Prevalence of Essential Fatty Acid Deficiency in Patients With Chronic Gastrointestinal Disorders

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Patients with chronic intestinal disorders causing malabsorption, nutritional losses through diarrhea, or catabolic illness would be expected to have essential fatty acid (EFA) deficiency (EFAD), but such deficiency has not been demonstrated in patients treated in accordance with the prevailing standard of care. We studied plasma fatty acid patterns of 56 reference or control subjects and 47 patients with chronic intestinal disorders (mostly Crohn's disease) using high-resolution capillary column gas-liquid chromatography. Patients exhibited a shift in fatty acid metabolism similar to that previously shown to be associated with EFAD. Compared with control subjects, patients had (1) decreased polyunsaturated fatty acid (PUFA) levels (43.7% *v* 50.4%, $P < .0001$), (2) increased monounsaturated fatty acid (MUFA) levels (25.8% *v* 22.0%, $P < .0001$), (3) higher ratios of mead (20:3ω9) to arachidonic (20:4ω6) acid (0.020 *v* 0.013, $P < .04$), and (4) lower concentrations of total (214 *v* 284 mg/dL, $P < .01$), saturated ([SFA] 63 *v* 75 mg/dL, $P < .001$), MUFA (56 *v* 63 mg/dL, $P < .001$), and PUFA (93 *v* 143 mg/dL, $P < .001$). Patients had metabolic shifts toward increased production of MUFA and an increased ratio of derivatives to precursors of ω6 fatty acids, shifts that occur when cells are EFA-deficient. More than 25% of the patients had biochemical evidence of EFAD according to at least one criterion. Optimal diagnosis requires a concurrent evaluation of concentrations of fatty acids in plasma and in lipoproteins (percent fatty acids). On indices of EFA status that depend on percents, ratios, or concentrations of fatty acids or on the production of abnormal fatty acids, the patients were between patients with severe whole-body EFAD and healthy subjects, a state referred to as absolute EFA insufficiency. Patients with chronic intestinal disease should be evaluated for likely EFA deficiencies and imbalances, and treated with substantial amounts of supplements rich in EFAs, such as oral vegetable and fish oils, or intravenous lipids if necessary.

PATIENTS WITH chronic intestinal disorders such as inflammatory bowel disease, sprue, and short bowel syndrome often suffer from fat malabsorption and nutritional deficiencies, as well as extraintestinal or systemic complications.[1] Fat malabsorption may occur in Crohn's disease due to loss of absorptive surface through disease or surgery. In ulcerative colitis, patients may lose weight due to poor intake and the catabolic stress of illness. One would suspect that these factors or the increased demand for essential fatty acids (EFAs) for tissue repair and membrane formation would lead to EFA deficiency (EFAD), abnormal fatty acid profiles, abnormal precursors of eicosanoids, and suboptimal cell function in these conditions. However, there have been few studies of fatty acid profiles in free-living patients. Previous studies either have not evaluated the nature or extent of EFAD or have not found evidence of EFAD. In fact, EFAD is considered a rare condition primarily limited to adults receiving fat-free parenteral alimentation and some newborns.[2] Patients with chronic intestinal disease are rarely tested or treated for fatty acid abnormalities because most physicians assume that it is highly unlikely that patients have EFAD. We propose that EFAD has not been found because methods previously used to detect it were not sensitive enough and that EFAD is highly prevalent among these patients.

