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## Maintenance of lower proportions of ( $n - 6$ ) eicosanoid precursors in phospholipids of human plasma in response to added dietary ( $n - 3$ ) fatty acids

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Competition between the ( $n - 3$ ) and ( $n - 6$ ) types of highly unsaturated fatty acids can diminish the abundance of ( $n - 6$ ) eicosanoid precursors in a tissue, which in turn can diminish the intensity of tissue responses that are mediated by ( $n - 6$ ) eicosanoids. The mixture of 20- and 22-carbon highly unsaturated fatty acids maintained in the phospholipids of human plasma is related to the dietary intake of 18:2 ( $n - 6$ ) and 18:3 ( $n - 3$ ) by empirical hyperbolic equations in a manner very similar to the relationship reported for laboratory rats (Lands, W.E.M., Morris, A. and Libelt, B. (1990) *Lipids* 25, 505–516). Analytical results from volunteers ingesting self-selected diets showed an inter-individual variance for the proportion of ( $n - 6$ ) eicosanoid precursors in the fatty acids of plasma phospholipids of about 5%, but the variance among multiple samples taken from the same individual throughout the day was less (about 3%), closer to the experimental variance of the analytical procedure (about 1%). The reproducibility of the results makes it likely that analysis of fatty-acid composition of plasma lipids from individuals will prove useful in estimating the diet-related tendency for severe thrombotic, arthritic or other disorders that are mediated by ( $n - 6$ ) eicosanoids. Additional constants and terms were included in the equations to account for the effects of 20- and 22-carbon highly unsaturated ( $n - 3$ ) fatty acids in the diet. A lower constant for the 20- and 22-carbon ( $n - 3$ ) fatty acids compared to that for the 18-carbon ( $n - 3$ ) fatty acid in decreasing the ability of dietary 18:2 ( $n - 6$ ) to maintain 20:4 ( $n - 6$ ) in tissue lipids confirmed the greater competitive effectiveness of the more highly unsaturated  $n - 3$  fatty acids in the elongation/desaturation process. Also, a lower constant for direct incorporation of 20-carbon fatty acids of the  $n - 6$  vs. the  $n - 3$  type indicated a greater competitive effectiveness of 20:4 ( $n - 6$ ) relative to 20:5 ( $n - 3$ ) in reesterification after release from tissue lipids. The equations may be used in reverse to estimate the dietary intakes of the ( $n - 3$ ) and ( $n - 6$ ) fatty acids by using the composition of the fatty acids that had been maintained in plasma lipids.

### Introduction

Interpretations of the well-documented fact [1] that dietary fats may precipitate human pathophysiology need to incorporate research results and concepts recognized by two recent Nobel Prize awards. The 1982

award to Drs. Bergstrom, Samuelsson and Vane emphasized the importance to human health of cellular mediators (prostaglandins, thromboxane and leukotrienes) formed from dietary ( $n - 6$ ) fatty acids. The 1988 award to Drs. Brown and Goldstein emphasized the importance of a receptor-mediated suppression of the HMG-CoA reductase that converts dietary materials into mevalonate, isoprenoids and cholesterol. Many research reports have documented the quantitative correlations of plasma cholesterol with dietary saturated fats and cardiovascular mortality, but the cellular mechanisms linking plasma cholesterol to mortality need further clarification. In contrast, many research reports document the cellular mechanisms whereby

Correspondence to (present address): W.E.M. Lands, Division of Basic Research, National Institute on Alcohol Abuse and Alcoholism, 5600 Fishers Lane/Rm.16C-06, Rockville, MD 20857, USA. Abbreviations: HUFA, 20- and 22-carbon highly unsaturated fatty acids; SFA, saturated fatty acids; UFA, 16- and 18-carbon unsaturated fatty acids; TG, triglycerides; PL, phospholipids; S.D., standard deviation.

eicosanoid mediators cause morbidity and mortality, but the quantitative correlations linking tissue levels of eicosanoid precursors to dietary fats need further clarification. Because the precursors of ( $n - 3$ ) and ( $n - 6$ ) eicosanoids can only be obtained from dietary sources, their relative abundance in tissues is linked to their abundance in the diet.

Eicosanoids are generally synthesized discontinuously in pulsatile responses to intermittent signals. The frequency of the signals may be relatively independent of nutritional factors, but the intensity of an  $n - 6$  eicosanoid-mediated response, once initiated, will be limited by the proportions of precursors and inhibitors in the tissue lipids. The intensity of formation of active ( $n - 6$ ) eicosanoids in response to extracellular signals can have an important rate-limiting role in many pathological situations (thrombotic heart attack and stroke, chronic immune-inflammatory conditions, dysmenorrhea, headache, etc., reviewed in Ref. 2). As a result, major research efforts have been expended to define pharmacological agents that help diminish the intensity of that formation. Also, considerable biomedical research is now under way to examine the degree to which dietary ( $n - 3$ ) fatty acids may moderate the pathological actions of the ( $n - 6$ ) eicosanoids [2-4].

Detailed studies of the proportions of ( $n - 3$ ) and ( $n - 6$ ) fatty acids that are maintained in tissue lipids of rats have described the degree to which they can be influenced by dietary supplies of ( $n - 3$ ) and ( $n - 6$ ) polyunsaturated fatty acids [5-9]. The fatty-acid composition of tissue lipids is influenced by competitive metabolic interactions of fatty acids of endogenous (( $n - 7$ ) and ( $n - 9$ ) types) and exogenous (( $n - 3$ ) and ( $n - 6$ ) types) origin. Analytical results for the fatty-acid composition of circulating plasma lipids of rats reflects those metabolic interactions and they, therefore, provide insight into the composition of intracellular lipids in other tissues [10]. Two algebraic relationships have been shown to describe quantitatively the principal interactions of the exogenous dietary 18:3 ( $n - 3$ ) and 18:2 ( $n - 6$ ) in rats [10], a linear equation for incorporation into tissue triglycerides, and a hyperbolic equation for conversion into the 20- and 22-carbon highly unsaturated fatty acids (HUFA) of phospholipids. For a wide range of dietary intakes of 18:3 ( $n - 3$ ) and 18:2 ( $n - 6$ ), these two general relationships successfully reflect the recognized general metabolic selectivities that maintain fatty-acid composition in tissue lipids [4,11,12].

Published data on average world-wide compositions of fatty acids in the lipids of human plasma (summarized in Refs. 3, 4, 12) indicate that about 80% of total fatty acids are the endogenous type of fatty acids (14:0, 16:0, 18:0, 16:1 ( $n - 7$ ), 18:1 ( $n - 7$ ) and 18:1 ( $n - 9$ )) with the exogenous fatty acids in typical samples showing a prevalence of the ( $n - 6$ ) type (18:2

( $n - 6$ ), 20:3 ( $n - 6$ ), 20:4 ( $n - 6$ ) and 22:4 ( $n - 6$ )) over the ( $n - 3$ ) type (18:3 ( $n - 3$ ), 20:5 ( $n - 3$ ), 22:5 ( $n - 3$ ) and 22:6 ( $n - 3$ )). The general patterns of fatty acids in lipids of human plasma (esp. in the USA [4]) resemble those for the lipids of rat plasma [10], supporting the concept that the general metabolic selectivities for fatty acid esterification into glycerolipids may be similar in rats and humans [12]. Quantitative nutrition studies with rats indicate that the proportions of ( $n - 6$ ) HUFA maintained in the HUFA of plasma phospholipids and other tissue phospholipids have similar relationships to dietary supplies [10]. Other reports indicate that the magnitude of the proportions in tissue lipids relate to the intensity of forming ( $n - 6$ ) eicosanoids (e.g., Refs. 7, 8, as discussed in Ref. 13). If a quantitative hyperbolic relationship were demonstrated for humans, as it was for rats [10,13], we could use that relationship to estimate the average dietary intake of ( $n - 3$ ) and ( $n - 6$ ) acids and to predict a probable intensity of formation and function of ( $n - 6$ ) eicosanoids. For this reason, we tested the degree to which the quantitative metabolic insights developed from the study of experimental animals [10] might be applied to data from humans.

This report describes empirical quantitative relationships between the abundance of dietary ( $n - 3$ ) and ( $n - 6$ ) fatty acids and the proportions of ( $n - 6$ ) eicosanoid precursors maintained in the plasma phospholipids of humans. We examined the fatty-acid composition in plasma from three separate groups of individuals who were participating in on-going nutrition studies. In this way, the plasma samples were associated with known dietary intakes, allowing a test of the limits of applicability of the equations for estimating the effect of the intake of ( $n - 3$ ) and ( $n - 6$ ) polyunsaturated fatty acids upon the levels of eicosanoid precursors that are maintained in the phospholipids of humans.

## Materials and Methods

The research blended detailed lipid-analytical methods with the professional efforts of nutritionists, dietitians, phlebotomists and physicians in interviewing, determining nutrient intake and acquiring plasma samples from the human subjects under carefully controlled conditions.

*Subjects for group A.* The study population consisted of 17 male and 4 female outpatients with a dyslipidemia most consistent with Frederickson's type IIa hyperlipoproteinemia (triglycerides  $< 300$  mg/dl, LDL cholesterol  $> 175$  mg/dl and HDL cholesterol  $< 45$  mg/dl). This determination was made within the year prior to screening and while the patients discontinued taking any anti-hyperlipidemic drugs or nutritional sup-

plements for at least 8 weeks prior to enrollment in the study. All members of this group were between 37 and 68 years old (mean = 55) and living in the Chicago area. The study protocol was approved by the Rush-Presbyterian-St.Luke's Medical Center Institutional Review Board.

