 431–441.
13. Fulton, M., M. Thomson, R. A. Elton, S. Brown, and A. Wood. 1998. Cigarette smoking, social class and nutrient intake: relevance to coronary heart disease. *Eur. J. Clin. Nutr.* **42:** 797–803.
14. Lieber, C. S., S. J. Robins, L. Jianjun, L. M. DeCarli, K. M. Mak, J. M. Fasulo, and M. A. Leo. 1994. Phosphatidylcholine protects against fibrosis and cirrhosis in the baboon. *Gastroenterology.* **106:** 152–159.
15. Raper, N. R., F. J. Cronin, and J. Exler. 1992. Omega-3 fatty acid content of the US food supply. *J. Am. Coll. Nutr.* **11:** 304–308.
16. Sprecher, H. 1972. Regulation of polyunsaturated fatty acid biosynthesis in the rat. *Fed. Proc.* **3:** 1451–1457.
17. Pawlosky, R. J., J. Hibbeln, Y. H. Lin, and N. Salem, Jr. 2003. n-3 Fatty acid metabolism in women. *Br. J. Nutr.* **90:** 993–995.
18. Burdge, G. C., A. E. Jones, and S. A. Wootton. 2002. Eicosapentaenoic and docosapentaenoic acids are the principal products of alpha-linolenic acid metabolism in young men. *Br. J. Nutr.* **88:** 355–364.
19. Nuttens, M. C., M. Romon, J. B. Ruidavets, D. Arveiler, P. Ducimetiere, J. M. Lecerf, J. L. Richard, J. P. Cambou, C. Simon, and J. L. Salomez. 1992. Relationship between smoking and diet: The MONICA-France project. *J. Intern. Med.* **231:** 349–356.
20. Bolton-Smith, C., M. Woodward, C. A. Brown, W. C. S. Smith, and H. Thunstall-Pedoe. 1991. Nutrient intakes from current, ex- and never smokers: results from the Scottish Heart Health Study. *Proc. Nutr. Soc.* **50:** 36A.
21. Pawlosky, R. J., J. R. Hibbeln, J. A. Novotny, and N. Salem, Jr. 2001. Physiological compartmental analysis of α-linolenic acid metabolism in adult humans. *J. Lipid Res.* **42:** 1257–1265.
22. Pawlosky, R. J., J. R. Hibbeln, and N. Salem, Jr. 2007. Compartmental analyses of plasma n-3 essential fatty acids among male and female smokers and nonsmokers. *J. Lipid Res.* **48:** 935–943.
23. Strogaad, H., S. D. Neilsen, and C. Gluud. 1994. The validity of the Michigan Alcohol Screening Test (MAST). *Alcohol Alcohol.* **29:** 493–502.
24. Eckardt, M. J., E. S. Parker, E. P. Noble, D. J. Feldman, and L. A. Gottsckalk. 1978. Relationship between neuropsychological performance and alcohol consumption in alcoholics. *Biol. Psychiatry.* **13:** 551–564.
25. Williams, J. B., M. Gibbon, M. B. First, R. L. Spitzer, M. Davies, J. Borus, M. J. Howes, J. Kane, H. G. Pope, Jr., B. Rounsaville, et al. 1992. The Structured Clinical Interview for DSM-III-R (SCID). II. Multisite test-retest reliability. *Arch. Gen. Psychiatry.* **49:** 630–636.
26. Hollingshead, A., and F. Redlick. 1958. Social Class and Mental Illness: A Community Survey. Wiley, New York, NY.
27. Kleiner, D. E., E. M. Brunt, M. Van Natta, C. Behling, M. J. Contos, O. W. Cummings, L. D. Ferrell, Y. C. Liu, M. S. Torbenson, A. Unalp-Arida, et al. 2005. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology.* **41:** 1313–1321.
28. Pawlosky, R. J., H. W. Sprecher, and N. Salem, Jr. 1992. High sensitivity negative ion GC-MS method for detection of desaturated and chain elongated products of deuterated linoleic and linolenic acids. *J. Lipid Res.* **33:** 1711–1717.