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The major fatty acid groups are the saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids. SFAs and MUFAs are nonessential because humans can derive them from proteins and carbohydrates.[3] Fatty acids undergo desaturation and elongation in the body via enzymes apparently shared among fatty acid families; their affinity for these enzymes follows the order, ω3 > ω6 > ω9 > ω7 fatty acids.[4] We abbreviate the four precursor or parent fatty acids (linolenic, linoleic, oleic, and palmitoleic) as PFAi (i = 3, 6, 9 and 7 for the ω3, ω6, ω9, and ω7 families), and their elongation and desaturation products are referred to collectively as derivatives, abbreviated DFAi (i = 3, 6, 9, and 7).[5] The term PFA3 refers to α-linolenic acid (18:3ω3) and DFA3 to all its derivative fatty acids such as eicosapentaenoic (20:5ω3) and docosahexaenoic (22:5ω3). Similarly, the term PFA6 refers to linoleic acid (18:2ω6) and DFA6 to all its derivative fatty acids such as arachidonic acid (20:4ω6). PFA3 and PFA6 constitute the EFAs. A useful formula to remember is PUFA = EFA + EFA derivatives = (PFA3 + PFA6) + (DFA3 + DFA6) = ω3 + ω6 (exceptions are small quantities of polyunsaturated derivatives of MUFAs produced in the presence of EFAD, such as 20:3ω9, and isomers or *trans* of the above fatty acids).

Absolute or whole-body EFAD refers to the condition in which whole-body amounts of EFAs are low (usually involving the ω6 family). The percent and concentration of plasma EFAs decline and the percents of ω7 and ω9 increase. These metabolic shifts lead to a higher percent of 20:3ω9 in plasma and a higher triene to tetraene ratio (T/T), 20:3ω9/20:4ω6.[6] Using methods developed by the first author, which improved the accuracy of 20:3ω9 1 order of magnitude,[7,8] plasma values of T/T above 0.02 indicate ω6 EFAD. These values are now used as the reference by many researchers, who cite them in their publications.

Absolute EFAD must be distinguished from relative

EFAD, found in patients with normal to elevated plasma concentration of EFAs.[9] EFA insufficiency refers to patients who have fatty acid profiles with abnormalities in the same direction as those found in EFAD subjects, namely (1) enhanced conversion of ω3, ω6, and ω9 fatty acids to their derivatives and (2) accumulation of MUFAs. However, the degree of the biochemical abnormality is smaller than in EFAD and there is less evidence of the traditional clinical signs associated with EFAD such as dermatitis, impaired wound healing, impaired growth, neurological abnormalities, decreased learning ability, and histological abnormalities in most tissues.[3,10]

The US Surgeon General recommended that studies be conducted to identify specific dietary factors that might influence the causation, prevention, and treatment of intestinal disease.[11] Although many nutritional deficiencies have been associated with intestinal disease, EFAD is not currently recognized by medical textbooks as a likely abnormality. There are good reasons to believe that patients with chronic intestinal disease may be at risk for EFAD. Such patients often have fat malabsorption and increased intestinal secretions and cell turnover.[12] However, severe EFAD with significant dermatitis is rarely found in humans and can be induced in animals only after long periods of EFA deprivation. Simply because acute manifestations of severe EFAD are not observed does not mean EFA insufficiency does not exist. EFA insufficiency is suspected of contributing to hyperlipidemia, hypertension, coronary heart disease, impaired wound healing and cell reproduction, and abnormal eicosanoid metabolism.[13] EFA insufficiency could exacerbate intestinal disease by affecting the eicosanoid balance (production and/or metabolism) and the maintenance of functional bowel by affecting cell reproduction. It is known that different fatty acids interact with each other, and the relative proportions are important determinants of cell metabolism.[14] Recent reviews identified major roles for ω3 fatty acids in health and disease,[15] described the need for balance between ω6 and ω3 fatty acids,[16] and stated the theoretical reasons that diseases may be associated with an imbalance of ω3 or ω6 fatty acids.[17] There are substantive unexplained variations in life expectancy and morbidity among patients with Crohn's disease.[18] The role of dietary fats in Crohn's disease mortality or morbidity is not known. Two studies reported abnormal fatty acids in selected cells in patients with Crohn's disease, but did not report biochemical evidence of EFAD.[19,20] It is now recognized that nutritional therapy has a fundamental role in the treatment of Crohn's disease.[21] EFAs play a major role in optimal membrane function.[22,23] An elemental diet has been found to be one of the best treatments for Crohn's disease,[24,25] but such a diet poses the problem of potential EFAD due to low levels of EFAs. The elemental diets used to treat Crohn's disease have little ω6 and practically no ω3 fatty acids (physicians are supposed to prescribe EFA supplements for patients on elemental diets). Severe ω3 deficiency has been associated with scaly, hemorrhagic dermatitis, impaired wound healing, and growth retardation.[26,27]