*Subjects for group B.* A group of 14 volunteer women, aged  $33 \pm 5$  years (mean  $\pm$  S.D.), premenopausal, healthy as evaluated by the UIC health service physician or the advisory committee physician were recruited to the study from the population of women (faculty, staff and students) on the UIC campus and nearby educational and medical institutions. The group had an average body mass index (BMI) of  $28 \pm 6$  kg/m<sup>2</sup> and were in the range of 25th to 90th percentile of body weight for women in the USA.

All subjects in this group were consuming a daily minimum of 1600 kcal and had fasting serum cholesterol levels above the 50th percentile for their age and race using the Lipid Research Clinics Data as a reference population [14]. All were willing to forego use of aspirin during the period of study, willing to consume only the meals and snacks provided for the 6 month experimental period and willing to provide blood samples at regular intervals (and provide other samples and measurements as per protocol) The study was approved by the Human Subjects Institutional Review Board of the University of Illinois at Chicago.

*Experimental design and diet protocols.* As part of the study protocol for Group A, patients were instructed by a registered dietician to adhere to the American Heart Association Diet which contained less than 300 mg of cholesterol/day, saturated fatty acids representing less than 9% of the total daily calories energy ( $< 9$  en%), polyunsaturated fatty acids representing at least 10 en% with total fat at about 30–32 en%. Dietary compliance was assessed using diet diaries collected for all food and beverage consumption for four days (Monday, Wednesday, Saturday and Sunday) prior to their scheduled visit when plasma specimens were collected for fatty acid analysis. The diaries were analyzed for calories (kcal), protein (g), fat (g), percent of calories from fat (en%), saturated fat (g), polyunsaturated fat (g) including 18:2 ( $n = 6$ ), 18:3 ( $n = 3$ ), 18:4 ( $n = 3$ ), 20:4 ( $n = 6$ ), 20:5 ( $n = 3$ ), 22:5 ( $n = 3$ ), 22:6 ( $n = 3$ ), and cholesterol (mg), using the University of Minnesota Nutrient Data System Version 2.1. Although variability was large, the group's mean daily intake conformed to the instructions, with cholesterol at 200 mg (125–301); total fat at 30 en% (14–49); 18:2 ( $n = 6$ ) at 6.8 en% (2–15) and 18:3 ( $n = 3$ ) at 0.68 en% (0.2–2.0).

For Group B, the Health Habits Questionnaire [15] was used to assess the level of energy and nutrient intake in the self-selected pre-study diets and the controlled, weighed diets (B38 and B30). Only samples

from women with reliable dietary questionnaires ( $n = 14$ ) were used in the analytical work. The diets consumed during the controlled diet period were developed using the Nutrition Data System Version 20D (University of Minnesota School of Public Health Nutrition Coordinating Center). All meals and snacks were prepared in the Nutrition and Metabolism Laboratory of the University of Illinois at Chicago and homogenized aliquots (500–700 g) of individual meals and 4-day composite samples were collected in plastic containers, labeled, dated and frozen at  $-20^{\circ}\text{C}$ . The frozen aliquots were packed in solid CO<sub>2</sub> and transported by air to Hazleton Laboratories in Madison, WI, where total calories were determined by bomb calorimetry, fat content by acid hydrolysis and fatty-acid composition by gas chromatography.

During enrollment into the study, subjects in Group B described in detail their daily food intake with their customary self-selected diet, which included an average of 2064 kcal, 37 en% total fat, 13.6 en% saturated fat, 5.9 en% 18:2 ( $n = 6$ ), 0.64 en% 18:3 ( $n = 3$ ) and 0.1 en% ( $n = 3$ ) HUFA.

During the first 28 days of the study with Group B, subjects ate a controlled reference diet consisting of about 38% fat with a P/S ratio of 0.5. This reference diet was designed to approximate the average diet typical of USA. Three sets of diets were prepared to meet different caloric needs and maintain body weight. The en% of linoleate and linolenate, respectively, was: 1600 cal diet, 5.60 and 0.71; 1900 cal diet, 5.84 and 0.48; 2200 cal diet, 7.52 and 0.57. Meal aliquots were collected for monitoring fat, fatty acid, and approximate nutrient composition of the meals served during the study.

From 29 days to 141 days, the subjects in Group B were fed an experimental diet of around 30% fat (P/S 1.0). The en% of linoleate and linolenate was as follows: 1600 cal diet, 7.44 and 0.79; 1900 cal diet, 7.54 and 0.84; 2200 cal diet, 7.56 and 0.79.

*Supplemented diets.* Group C consisted of eight patients (2 females, 6 males; average age 59 years) with hyperlipoproteinemia of either type IIb (5 persons) or type IV (3 persons) were studied as outpatients while consuming marine oil as part of their prescribed lipid-lowering therapy. At the time of analysis, the patients had been consuming 3, 6 or 9 1-g capsules per day of Superepa (52% ( $n = 3$ ) HUFA; Pharmacaps, Elizabeth, NJ) for at least 12 months. Three-day dietary records from each patient, collected within one week prior to the plasma specimen collection, confirmed that mean nutrient intakes for the group were in compliance with the National Cholesterol Education Program Step 1 diet for which all patients had been previously instructed by a registered dietician. The average diet contained 30 en% fat (of which 1/3 or less was saturated) and  $< 300$  mg of cholesterol. The study protocol



was approved by the Rush-Presbyterian-St. Luke's Human Subjects Institutional Review Board.

**Plasma preparation.** Blood was drawn prior to the diet intervention and at specified dates afterward. Fasting blood samples obtained after a 12–14 h overnight fast were routinely collected. Another series of 16 sequential samples was collected at 30–60 min intervals throughout the day while subjects maintained a normal meal schedule. At the University of Illinois, blood was placed in plastic tubes containing citrate as anticoagulant, mixed and centrifuged for 15 min at room temperature. Thereafter plasma was transferred to the plastic vials, frozen at  $-20$  or  $-40^{\circ}\text{C}$  and kept in this form until analysis. At the Chicago Center for Clinical Research, the blood was placed in plastic tubes containing EDTA as anticoagulant, mixed, and centrifuged at  $1500 \times g$  for 15 min at room temperature. Plasma was then transferred to smaller tubes for storage at  $-70^{\circ}\text{C}$  until analysis.

**Fatty-acid analysis.** A mixture of standards in  $10 \mu\text{l}$  [16] was spotted on a 5-cm wide lane of the TLC plate, and then  $100 \mu\text{l}$  of plasma was applied and allowed to air dry. Plates were partially developed to 1.5 cm first in methanol, then in chloroform/methanol (1:1), to extract lipids from the plasma protein that remained at the origin [16]. After evaporating these solvents, each plate was fully developed to 18 cm in hexane/diethyl ether/acetic acid (80:20:1). The resulting lipids bands were visualized by spraying the plate with rhodamine (0.02% in 95% ethanol). Lipid fractions were scraped into tubes and methyl esters were prepared by transesterification with  $\text{BF}_3$ /methanol. The hexane solutions of methyl esters (with  $25 \mu\text{l}$  of decane to prevent spontaneous evaporation to dryness) were placed on an autosampler and analyzed by gas-liquid chromatography on a Hewlett Packard Model 5890A chromatograph fitted with a split injection system and a flame ionization detector using a silica capillary column Durabond-225 ( $30 \times 0.25 \text{ mm i.d.}$ ,  $0.25 \mu\text{m}$  thickness). Hydrogen was used as carrier gas at a flow rate of 1.3 ml/min and the oven temperature was programmed from  $140$ – $240^{\circ}\text{C}$  as described earlier [10,16]. Peak areas were integrated, stored on hard disk of a Hewlett Packard Vectra AT and the stored results were converted electronically to a final spreadsheet format (Lotus 1-2-3) for display [10]. Mean values are given with standard deviations (noted S.D. in the tables).

## Results and Discussion

### *Fatty-acid composition of plasma triglycerides*

Table I provides results for plasma triglycerides of the volunteers in Group A and Group B (diets B38 and B30). In accord with previously described general acyl-group selectivities in the formation of glycerolipids [11], saturated fatty acids (SFA), which are esterified at

position 1 of the three glycerol hydroxyls, were approx. one-third (36%) of total fatty acids in plasma triglycerides. In contrast, the 16- and 18-carbon unsaturated fatty acids (UFA), abundant at positions 2 and 3, were about 60% of the triglyceride fatty acids and the 20- and 22-carbon highly unsaturated fatty acids (HUFA) were only about 3.4%. The values are similar to the 31% SFA, 65% UFA, and 4.7% HUFA reported for plasma triglycerides of rats raised on corn oil-supplemented diets [10]. The average linoleic acid content (Table I) was 20% (range of 17.9 to 25.5) and linolenic acid averaged only about 0.9% (range of 0.7 to 1.1). The average ratio for the weight percentage (wt %) of each of these two acids in plasma triglycerides relative to its percentage of daily caloric intake (en%) is shown in the two bottom rows): 2.84 for 18:2 ( $n - 6$ ) and 1.31 for 18:3 ( $n - 3$ ). These ratios are similar, but not identical, to those reported for rats: 2.95 and 1.84, respectively. Such similarity was not expected initially, but it was subsequently joined by many other experimental results that repeatedly confirmed an appreciably similar metabolic selectivity for fatty acid esterification in humans and rodents.