29. Campra, J., and T. Reynolds. 1982. The hepatic circulation. *In* The Liver Biology and Pathobiology. Arias, I. M., H. Popper, D. Schachter, and D. Shafritz, editors. Raven Press, New York, NY. 635–636.
30. Green, M. H., L. Uhl, and J. B. Green. 1985. A multicompartmental model of vitamin A kinetics in rats with marginal liver vitamin A stores. *J. Lipid Res.* **26:** 806–818.
31. Morrow, J. D., B. Frei, A. W. Longmire, J. M. Gaziano, S. M. Lynch, Y. Shyr, W. E. Strauss, J. A. Oates, and L. J. Roberts 2nd. 1995. Increase in circulating products of lipid peroxidation (F2-isoprostanes) in smokers — smoking as a cause of oxidative damage. *N. Engl. J. Med.* **332:** 1198–1203.
32. Murdeach, R., D. Norman, J. A. Lawson, and G. A. FitzGerald. 1996. Modulation of oxidant stress in vivo in chronic cigarette smokers. *Circulation.* **94:** 19–25.
33. Kamimura, S., K. Gaal, R. S. Britton, B. R. Bacon, G. Triadafilopoulos, and H. Tsukamaoto. 1992. Increased 4-hydroxynonenal levels in experimental alcoholic liver disease: association of lipid peroxidation with liver fibrogensis. *Hepatology.* **16:** 448–453.
34. Meagher, E. A., O. P. Barry, A. Burke, M. R. Lucy, J. A. Lawson, J. Rokach, and G. A. FitzGerald. 1999. Alcohol-induced generation of lipid peroxidation products in humans. *J. Clin. Invest.* **104:** 805–813.
35. Moser, J., D. Bagghi, P. I. Akubue, and S. J. Stohs. 1993. Excretion of malondialdehyde, formaldehyde, acetaldehyde and acetone in the urine of rats following acute and chronic administration of ethanol. *Alcohol Alcohol.* **28:** 287–295.
36. Nordmann, R., C. Ribiere, and H. Rouach. 1992. Implication of free radical mechanisms in ethanol-induced cellular injury. *Free Radic. Biol. Med.* **12:** 219–240.
37. Tuma, D. J., G. M. Thiele, D. Xu, L. W. Klassen, and M. F. Sorrell. 1996. Acetaldehyde and malondialdehyde react together to generate distinct protein adducts in the liver during long-term ethanol administration. *Hepatology.* **23:** 872–880.
38. Nervi, A. M., R. O. Peluffo, R. R. Brenner, and A. I. Leikin. 1980. Effect of ethanol administration on fatty acid desaturation. *Lipids.* **15:** 263–268.
39. Nakamura, M. T., A. B. Tang, J. Villanueva, C. H. Halsted, and S. D. Phinney. 1994. Selective reduction of delta 6 and delta 5 desaturase activities but not delta 9 desaturase in micropigs chronically fed ethanol. *J. Clin. Invest.* **93:** 450–454.
40. Wang, D. L., and R. C. Reitz. 1983. Ethanol ingestion and polyunsaturated fatty acids: effects on the acyl-CoA desaturases. *Alcohol. Clin. Exp. Res.* **7:** 220–226.
41. Venkatesan, S., J. M. Rideout, and K. J. Simpson. 1990. Microsomal delta 9, delta 6 and delta 5 desaturase activities and liver membrane fatty acid profiles in alcohol-fed rats. *Biomed. Chromatogr.* **4:** 234–238.
42. Narce, M., J. P. Poisson, J. Bellenger, and S. Bellenger. 2001. Effect of ethanol on polyunsaturated fatty acid biosynthesis in hepatocytes from spontaneously hypertensive rats. *Alcohol. Clin. Exp. Res.* **25:** 1231–1237.
43. Pawlosky, R. J., and N. Salem, Jr. 2004. Perspectives on alcohol consumption: liver polyunsaturated fatty acids and essential fatty acid metabolism. *Alcohol.* **34:** 27–33.
44. Cho, H. P., M. T. Nakamura, and S. D. Clarke. 1999. Cloning, expression, and nutritional regulation of the mammalian δ-6 desaturase. *J. Biol. Chem.* **274:** 471–477.