Eicosanoids are being recognized as mediators of inflammation in Crohn's disease,[28,29] and they are in turn influenced by fatty acid patterns.[30-32] Because the inflammatory response is a key element of inflammatory bowel disease, the relative proportions of each fatty acid in patients with chronic intestinal disease should play a major role in the course of the disease. Altered fatty acid profiles have been associated with other inflammatory processes.[33] Moreover, chronic malnutrition caused by diets low in EFAs may induce EFA abnormalities.[34,35]

There are few studies on fatty acid profiles and the existence of EFAD in patients with intestinal disease treated by their physicians in accordance with the conventional standard of care of the 1980s. Fatty acids play such a ubiquitous and fundamental role in practically all cell functions that specific clinical abnormalities due to EFAD may be impossible to detect, but improved well-being may be expected as the result of diagnosis and correction of EFA abnormalities. Early diagnosis of EFAD leads to a diet or supplements that might prevent some of the complications of intestinal disease. We hypothesized that patients with chronic intestinal disorders, including Crohn's disease, ulcerative colitis, sprue, and short bowel syndrome, have unrecognized EFAD.

Two studies by Esteve-Comas et al[36,37] found that patients with inflammatory bowel disease had elevated plasma levels of 18:3ω3 and 22:6ω3 and decreased levels of 20:3ω6. The investigators did not measure 20:3ω9 levels and found only small changes in 18:2ω6. In other words, they found elevated levels of ω3 EFAs, mild reduction of ω6 EFAs, and conflicting evidence of EFAD. Our results are different and indicate that patients have reduced EFA levels and highly significant biochemical evidence of EFAD. Our data are consistent with a previous study of patients with Crohn's disease that showed deficiencies of ω6 and ω3 fatty acids.[38]

There are many variables that characterize patients with chronic intestinal disease, such as disease type, extent of surgery, medications, diet, etc., to such an extent that it is practically impossible to predict fatty acid profiles from medical or nutritional history. Regardless of their condition or etiology, fatty acid analyses can identify treatable EFADs. We studied free-living patients with different types of chronic intestinal disease who followed their usual treatment as prescribed by their gastroenterologists. We wanted to know whether significant EFA abnormalities were present, regardless of the cause. In a future report, we shall describe how plasma fatty acids correlate with other nutritional deficiencies, specific disease entities, and indicators of disease status.

SUBJECTS AND METHODS

We analyzed 47 patients with chronic intestinal disease or resection using plasma left over from a previously reported study of vitamin K deficiency.[39] Leftover samples were kept frozen at less than −60°C and were analyzed in a timely manner shortly after collection. Samples and controls were stored under similar conditions. Our assay can identify chemicals associated with fatty acid deterioration caused by poor storage conditions or contamination with plastic storage containers (which causes unusual chromatographic peaks). None of the samples used exhibited evidence of deterioration. Fasting samples were drawn from subjects who