The controlled diets, B38 and B30, were developed to provide nutrient intakes typical for Americans. As a result, the fatty-acid compositions maintained in the plasma lipids for the group with the self-selected diets were very similar to the overall combined average results obtained with all dietary regimens. Although the average values for groups of individuals were similar, there was among the individual subjects an appreciable interindividual variance in the amounts of specific fatty acids, regardless of the diet. These large inter-individual variances were evident in the 18:2 ( $n - 6$ ) and 18:3 ( $n - 3$ ) of plasma triglycerides for all diet groups even though less variance occurred with controlled diets than self-selected diets. The differences may reflect individual metabolic differences of genetic origin, as well as the cumulative effects of different prior diets that would have affected the adipose tissue stores of 10 to 20 kg of fat per person, which are continuously mixing with the other precursors of the plasma lipids produced by the liver. The overall variance among different individuals and different diets for the ratio of the weight percent of a polyunsaturated fatty acid in triglycerides to the en% of that acid in the diet was about 15% for 18:2 ( $n - 6$ ) ( $2.84 \pm 0.37$ ) and about 25% for 18:3 ( $n - 3$ ) ( $1.31 \pm 0.32$ ).

### *Postprandial variance in fatty-acid composition*

To see if intra-individual sampling variances might make a significant contribution to the range of values obtained, three subjects in Group B were examined at 16 to 17 different times throughout the day. In this way, the differences between the values for the plasma



TABLE III

*Intra-individual variance in fatty acid composition of plasma phospholipids*

Fatty acid	Subject 1		Subject 2		Subject 3		Overall average					
	0-4.5 h n = 7		0-4.5 h n = 9		0-4.5 h n = 8		n = 50					
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.				
14:0	5.84	1.85	0.54	0.04	0.59	0.08	0.86	0.16	0.92	0.15	1.86	1.98
16:0	32.34	0.81	31.73	0.57	32.56	0.49	34.70	1.00	35.77	0.36	33.49	1.54
18:0	11.09	1.16	14.19	0.30	14.04	0.37	11.83	0.47	10.48	0.42	12.54	1.52
16:1 (n-7)	0.48	0.07	0.45	0.08	0.55	0.06	1.24	0.21	1.09	0.16	0.72	0.34
18:1 (n-9)	7.47	0.24	7.65	0.29	7.68	0.45	9.96	0.68	9.93	0.81	8.50	1.20
18:1 (n-7)	1.40	0.06	1.40	0.03	1.38	0.01	1.33	0.51	1.61	0.04	1.40	0.23
18:2 (n-6)	25.15	0.20	25.83	0.76	29.30	0.31	25.80	0.52	27.62	1.09	27.06	1.64
18:3 (n-6)	0.21	0.23	0.08	0.03	0.07	0.04	0.03	0.07	0.01	0.03	0.07	0.11
18:3 (n-3)	0.28	0.03	0.27	0.03	0.21	0.04	0.23	0.03	0.25	0.03	0.23	0.05
20:1 (n-9)	0.04	0.05	0.11	0.03	0.07	0.02	0.17	0.13	0.16	0.20	0.14	0.11
20:3 (n-9)	0.02	0.03	0.08	0.02	0.18	0.02	0.00	0.00	0.00	0.00	0.03	0.04
20:2 (n-6)	0.23	0.05	0.24	0.02	0.29	0.03	0.27	0.04	0.25	0.01	0.26	0.04
20:3 (n-6)	1.97	0.17	1.92	0.03	1.44	0.05	2.26	0.14	2.21	0.08	1.88	0.32
20:4 (n-6)	9.40	0.66	8.65	0.17	8.06	0.20	7.25	0.42	6.36	0.16	8.04	1.03
22:4 (n-6)	0.28	0.06	0.25	0.05	0.18	0.00	0.27	0.04	0.24	0.03	0.06	0.07
20:3 (n-3)	0.12	0.08	0.10	0.05	0.03	0.04	0.42	0.03	0.38	0.01	0.24	0.05
20:5 (n-3)	0.59	0.04	0.49	0.03	0.45	0.02	0.54	0.10	0.45	0.03	0.47	0.07
22:5 (n-3)	0.60	0.08	0.48	0.03	0.45	0.01	0.35	0.10	0.30	0.06	0.50	0.08
22:6 (n-3)	2.49	0.28	2.14	0.08	2.56	0.11	2.47	0.44	1.98	0.09	2.41	0.37
Total SFA (%)	49.26	0.96	49.87	1.09	47.20	0.71	47.40	0.97	47.17	0.47	47.89	1.49
Total UFA (%)	34.99	0.41	35.68	0.89	39.17	0.64	38.60	1.22	40.51	0.39	38.01	2.09
Total HUFA (%)	15.58	1.21	14.24	0.34	13.46	0.35	13.65	1.16	12.02	0.28	13.87	1.28
20:4 as %												
(n-6) HUFA	79.14	0.42	78.23	0.32	80.86	0.33	72.08	0.50	70.22	0.87	76.69	4.12
22:6 as %												
(n-3) HUFA	67.58	1.47	68.88	0.29	74.10	0.25	71.96	1.60	70.40	0.66	71.24	2.59
(n-7) as %												
of UFA	5.37	0.14	5.19	0.24	4.70	0.18	6.65	1.28	6.67	0.43	5.57	0.97
20:5 + 22:5												
as % HUFA	7.66	0.48	6.80	0.16	6.57	0.46	6.97	0.22	6.91	0.22	6.96	0.39
20:4 + 20:3												
as % HUFA	72.97	0.73	74.18	0.61	70.66	0.48	69.87	2.57	71.32	1.47	71.49	2.07

triglycerides obtained throughout the day for each individual (Table II) reflected food influx and different metabolic stages as well as the variances due to the analytical techniques employed. The variance in triglyceride fatty acids was low for the eight sequential samples obtained throughout the morning (0 to 4.5 h), before the absorbed dietary fat began to influence the composition of the circulating triglycerides. The intra-individual variance for the morning samples approached that expected for the analytical method [16]. The ratio of 18:3 ( $n - 3$ )/18:2 ( $n - 6$ ) in the morning samples was also reproducible with little intra-individual variance. The eight samples obtained in the afternoon (4.5 to 9 h) had lower percentages of ( $n - 7$ ) UFA and total HUFA, whereas the percent of 18:3 ( $n - 3$ ) tended to be slightly higher in the afternoon compared to the morning samples. Also, the afternoon values for the fatty-acid composition tended to have higher variances, indicating the influence of changing

levels of exogenous fat entering the plasma triglycerides during this time period. The combined overall results that included both intra- and inter-individual differences (right-hand column in Table II) had the greatest variance.

Intra-individual variations in fatty-acid composition of plasma phospholipids (Table III) were less than those for triglycerides (Table II), although differences among individuals were significant. For example, ( $n - 7$ ) acids as a percent of phospholipid UFA and 22:6 ( $n - 3$ ) as a percent of ( $n - 3$ ) HUFA differed among the three subjects, irrespective of the time of day. However, relatively small difference among these individuals (who were ingesting the same diet) was noted for the two parameters that were selected to interpret the dietary effects on eicosanoid precursors: 20:5 + 22:5 ( $n - 3$ ) and 20:3 + 20:4 ( $n - 6$ ) as a percent of total phospholipid HUFA. The low variance for these parameters supports the concept that they may be

TABLE IV

Fatty-acid composition of plasma phospholipids

Diet:	A		B		B38		B30		Overall average	
en% 18:2 ( $n - 6$ )	7.62	3.29	5.98	0.30	5.94	0.32	7.49	0.05		
en% 18:3 ( $n - 3$ )	0.74	0.40	0.73	0.03	0.72	0.03	0.81	0.02		
	$n = 21$		$n = 18$		$n = 16$		$n = 18$		$n = 73$	
Fatty acid	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
14:0	0.75	0.30	0.65	0.16	1.39	0.47	0.72	0.19	0.87	0.41
16:0	34.25	1.52	32.99	1.42	37.54	3.11	32.62	2.43	34.22	2.87
18:0	14.20	1.44	13.15	0.96	13.49	1.03	14.69	1.45	13.91	1.44
16:1 ( $n - 7$ )	1.00	0.36	0.84	0.16	0.66	0.15	1.19	0.47	0.93	0.37
18:1 ( $n - 9$ )	10.89	2.52	9.95	1.52	8.05	0.86	10.44	1.38	9.94	2.02
18:1 ( $n - 7$ )	1.53	0.30	1.64	0.14	1.54	0.13	1.55	0.19	1.57	0.21
18:2 ( $n - 6$ )	23.06	3.07	23.25	3.32	22.86	2.62	24.03	2.92	23.29	2.97
18:3 ( $n - 6$ )	0.18	0.09	0.08	0.03	0.09	0.07	0.15	0.12	0.13	0.10
18:3 ( $n - 3$ )	0.23	0.09	0.16	0.04	0.26	0.05	0.21	0.06	0.21	0.07
20:1 ( $n - 9$ )	0.17	0.05	0.20	0.07	0.16	0.10	0.19	0.05	0.18	0.07
20:3 ( $n - 9$ )	0.08	0.08	0.00	0.00	0.07	0.06	0.09	0.02	0.06	0.06
20:2 ( $n - 6$ )	0.31	0.07	0.31	0.07	0.23	0.04	0.28	0.07	0.29	0.07
20:3 ( $n - 6$ )	2.23	0.57	2.37	0.81	1.80	0.34	1.98	0.47	2.13	0.60
20:4 ( $n - 6$ )	7.56	1.62	10.30	2.12	8.10	1.31	8.14	1.19	8.54	1.92
22:4 ( $n - 6$ )	0.25	0.07	0.37	0.13	0.34	0.22	0.28	0.09	0.30	0.14
20:5 ( $n - 3$ )	0.42	0.14	0.31	0.17	0.41	0.14	0.40	0.10	0.39	0.14
22:5 ( $n - 3$ )	0.56	0.20	0.57	0.12	0.46	0.15	0.64	0.38	0.56	0.25
22:6 ( $n - 3$ )	1.84	0.50	2.34	0.49	2.03	0.60	1.84	0.39	1.97	0.53
24:0	0.49	0.30	0.51	0.23	0.53	0.27	0.47	0.35	0.49	0.29
Total SFA (%)	49.20	2.35	46.79	1.02	52.42	3.22	48.04	2.26	49.12	3.25
Total UFA (%)	36.91	4.16	35.92	3.28	33.46	3.10	37.57	3.33	36.11	3.80
Total HUFA (%)	13.23	2.25	16.57	2.54	13.94	2.14	13.66	1.68	14.24	2.55
20:4 as % ( $n - 6$ )										
HUFA	72.94	4.37	76.68	7.71	77.36	2.50	76.02	5.38	75.61	5.35
22:6 as % ( $n - 3$ )										
HUFA	65.05	5.98	72.97	3.72	69.45	5.10	64.68	6.66	67.31	7.23
( $n - 7$ ) as % of UFA	6.96	1.48	6.96	0.85	6.64	0.77	7.37	1.81	6.98	1.35
20:5 + 22:5 as %										
HUFA	7.54	2.44	5.21	0.96	6.53	1.39	7.45	2.11	6.77	2.12
20:4 + 20:3 as %										
HUFA	73.64	5.26	76.50	2.31	73.77	3.73	74.28	3.11	74.72	4.11

reliable indices, useful in estimating the relative abundance of eicosanoid precursors and the potential intensity of eicosanoid-mediated responses for an individual as discussed earlier [13]. The low variance means that a gas chromatographic analysis of the fatty acids maintained in plasma lipids may give more reliable insight into the average proportions of ingested ( $n-3$ ) and ( $n-6$ ) fatty acids than is available from less reliable dietary recall procedures that have high variance [17,18].

#### Fatty-acid composition of plasma phospholipids

The average composition of fatty acids in the phospholipids in human plasma followed the general pattern of metabolic selectivity for fatty acids reviewed earlier [4,11], averaging approx. 49% SFA, 36% UFA, and 14.6% HUFA (Table IV). This distribution follows the widely recognized metabolic selectivity that places SFA at one of the two phospholipid hydroxyls (position 1) and UFA and HUFA at the other (position 2), giving 50% SFA and 50% UFA + HUFA. No significant differences were observed for the proportions of these general categories of fatty acids for the four different groups of subjects in this study and the pattern was similar to that seen with rats [10]: 45% SFA; 29% UFA; 27% HUFA. In addition, the four sets of data in Table IV showed no significant difference in either the proportion of 20:4 ( $n-6$ ) in the ( $n-6$ ) HUFA or the proportion of 22:6 ( $n-3$ ) in the ( $n-3$ ) HUFA, confirming the reproducibility of the selectivities for elongation, desaturation and esterification. The average proportion of phospholipid HUFA in the form of 20:5 + 22:5 ( $n-3$ ) rose steadily for Group B as the subjects sequentially ingested the controlled diets, B38 and B30, reaching a final value close to that for Group A. Average intakes of ( $n-3$ ) HUFA were well-documented for the controlled diets, whereas the self-selected diets had a high intra-individual variance in

the intake of ( $n-3$ ) HUFA as well as a well-recognized imprecision in reporting actual intakes. Perhaps this slow shift originated from traditionally low intakes of ( $n-3$ ) fatty acids in the self-selected diets for Group B prior to the controlled feeding study which systematically included modest amounts of tuna and salmon. Patients in Group A had been counselled previously by nutrition specialists and the resulting self-selected diets appeared to contain slightly more seafood than the self-selected diets of uncounselled individuals who enrolled in Group B.

#### Predictions of fatty acids in phospholipids

Two different types of equations relate the fatty acid compositions of diets with those in plasma lipids: a linear relationship for triglycerides and a hyperbolic relationship for phospholipid HUFA (10). Since each type of equation relates to the en% of 18:2 ( $n-6$ ) and 18:3 ( $n-3$ ) in the diet, the composition of fatty acids in plasma triglycerides can be used to predict the composition of ( $n-3$ ) and ( $n-6$ ) HUFA in plasma phospholipids. The values obtained from such predictions can be compared to the experimentally determined values to test the appropriateness of the four constants ( $C_3$ ,  $C_6$ ,  $C_O$  and  $K_S$ ) employed in the empirical hyperbolic equations [10]

( $n-3$ ) as %HUFA

$$= \frac{100}{1 + C_3 / \text{en}\%3(1 + \text{en}\%6 / C_6 + \text{en}\%O / C_O + \text{en}\%3 / K_S)} \quad (1)$$

( $n-6$ ) as %HUFA

$$= \frac{100}{1 + C_6 / \text{en}\%6(1 + \text{en}\%3 / C_3 + \text{en}\%O / C_O + \text{en}\%6 / K_S)} \quad (2)$$

These equations include constants,  $C_3$  and  $C_6$ , that characterize the competitive interactions of dietary

TABLE V

#### Predicted composition of HUFA in phospholipids

Average values (wt%;  $n = 18$ ): 18:2 ( $n-6$ ) in TG, 19.3; 18:3 ( $n-3$ ) in TG, 0.8. Estimated supply (en%;  $n = 18$ ): 18:2 ( $n-6$ )/2.84, 6.8 and 18:3 ( $n-3$ )/1.31, 0.6.

	HUFA composition (% HUFA)		
	Predicted in PL based on constants <sup>a</sup>	observed in plasma PL	predicted based on new constants <sup>b</sup>
20:3 + 20:4 ( $n-6$ )	73.1	77.1	77.1
20:5 + 22:5 ( $n-3$ )	5.0	5.3	5.3
Sum		82.5	82.4

<sup>a</sup>  $C_6 = 0.0400$ ;  $C_3 = 0.0600$ ;  $C_O = 5.0000$ ;  $K_S = 0.1500$ .

<sup>b</sup>  $C_6 = 0.0405$  0.0357 0.0305 0.0215 0.0325 0.0590  
 $C_3 = 0.0580$  0.0510 0.0435 0.0310 0.0475 0.0850  
 $C_O = 5.0000$  5.0000 5.0000 5.0000 9.0000 9.0000  
 $K_S = 0.2100$  0.1800 0.1500 0.1000 0.1500 0.3000

18:3 ( $n - 3$ ) and 18:2 ( $n - 6$ ) during elongation, desaturation and incorporation into the HUFA of phospholipids. The values of  $C_3$  and  $C_6$  represent a standard effective concentration of dietary 18:3 ( $n - 3$ ) and 18:2 ( $n - 6$ ) expressed as a percent of total calories (en%). Additional constants,  $C_O$  and  $K_S$ , were included to adjust for a small effect of other dietary fatty acids ( $C_O$ ) and for shape fitting ( $K_S$ ), respectively, as explained in detail in an earlier publication [10]. The equations do not include terms for dietary HUFA which were not provided in the diets of the rats [10] and are also not appreciable components of the average self-selected diets of typical Americans or the subjects in this study (being near 0.1 en% for ( $n - 3$ ) and ( $n - 6$ ) HUFA [19]).

Eqns. 1 and 2 provided a close estimate of the observed and predicted values for an independent set of samples (Table V) by first using the proportionality constants from Table I (2.84 and 1.31) and the weight percent values of 18:2 ( $n - 6$ ) and 18:3 ( $n - 3$ ) in plasma triglycerides to estimate an approximate dietary influx. The unexpectedly close similarity of the values for the two constants to those obtained earlier for rat data [10] helps emphasize the similarity in the general selectivities for fatty acids in the synthesis of glycerolipids with rats and humans. The values for the estimated supply of 18:2 ( $n - 6$ ) and 18:3 ( $n - 3$ ) were then used with Eqns. 1 and 2 and the constants from the rat study ( $C_6$ , 0.04;  $C_3$ , 0.06;  $C_O$ , 5.0;  $K_S$ , 0.15) to predict the proportion of 20:3 + 20:4 ( $n - 6$ ) and 20:5 + 22:5 ( $n - 3$ ) in the phospholipid HUFA.