followed free-living diets. Subject selection was independent of fatty acid levels. The patient group (GI) consisted of 25 patients with Crohn's disease, 11 with ulcerative colitis, four with celiac sprue, and seven with short bowel syndrome. There were 27 males and 20 females (aged 16 to 64 years). None of these patients had been previously analyzed for fatty acid composition or were treated differently because of suspected fatty acid abnormalities. These patients were treated by board-certified gastroenterologists and were representative of free-living patients with intestinal disease (under a standard of care prior to 1986, which we now consider inappropriate). These patients were compared with a previously reported control group of 56 non-obese subjects (aged 25 to 65 years; 29 men and 27 women).[7] This group consisted of 24 healthy reference volunteers without any known disease and 32 randomly selected subjects from the Framingham Cardiovascular Offspring Study, cycle 3, who provide a rough indicator of prevalent levels of fatty acids. We also included in one graph (Fig 3) data from 10 patients (aged 27 to 51 years; six men and four women) with chronic malabsorption[8] who required total parenteral nutrition but received little or no intravenous lipids (patients on total parenteral nutrition not receiving intravenous lipids are now rarely found in the United States). These patients had biochemical evidence of marked EFAD. Fatty acid plasma levels were measured by capillary gas-liquid chromatography as previously described.[7] Different tissues provide different indications of fatty acid status. As a single test, a whole-plasma fatty acid profile (on fasting plasma) reporting individual and group fatty acids, ratios, and concentrations is the most effective indicator of EFA body levels *and* metabolism. As a predictor of health status, we found that such a profile provides practically all the information needed to treat patients. Adipose tissue is a better measure of body fat composition, but it provides almost no information on EFA metabolism because EFA derivatives are practically nonexistent in adipose tissue. From adipose tissue analysis, one can estimate the whole-body deficit of EFAs, but not whether the patient requires EFA derivatives to bypass a metabolic block. Red blood cell (RBC) or phospholipid fatty acids exhibit a narrower range of levels than plasma fatty acids (probably due to body homeostasis). We find that RBCs can diagnose only the more severe abnormalities. It is possible, although not proven, that RBCs are better indicators than whole plasma in cases of severe hyperlipidemia or nonfasting blood samples. Lepage et al[40] reported that "total fatty acid analysis on 0.100 mL of plasma provides as good an assessment of EFA status as the fatty acid profile of plasma, red cell, and platelet phospholipids." Whole plasma combines fatty acids from all major lipoproteins and lipid fractions and summarizes the body's regulatory response (such as increasing or decreasing lipoprotein levels); whole plasma can identify subjects with EFA abnormalities[41] and the correction of those abnormalities in response to treatment,[8] and can differentiate healthy from diseased individuals because the plasma fatty acid profile indicates the net result of factors such as drugs, diet, exercise, and antioxidants.[42,43] What distinguishes our method is the use of a long capillary column (60 m), a long-duration chromatographic method (>60 minutes) with far greater separation of very small peaks like 16:1ω7 and 20:3ω9 than previously reported, and the use of a computer-based integrating system for manual correction of improperly integrated peaks based on adjustment of baselines while visualized on a monitor (something impossible with integrators). Integrators can make substantial errors in more than 10% of peaks representing less than 5% of total fatty acids. Integration errors in small peaks can be as high as 100%.[44] These errors are caused by a combination of poor peak separation, fluctuating baselines, noise, and mathematical errors in the integration algorithm. Correct small-peak integration requires computer-controlled enhancement to visualize the baseline noise and integration baseline. Differences in separation and integration methods may account for significant differences between our results and those reported by previous investigators.

Diagnosis of EFAD

The word "concentration" has different meanings when used in the context of plasma lipids. The most familiar meaning refers to the total concentration of a particular substance in whole plasma. When we speak of fatty acid concentrations, we speak of concentrations in milligrams per deciliter of total or whole plasma. We also measured the (relative) concentration of fatty acids in whole plasma, which is approximately the relative concentration within the lipoprotein or lipid fraction of the plasma. This relative concentration is equivalent to the amount of each fatty acid as a proportion of total fatty acids. We called these values "percents" or "percentages," as they are commonly referred to in other studies. The percents depend on how many fatty acids are measured and thus may vary from one laboratory to another. The supply of EFAs and other fatty acids to cells depends on both the relative concentration in lipoproteins (percent of individual fatty acids) and the absolute concentration in plasma. Cells obtain their EFAs mostly from lipoproteins. Their ability to meet their EFA needs is limited by the number of lipoproteins they can "eat" per unit of time and how many EFAs are within each lipoprotein. Because cells acquire EFAs from lipoproteins, it is likely that the ability of fatty acids to enter into reactions is determined by their percents in lipoproteins and at the interfaces between lipids and membranes rather than in the total aqueous fraction (whole plasma). As a general rule, the higher the concentration and the percent of EFAs, the easier it is for cells to meet their EFA needs. When the needs are not met, cells shift toward production of 20:3ω9 and 16:1ω7. The ability to produce these fatty acids, particularly 20:3ω9, depends on overall nutritional status because production depends on enzyme activity, which depends on protein, vitamin, and mineral status (in a way unknown at present). These issues are particularly important in patients with intestinal disease. In the past, researchers used the T/T ratio, 20:3ω9/20:4ω6, as a measure of EFAD. However, the T/T ratio would be misleading if patients cannot make 20:3ω9. We looked at several indices described later. A healthy patient should be normal in all indices—any deviation indicates EFA abnormality. The more indices that are abnormal, the more severe the abnormality.