For example, in Table V the experimentally determined average compositions of HUFA in plasma phospholipids of early morning samples obtained on day 28 of the study for 18 subjects in Group B are compared with the values predicted by the hyperbolic equations and constants that were used in the dietary studies with rats [10]. The average predicted values (column 1) were similar to those experimentally determined by gas chromatography (column 2). The similarity indicated that the dietary 18:3 ( $n - 3$ ) and 18:2 ( $n - 6$ ) were exerting an influence on the levels of ( $n - 3$ ) and ( $n - 6$ ) HUFA maintained in phospholipids of humans that was very similar to that for rats. Although the fit of observed and predicted values was close, an empirical adjustment of the constants to fit this set of data more closely was achieved by starting with the values for rats [10] and then selecting new values by trial and error that fit best. For example, values that fit data in Table V were:  $C_6$ , 0.0405;  $C_3$ , 0.0580;  $C_O$ , 5;  $K_S$ , 0.21. This set of values, however, was not a unique solution to the equations and several other sets of constants that are shown in Table V also permitted the same good fit to this set of data.

The applicability of this empirical approach was confirmed for all four dietary groups in this study as demonstrated for fatty acids in the HUFA of plasma phospholipids in Table VI, which compares the average values observed and the values predicted using the analyzed fatty acid composition of plasma triglycerides and equations and constants similar to those noted above. Table VI indicates that the linear equation and

TABLE VI

*Relationships between triglycerides and phospholipids of human plasma*

Constants for PL of human plasma:  $C_3$ , 0.0555;  $C_6$ , 0.0441;  $C_O$ , 5.00;  $K_S$ , 0.20.

	Diets							
	Diets self selected				Controlled diets			
	Group A ( $n = 21$ )		Group B ( $n = 18$ )		Group B38 ( $n = 18$ )		Group B30 ( $n = 18$ )	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
<b>Diet composition</b>								
en% 18:2 ( $n - 6$ )	7.62	3.20	—	—	5.94	0.32	7.49	0.05
en% 18:3 ( $n - 3$ )	0.74	0.40	—	—	0.72	0.03	0.81	0.02
<b>Plasma triglyceride composition</b>								
wt% 18:2 ( $n - 6$ )	20.31	3.82	17.29	5.08	18.51	2.29	20.48	4.45
wt% 18:3 ( $n - 3$ )	0.99	0.35	0.73	0.28	0.9	0.23	0.81	0.15
<b>Predicted diet composition</b>								
en% 18:2 ( $n - 6$ )	7.23	1.36	6.06	1.91	6.59	0.81	7.29	1.58
en% 18:3 ( $n - 3$ )	0.75	0.27	0.69	0.25	0.69	0.17	0.62	0.11
<b>Predicted HUFA composition in PL</b>								
( $n - 3$ ) as % HUFA	7.57	1.54	8.16	1.55	7.94	1.73	6.6	1.42
( $n - 6$ ) as % HUFA	73.38	0.93	68.37	0.92	75.11	1.23	74.04	1.41
<b>Observed HUFA composition in PL</b>								
( $n - 3$ ) as % HUFA	7.36	2.39	5.88	1.43	6.33	1.73	7.27	1.76
( $n - 6$ ) as % HUFA	73.57	4.94	76.17	2.33	73.84	1.23	73.88	2.96

the constants from Table I, relating the weight percent of 18:2 ( $n - 6$ ) and 18:3 ( $n - 3$ ) in plasma triglycerides to the dietary energy percent of these acids performed well for Groups A and B30. The predicted amounts of 18:2 ( $n - 6$ ) and 18:3 ( $n - 3$ ) in the self-selected diets for Group A (7.23 and 0.75) closely fit the actual dietary intake (7.62 and 0.74). For Group B30, after weeks of consistent dietary intake, the fit for 18:2 ( $n - 6$ ) was 7.29 compared to 7.49 in the diet. Predicting the proportions of ( $n - 3$ ) and ( $n - 6$ ) in the HUFA of plasma phospholipids using the constants from Table V permitted estimates that were close to observed proportions, but trial-and-error fitting gave slightly modified values that fit better for the larger overall combined set of data in Table VI:  $C_6$ , 0.0441;  $C_3$ , 0.0555;  $C_O$ , 5;  $K_S$ , 0.20. These constants resemble those that fit Table V ( $C_6$ , 0.0405;  $C_3$ , 0.0580;  $C_O$ , 5;  $K_S$ , 0.21) and are similar to those used to describe results from rats [10]:  $C_6$ , 0.04;  $C_3$ , 0.06;  $C_O$ , 5;  $K_S$ , 0.15.

The diets consumed by the subjects in this study were typical of those consumed by US populations which include very small quantities of ( $n - 3$ ) fatty acids and relatively little HUFA [19]. Therefore, it is likely to be difficult to test the equations further by finding large numbers of people in the USA who are habituated to diets that cover a wide range of ( $n - 3$ ) fatty acids and total en% of fat. In contrast to this experiment with a narrow range of data available for humans on different diets, the equations and constants developed for predicting results with rats were generated using a wide range of diets with the rats habituated to these diets through their lifetime (the corresponding dams were even fed the same diets before gestation). That experimental design ensured that the liver, adipose and plasma fatty acids in the animals that were analyzed were probably in dynamic equilibrium with the dietary supply of fatty acids. Although such an equilibrated state seems less likely with human subjects, the similarity of analytical values in Tables I–V indicate that Diet B was similar to the typical self-selected diet in the USA and that the plasma and adipose fatty acids were in near equilibrium with regard to the diet.

#### *Fatty-acid composition maintained with fish oil supplements*

An important consideration in relating the average fatty-acid composition of diet with the average composition maintained in tissues is the period of time over which the average diet has been ingested. The animal studies discussed in this report had maintained diets for a major portion of the life of the animal so that tissue lipids were probably in dynamic equilibrium with the dietary supply of fatty acids. Other studies for shorter periods produced similar trends, although a

lesser degree of change suggests that equilibration had not yet been achieved (e.g., Ref. 20) reviewed in Ref. 10). Results in this report for humans ingesting supplemental ( $n - 3$ ) HUFA were only from patients who had maintained the prescribed supplementation for at least 12 months. Other studies for shorter periods (e.g., Refs. 21, 22) reported similar trends with supplemental ( $n - 3$ ) HUFA, although the extent of equilibration may have been less than achieved with the greater than 12-month regimen in this study.

The average overall composition of fatty acids in triglycerides of human plasma in this ( $n - 3$ ) HUFA study resembled a typical American average (from several studies tabulated in Ref. 4), containing about 33% SFA (vs. 34.1), 61% UFA (vs. 60) and 5% HUFA (vs. 3.2). Although the total HUFA in triglycerides remained low when supplemental HUFA were ingested, the dietary ( $n - 3$ ) HUFA caused a several-fold increase in the average ratio of ( $n - 3$ )/( $n - 6$ ) HUFA (from 0.6 to 2.8). Linoleic acid (18:2 ( $n - 6$ )) and linolenic acid (18:3 ( $n - 3$ )) were both at higher levels in the triglycerides of patients than observed for control subjects, reflecting a greater-than-typical intake of dietary polyunsaturated fatty acids by the patients in the prescribed ongoing lipid-lowering therapy. All values for 18:2 ( $n - 6$ ) in plasma triglycerides in this study exceeded those obtained in 1973 to 1975 [23], reflecting rising intakes of 18:2 ( $n - 6$ ) in the USA (discussed in Ref. 16).

For the patients taking fish oil supplements, the average overall fatty-acid composition of plasma phospholipids resembled the USA averages, including about 48% SFA, 35% UFA and 18% HUFA. However, the HUFA in phospholipids of the patients contained approx. 51% 20:3 + 20:4 ( $n - 6$ ) and 25% 20:5 + 22:5 ( $n - 3$ ), whereas average results typical of the USA are 75% and 10%, respectively. The proportion of 20:5 within the ( $n - 3$ ) HUFA of phospholipids increased from 19% to about 40% with supplemental fish oil in the diet while the proportion of 22:6 ( $n - 3$ ) in the ( $n - 3$ ) HUFA remained at about 50%. In contrast, the proportions of 20:3 and 20:4 within the ( $n - 6$ ) HUFA (22% and 77%, respectively) were not appreciably influenced by supplementation with ( $n - 3$ ) HUFA. This stability in the proportions of two major ( $n - 6$ ) HUFA helps simplify the considerations of forming ( $n - 6$ ) eicosanoid precursors and competition between the ( $n - 3$ ) and ( $n - 6$ ) types of fatty acids is the predominant consideration. The accumulation of 18:2 ( $n - 6$ ) and the near exclusion of 18:3 ( $n - 3$ ) in phospholipids in all tissues (while both are accumulated in triglycerides) has been observed consistently in all reports of rats and humans, but the metabolic basis for this discrimination between the two fatty acids remains unknown. Because this discrimination occurs, it is important to separate the phospholipids from the trigly-

cerides when attempting to use plasma lipid composition to estimate the amounts of 18:2 ( $n-6$ ) and 18:3 ( $n-3$ ) in the diet.

#### New equations for predicting phospholipid HUFA

The weight percent of 18:2 ( $n-6$ ) and 18:3 ( $n-3$ ) in plasma triglycerides listed in Table VII and the proportionality constants obtained earlier (2.84 and 1.31; Table I) to relate those values to dietary supply for humans were used to estimate a probable average proportion of dietary calories of these two fatty acids for each person's daily intake (Rows 3 and 4 in Table VII). These estimates, with the hyperbolic equations 1 and 2 and the constants ( $C_6$ , 0.0441;  $C_3$ , 0.0555;  $C_O$ , 5.00;  $K_S$ , 0.20) that were used successfully in the earlier studies of this report were then employed to predict the proportion of 20:5 + 22:5 ( $n-3$ ) and 20:3 + 20:4 ( $n-6$ ) in the HUFA of plasma phospholipids of the patients (Rows 7 and 10, Table VII). Control values averaged from reports of subjects eating typical American diets are shown in the right-hand column of Table VII for comparison. Although the predicted values for the USA subjects for ( $n-3$ ) and ( $n-6$ ) in HUFA (rows 7 and 10) were similar to the experimentally measured values (determined by gas chromatography; rows 9 and 12), the predicted values for ( $n-3$ ) HUFA were not at all similar for the patients taking fish oil supplements. This discrepancy indicated that the supplementary ( $n-3$ ) HUFA ( $H_3$ ) were exerting a competitive effect that did not occur with typical American dietary fatty acid intakes for which the two 18-carbon UFA, 18:3 ( $n-3$ ) and 18:2 ( $n-6$ ) ( $P_3$  and  $P_6$ ), were the predominant polyunsaturated fatty acids

(about 0.6 en% ( $n-3$ ) and 5.6 en% ( $n-6$ )) and HUFA are minor components ( $<0.1$  en% ( $n-3$ ) and  $<0.1$  en% ( $n-6$ ), Ref. 19).

To accommodate the influence of dietary ( $n-3$ ) HUFA ( $H_3$ ), terms were included in Eqns. 3 and 4 to account for the competitive interactions of the HUFA ( $H_3$  and  $H_6$ ) in direct esterifications, as well as in the elongation/desaturation process.

( $n-3$ ) as %HUFA

$$= [100] \left[ 1 + PC_3 / \text{en}\%P_3 (1 + \text{en}\%P_6 / PC_6 + \text{en}\%H_6 / HI_6 + \text{en}\%O / C_O + \text{en}\%P_3 / K_S) \right]^{-1} + \frac{100}{1 + HC_3 / \text{en}\%H_3 (1 + \text{en}\%H_6 / HC_6)} \quad (3)$$

( $n-6$ ) as %HUFA

$$= [100] \left[ 1 + PC_6 / \text{en}\%P_6 (1 + \text{en}\%P_3 / PC_3 + \text{en}\%H_3 / HI_3 + \text{en}\%O / C_O + \text{en}\%P_6 / K_S) \right]^{-1} + \frac{100}{1 + HC_6 / \text{en}\%H_6 (1 + \text{en}\%H_3 / HC_3)} \quad (4)$$

The new empirical equations include constants ( $HC_3$  and  $HC_6$ ) for the efficiency of direct esterification of dietary ( $n-3$ ) and ( $n-6$ ) HUFA ( $H_3$  and  $H_6$ ) as well as constants ( $HI_3$  and  $HI_6$ ) for the competitive inhibition by the dietary HUFA in elongation and desaturation of the ( $n-3$ ) and ( $n-6$ ) dietary UFA. Assignment of the values of the constants was achieved by starting with the values obtained from the study of

TABLE VII

Estimates of HUFA and PUFA intake for human subjects

	Subject: 1	2	3	4	5	6	7	8	USA
(1) wt% 18:2 ( $n-6$ ) in TG	17.0	22.4	14.5	27.4	24.4	26.9	13.0	27.9	21.0
(2) wt% 18:3 ( $n-3$ ) in TG	1.3	0.7	1.1	2.6	1.4	1.3	1.0	1.6	0.9
(3) est. en% 18:2 ( $n-6$ )	6.0	7.9	5.1	9.6	8.6	9.5	4.6	9.8	7.4
(4) est. en% 18:3 ( $n-3$ )	1.0	0.5	0.8	2.0	1.1	1.0	0.7	1.2	0.7
(5) est. en% ( $n-6$ ) HUFA	0.08	0.06	0.08	0.04	0.09	0.15	0.13	0.03	0.05
(6) est. en% ( $n-3$ ) HUFA	1.89	1.89	2.84	0.95	0.95	1.89	1.89	2.84	0.05
20:5 + 22:5 ( $n-3$ ) as% HUFA in plasma PL									
(7) Pred. Eqn. 1	10.6	4.9	10.4	13.4	8.4	7.5	10.6	8.5	6.5
(8) Pred. Eqn. 3	26.3	21.1	32.2	22.4	16.7	21.7	25.0	32.0	7.0
(9) obs. ( $n-3$ ) as % HUFA	24.2	20.6	30.1	28.6	13.2	20.7	25.4	36.6	7.1
20:3 + 20:4 ( $n-6$ ) as% HUFA in plasma PL									
(10) Pred. Eqn. 2	71.4	76.1	71.0	70.5	73.9	74.7	70.5	74.0	74.8
(11) Pred. Eqn. 4	42.7	46.3	32.6	57.2	64.8	60.0	44.3	37.9	80.5
(12) obs. ( $n-6$ ) as % HUFA	46.2	52.3	46.6	44.4	62.7	58.3	49.9	32.7	76.9
Estimates of ( $n-3$ ) HUFA intake based upon fatty-acid analyses									
(13) Estimated en% $H_3$	1.74	2.02	2.70	1.98	0.55	1.66	1.92	4.25	0.08
(14) Estimated capsules	6	6	9	6	2	5	6	13	0
(15) Prescribed capsules	6	6	9	3	3	6	6	9	0

unsupplemented diets and adjusting the new constants by trial and error to fit the specific data set in this report.

#### Estimates of values for constants

Empirical fits of the fatty-acid composition of diets with those maintained in tissue lipids were obtained with sets of inter-related constants without providing absolute or unique values to be assigned (Table V). Successive approximation of appropriate values for the additional constants of Eqns. 3 and 4 was achieved following initial estimates of the dietary intake of ( $n-3$ ) and ( $n-6$ ) fatty acids. The average value for the supplemented intake of ( $n-3$ ) HUFA (en%  $H_3$ ) was set initially at the prescribed therapeutic level, since the supplements greatly exceeded the 0.05 en% ( $n-3$ ) HUFA obtained in the foods. Dietary ( $n-6$ ) HUFA (en%  $H_6$ ) was set at the values estimated by nutrient assessment questionnaire for each individual (ranging from 0.03 to 0.15 en%). The 'other' (non-polyunsaturated fatty acids) dietary fatty acids were set at 25 en% and the intakes of 18:2 ( $n-6$ ) (en%  $P_6$ ) and 18:3 ( $n-3$ ) (en%  $P_3$ ) were estimated from the composition of plasma triglycerides using the factors of 2.84 and 1.31 from Table I. These estimates permitted Eqns. 3 and 4 to be used for empirical trial-and-error estimates of values for the constants. From this process, a set of suitable values was identified for the constants:  $PC_3$ , 0.0555;  $PC_6$ , 0.0441;  $C_O$ , 5.00;  $K_S$ , 0.175;  $HC_3$ , 8.75;  $HC_6$ , 0.5;  $HI_3$ , 0.008;  $HI_6$ , 0.040. These constants with the expanded Eqns. 3 and 4 provided much better predictions of the HUFA composition for the individuals in this study (rows 8 and 11, Table VII) than did Eqns. 1 and 2.

The usefulness of the newly derived equations and constants became evident when they were applied to interpreting results from an independent diet-eicosanoid study of rats [24] which included both ( $n-3$ ) UFA and ( $n-3$ ) HUFA in the diet and a study of mice ingesting supplemental ( $n-3$ ) HUFA [25]. There was excellent agreement of the observed formation of thromboxane by rat platelets with values predicted using the same equations and constants that describe fatty acid maintenance in the lipids of human plasma (setting the numerator to 175 ng TXB/ml rather than 100). The close agreement in Fig. 1 for the formation of thromboxane, the principal eicosanoid formed by platelets, demonstrates an unexpected ability of the new equations and constants to predict the effects on eicosanoid formation by diets that contain ( $n-3$ ) acids of either the UFA or HUFA type. It also confirms the utility of employing the analytically determined proportion of 20:3 + 20:4 ( $n-6$ ) in the phospholipid HUFA as a predictor of the probable intensity of ( $n-6$ ) eicosanoid formation. As in Fig. 1, a good fit of observed and predicted values over a wide range of

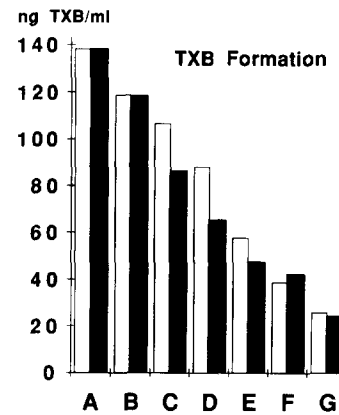


Fig. 1. Predicted and observed formation of thromboxane by rat platelets. The predicted thromboxane formation (open bars) was obtained using published diet information [24] with Eqn. 4 and the same constants developed in Table VII for humans with a numerator value of 175 ng thromboxane per ml. Values for the measured thromboxane are indicated by solid bars.

dietary ( $n-3$ ) HUFA was obtained in Fig. 2, although it required  $K_S$  to be adjusted to 0.090 and  $HI_3$ , 0.03. The good prediction indicates that the general metabolic selectivities that maintain tissue HUFA in rats and humans also prevail in mouse peritoneal cells. Expressing the ( $n-6$ ) HUFA as a proportion of the total phospholipid HUFA provided a better fit than expected, irrespective of different types of glycerophospholipid that are contained within the total tissue lipids. The lower values for  $HI_3$  needed to fit the results in Figs. 1 and 2 compared to that for  $PC_3$  (0.0555 en%) confirm earlier estimates [13,24] that the ( $n-3$ ) HUFA are more effective than ( $n-3$ ) UFA as competitors in diminishing the entry of dietary 18:2 ( $n-6$ ) into the ( $n-6$ ) HUFA of tissue lipids. The successful independent tests of the suitability of the

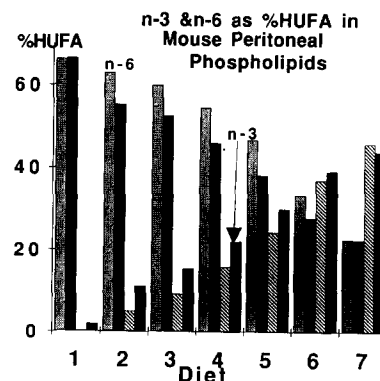


Fig. 2. Predicted and observed levels of eicosanoid precursors in mouse peritoneal cells. The predicted proportions of 20:3 + 20:4 ( $n-6$ ) (light stippled bars) and 20:5 + 22:5 ( $n-3$ ) (hatched bars) in the HUFA of cellular phospholipids were calculated using published diet information [25] and Eqns. 3 and 4 with constants similar to those developed for humans as described in the text. The corresponding observed analytical values are solid bars and dark stippled bars, respectively.

hyperbolic equations indicates that further refinement and use of this approach will probably improve our ability to interpret diet-eicosanoid relationships in animals and humans. Although a unique assignment of the absolute values for the constants is not yet possible at this time, the empirically derived equations and sets of values provided in this report are useful ways to assemble and summarize the existing knowledge about human metabolism in ways that predict future results in studies of lipids in humans and that may indicate the probable intensity of  $(n-6)$  eicosanoid-mediated events.

Future controlled studies of humans with a wide range of en% fat and a widely varied intake of  $(n-3)$  and  $(n-6)$  fatty acids could provide data that might permit a unique solution for the constants. However, recruiting volunteers for rigorously controlled long-term human studies with a wide range of en% fat, en%  $(n-3)$  HUFA and en%  $(n-6)$  HUFA seems not very feasible at this time with the low general level of awareness of the utility of such information. Fortunately, this report adds to the growing evidence of extensive similarities between rats and humans in the selectivity of fatty-acid metabolism. The similarities will eventually permit some of the broader range-finding studies with rats to develop more certain knowledge of the absolute values for the constants involved. Those values can then be applied to human studies to help define more precise limits for the constants when applied to humans.

#### *Estimation of dietary intake for humans*

Once an approximate set of constants was assigned, they were tested with a rearranged form of Eqn. 3 using the observed analytical results and starting with an arbitrary rough estimate of typical HUFA intake of 0.12 en%  $(n-6)$  HUFA and 0.1 en%  $(n-3)$  HUFA to calculate a probable amount of dietary  $(n-3)$  HUFA (en%  $H_3$ ). The calculated value (row 13, Table VII) was then used to calculate the probable number of capsules ingested (row 14, Table VII) by the patients (and the typical USA controls). The result agreed closely with the prescribed dosage for all patients except subjects 4 and 8. Subject 8 was the lightest female in the study (148 pounds, 77 years old) who had been advised to ingest 9 capsules per day for two years. We remain uncertain as to whether she had taken extra supplements. Subject 4 was the lightest male in the study (138 pounds) and he had higher than typical amounts of 18:3  $(n-3)$  in plasma triglycerides. The values for  $(n-3)$  as %HUFA in plasma phospholipids fitted an ingestion of 6 fish oil capsules per day, even though he claimed only three per day was the regular supplement. When this individual was questioned further by the nutritionist in the study, he confirmed that he was taking only 3 fish oil capsules daily, but admit-

ted that he was including two additional slices of flax-bran bread (Manitowoc Ovens, Manitowoc, WI) that contained 950 mg 18:3  $(n-3)$ , explaining the elevated 18:3  $(n-3)$  in plasma triglycerides. He was also supplementing his diet with non-marine oil sources of extra  $(n-3)$  HUFA (e.g., seaweed, etc.) that had not been declared in the dietary records because it was not fish oil. This interaction illustrates the well-known difficulty of standard recording procedures for obtaining valid estimates of dietary intake by free-living individuals on self-selected diets [17,18]. The ability of the gas chromatographic analysis to detect non-compliance not reported by standard nutritional assessment interview methods illustrates the usefulness of the equations and constants and the power of the direct gas chromatographic method for assessing dietary intake of  $(n-3)$  and  $(n-6)$  polyunsaturated fatty acids. This successful application of the equations, combined with the recently developed ability to perform the analysis on 50  $\mu$ l samples of blood [16], opens the possibility of a large-scale study of diet-disease relationships such as were proposed recently for Japan [16,26].

#### *Interpreting the equations and constants*

The extensive set of data with rats that was provided by Mohrhauer and Holman [5,6] was confirmed and extended by the recent set from this laboratory [10]. The results consistently indicated a competitive hyperbolic interaction of dietary 18:2  $(n-6)$  and 18:3  $(n-3)$  in forming tissue HUFA [10,12,13]. The constants (about 0.05 en%) employed in fitting the dietary  $(n-3)$  and  $(n-6)$  fatty acids are analogous to Michaelis constants used in describing the hyperbolic interactions of substrates with enzymes. As a result, the magnitude of the constant represents the dietary supply that achieves a standard degree of effectiveness. With supplies of material at 10-times that value (about 0.5 en%), the system can be expected to approach a maximal response. The low values of the constants confirm the long-standing evidence that a biologically adequate level of 18:2  $(n-6)$  in the diet of rats is 0.3 en% [5,6]. In fact, the corresponding adequate dietary level for human infants was indicated in 1965 to be near 0.5 en% (Ref. 27, as discussed in Ref. 13).

#### *Application of equations to diet-disease relationships*

Because many chronic disease processes are associated with an overproduction of  $(n-6)$  eicosanoids [2], it is appropriate to consider carefully what level of  $(n-6)$  eicosanoid precursors is desirable to be maintained in human tissues. The equations and constants developed in this report were used to predict the multiple curvilinear patterns for  $(n-6)$  eicosanoid precursors that we should expect for humans with different dietary intakes of 18:2  $(n-6)$  and  $(n-3)$  HUFA (see Fig. 3). The same proportion of  $(n-6)$

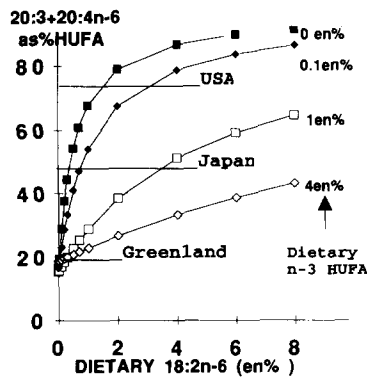


Fig. 3. Proportion of  $(n-6)$  eicosanoid precursors in the HUFA of plasma phospholipids. The curves represent values calculated by Eqn. 4 using the constants developed for Table VII. The three values on the ordinate axis for populations in the USA, Japan and Greenland were reviewed in Ref. 4. This figure was modified from a similar one in FASEB J. [28].

eicosanoid precursors in phospholipid HUFA can be achieved by different combinations of  $(n-3)$  and  $(n-6)$  acids without changing the total fat intake. As dietary 18:2  $(n-6)$  increases from 0 to 2 en%, the proportion of 20:3 + 20:4  $(n-6)$  that is accumulated in tissue phospholipids rises to near maximal values when  $(n-3)$  fatty acids are minor components of the diet. Increasing the amount of dietary  $(n-3)$  fatty acids diminishes the accumulation of  $(n-6)$  eicosanoid precursors. The equation predicts that Greenlanders with diets of about 2 en% 18:2  $(n-6)$  and 4 en%  $(n-3)$  HUFA would have about 20% of their phospholipid HUFA as 20:3 + 20:4  $(n-6)$ . This agrees well with the value of 22% reported in the literature (reviewed in Refs. 4 and 13), confirming the applicability of the equations and constants in this report. Similar confirmation came from results for adult Japanese with diets averaging about 5 en% 18:2  $(n-6)$ , 0.6 en% 18:3  $(n-3)$  and 2 en%  $(n-3)$  HUFA who would be expected to have about 50% of their phospholipid HUFA as  $(n-6)$  eicosanoid precursors. This agrees well with the 50% reported (reviewed in Ref. 13). However, the rapid shift towards lower  $(n-3)/$  $(n-6)$  ratios in the diet of younger Japanese [26] means that a future rise can be expected for the proportion of  $(n-6)$  HUFA in the total HUFA of plasma phospholipids of typical Japanese. Finally, Americans with an average intake of 6 en% 18:2  $(n-6)$ , 0.7 en% 18:3  $(n-3)$  and  $<0.1$  en%  $(n-3)$  HUFA [20] would be expected to have about 75% of their phospholipid HUFA as  $(n-6)$  eicosanoid precursors, as confirmed by the average analytical values for USA (Table VII). The successful fit for a wide range of dietary levels of  $(n-3)$  HUFA for these three groups of humans demonstrates the validity of the quantitative approach described in this report.

The quantitative metabolic link between diet and accumulated  $(n-6)$  eicosanoid precursors in tissues can now be applied to interpreting the impact of dietary polyunsaturated fatty acids upon the frequency and severity of pathological disorders that are known to be mediated by  $(n-6)$  eicosanoids (for example, thromboxane mediating thrombotic death or prostaglandins and leukotrienes mediating rheumatoid arthritis; reviewed in Ref. 2). When tissue HUFA are mobilized during pathological processes in these disorders, the intensity of the process will be dependent upon the proportion of  $(n-6)$  fatty acids in the non-esterified HUFA pool from which the oxygenases obtain the substrates for eicosanoid biosynthesis (Ref. 29, reviewed in Ref. 4). Since 1973 [30], it is evident that competition with  $(n-3)$  HUFA for these enzymes can diminish the conversion of the  $(n-6)$  HUFA into active eicosanoids and thereby diminish the pathophysiology. The present report provides an algorithm for evaluating quantitatively the influence of dietary fatty acids upon the physiology and epidemiology of diseases that are mediated by  $(n-6)$  eicosanoids. In this manner, the values on the ordinate axis in Fig. 3 reflect the probable intensity of an  $(n-6)$ -mediated process, and the curves indicate its dependency upon dietary supplies of 18:2  $(n-6)$  and  $(n-3)$  HUFA.

An extensive longitudinal study [19] showed that Americans in the upper quintile of  $(n-3)$  HUFA intake (about 0.66 g per day, approx. 0.3 en%) had only 60% of the relative risk for cardiovascular death for men in the USA. Similarly, Japanese ingesting approximately 5–10-times more  $(n-3)$  HUFA had 20% of the typical USA rate for ischemic heart disease mortality [26]. Finally, the corresponding mortality rate for Greenlanders was only 10% that for typical Danes (reviewed in Ref. 2), who have diets and cardiovascular mortality rates similar to those for Americans. Similarly, the incidence of rheumatoid arthritis in Japan is much less than would be expected on the basis of HLA genotypes [31]. Recent intervention trials have confirmed the tendency for supplemental  $(n-3)$  HUFA in the diet to diminish the rate of cardiovascular mortality [32] and diminish the severity of rheumatoid arthritis [33].

Our understanding of diet-disease relationships has now evolved so that the quantitative impact of dietary  $(n-3)/$  $(n-6)$  fatty acids upon the probable synthetic capacity of  $(n-6)$  eicosanoids can be added to our knowledge of the  $(n-6)$  eicosanoids in detailed cellular mechanisms for the disease processes. Important in interpreting these events, is the fact that tissues in rats [10,34] and humans [16] have a tendency to maintain higher ratios of  $(n-3)/$  $(n-6)$  HUFA in the non-esterified fatty acid eicosanoid precursor pools than is maintained in tissue phospholipids. This tendency provides a downward adjustment in the probable  $(n-6)$

icosanoid synthetic capacity that is predicted from the proportions of  $(n-3)/(n-6)$  eicosanoid precursors in the phospholipids. This downward adjustment of the  $(n-6)$  eicosanoid synthetic capacity from the phospholipid precursors that can be predicted from dietary  $(n-3)/(n-6)$  data permits an even closer correlation of dietary intake with the observed epidemiological data, and it emphasizes the physiological importance of dietary  $(n-3)$  fatty acids. The combined use of the knowledge of cellular signalling mechanisms of eicosanoids and the knowledge of quantitative relationships of eicosanoid precursor abundance with diet now puts us in an improved position to interpret the probable impact of our daily intakes of  $(n-3)/(n-6)$  fatty acids upon the quality of daily life, which is so strongly influenced by  $(n-6)$  eicosanoids.

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### References

- 1 The Surgeon General's Report on Nutrition and Health (1988) DHHS (PHS) Publication No. 88-50210.
- 2 Lands, W.E.M. (1986) *Fish and Human Health*, pp. 1-170, Academic Press, Orlando, FL.
- 3 Lands, W.E.M. (1989) *J. Int. Med.* 225 (Suppl. No.731), 11-20.
- 4 Lands, W.E.M. (1991) *Annu. Rev. Nutr.* 11, 41-60.
- 5 Mohrhauer, H. and Holman, R.T. (1963) *J. Lipid Res.* 4, 151-159.
- 6 Mohrhauer, H. and Holman, R.T. (1963) *J. Nutr.* 81, 67-74.
- 7 Hwang, D.H. and Carroll, A.E. (1980) *Am. J. Clin. Nutr.* 33, 590-597.
- 8 Hwang, D.H., Boudreau, M. and Chanmugam, P. (1988) *J. Nutr.* 118, 427-437.
- 9 Prasad, M.R., Culp, B. and Lands, W.E.M. (1987) *J. Biosci.* 11, 443-453.
- 10 Lands, W.E.M., Morris, A. and Libelt, B. (1990) *Lipids* 25, 505-516.
- 11 Lands, W.E.M. and Crawford, C.G. (1977) in *Membrane Bound Enzymes* (Martinosi, A., ed.), pp. 3-185, Plenum, New York.
- 12 Lands, W.E.M., Morris, A. and Libelt, B. (1991) in *AOCS Short Course on Health Effects of Dietary Fatty Acids* (Nelson, G., ed.), pp. 21-41, American Oil Chemists' Society, Champaign.
- 13 Lands, W.E.M. (1991) in *World Review of Nutrition and Diet* (Simopoulos, A.P., Kifer, R.E., Martin, R.R. and Barlow, S.E., eds.), Vol. 66, pp. 177-194, Karger, Basel.
- 14 *Lipid Research Clinics Population Studies Data Book* (1980) I. The Prevalence Study, National Institutes of Health, Washington, DC.
- 15 Block, G., Coyle, L., Smucker, R., Harlan, L., Hartman, A. and Kessler, L. (1989) *Health Habits and History Questionnaire: Diet History and other Risk Factors*. Personal Computer System Packet, National Cancer Institute, Division of Cancer Prevention and Control, Bethesda, MD.
- 16 Ohta, A., Mayo, M.C., Kramer, N. and Lands, W.E.M. (1990) *Lipids* 25, 742-747.
- 17 Basiotis, P.P., Welsh, S.O., Cronin, F.J., Kelsay, J.L. and Mertz, W. (1987) *J. Nutr.* 117, 1638-1641.
- 18 Liu, K., Stamler, J., Dyer, A., McKeever, J. and McKeever, P. (1989) *J. Chronic Dis.* 31, 399-418.
- 19 Dolecek, T.A. and Grandits, G. (1991) in *World Review of Nutrition and Diet*, (Simopoulos, A.P., Kifer, R.E., Martin, R.R. and Barlow, S.E., eds.), Vol. 66, pp. 205-216, Karger, Basel.
- 20 Iritani, N. and Fujikawa, S. (1982) *J. Nutr. Sci. Vitaminol.* 28, 621-629.
- 21 Von Schacky, C., Fischer, S. and Weber, P.C. (1985) *J. Clin. Invest.* 76, 1626-1631.
- 22 Sinclair, A.J., O'Dea, K., Dunstan, G., Ireland, P.D. and Niall, M. (1987) *Lipids* 22, 523-529.
- 23 Holman, R.T., Smythe, L. and Johnson, S. (1979) *Am. J. Clin. Nutr.* 32, 2390-2399.
- 24 Hwang, D.H., Boudreau, M. and Chanmugam, P. (1988) *J. Nutr.* 118, 427-437.
- 25 Broughton, K.S., Whelan, J., Hardardotter, I. and Kinsella, J.E. (1991) *J. Nutr.* 121, 155-164.
- 26 Lands, W.E.M., Hamazaki, T., Yamazaki, K., Okuyama, H., Sakai, K., Goto, Y. and Hubbard, V.S. (1990) *Am. J. Clin. Nutr.* 51, 991-993.
- 27 Cuthbertson, W.F.J. (1976) *Am. J. Clin. Nutr.* 29, 559-568.
- 28 Lands, W.E.M. (1992) *FASEB J.* 6, 2530-2536.
- 29 Lands, W.E.M. and Samuelsson, B. (1968) *Biochim. Biophys. Acta* 164, 426-429.
- 30 Lands, W.E.M., LeTellier, P.R., Rome, L.H., and Vanderhoek, J.Y. (1973) *Adv. Biosci.* 9, 15-27.
- 31 Shichikawa, K., Takenaka, Y., Maeda, A., Yoshino, R., Tsujimoto, M., Ota, H., Kashiwade, T. and Hongo, I. (1981) *Ryumachi* 21 (Suppl.), 35-43.
- 32 Burr, M.L., Fehily, A.M., Gilbert, J.F. et al. (1989) *Lancet* ii, 757-761.
- 33 Cleland, L.G., French, J.K., Betts, W.H., Murphy, G.A. and Elliott, M.J. (1988) *J. Rheumatol.* 15, 1471-1475.
- 34 Lands W.E.M. (1988) *Adv. Prostaglandin, Thromboxane Leukotriene Res.* 19, 602-605.