The concentration and percent of fatty acids in whole plasma measure the efficiency of the fatty acid transport and utilization mechanism.[5] The percents of fatty acids in whole fasting plasma reflect the way the body produces different lipids to meet cell needs. It is a summary of the relative amount in each lipid fraction (such as phospholipids, cholesterol esters, and triglycerides), and it provides the best indicator of EFA status. In our experience and that of other researchers, fatty acids in plasma lipid fractions are not sensitive enough, cost more money to analyze, and are subject to greater experimental error. For example, the data obtained by the first author (unpublished) show that EFAs within phospholipids remain relatively constant over a wide range of EFA status. Thus, phospholipids are poor indicators of EFA status except in severe EFAD.

Because accurate diagnosis requires a concurrent consideration of lipid and fatty acid concentrations in whole plasma and lipoproteins (plasma percents), we use two- or higher-dimension relationships to identify metabolic abnormalities. For these purposes, we present several figures that illustrate the two-dimensional relationships among relevant indices.

Statistics

We used nonpaired Student's *t* test analysis, adjusted for equal or unequal variance as appropriate, to compare the control population with the patients, Pearson correlation coefficient analysis, and multivariate analyses with dummy variables (equivalent to ANOVA/covariance analysis).[45] Statistical and graphics analyses were made using EXCEL (version 4.0; Microsoft, Redmond, VA) and SYSTAT (version 5.0; SYSTAT, Evanston, IL). An expert program, "Statistical Navigator" (Idea-Works, Columbia, MO), further assisted in the selection of statistical techniques. We calculated various ratios of fatty acids that correct for possible measurement errors due to water evaporation from plasma (ie, a ratio of two fatty acids compensates for potential concentration measurement errors). No significant sex differences were found in major plasma fatty acids, and therefore the data were combined for males and females in all groups (differences nonsignificant by *t* test and Mann-Whitney *U* test). To illustrate group differences, the graphs present data by group; to save space, groups are combined for statistical analyses and tabular presentation.

RESULTS

Table 1 lists the mean ± SE of the percentages of the total yield of identified fatty acids. The main pattern was that patients had higher levels (percents) of SFA and MUFA (ω9 and ω7) and lower levels of ω6 and ω3. Specifically, patients had statistically significantly higher percents of 16:0, 16:1ω7, 18:1ω7, 18:1ω9, and 20:3ω9, which were 116%, 142%, 112%, 117%, and 145% of values observed in the control population, respectively. However, the percent of 18:0 in patients was almost the same as in controls.

In both groups, most ω6 fatty acids are in the form of linoleic acid and most ω3 fatty acids are in the form of derivatives of linolenic acid. Patients had mean values for the EFAs, linoleic and linolenic acid, that were 85% and 104% of control values, and derivatives of these fatty acids such as arachidonic acid and docosahexaenoic acid were 94% and 69% of control values. The percentage of linolenic acid was small in both groups (as it is in the US population), and individual variations were large; hence, reference ranges are broad and linolenic acid differences between groups are not clinically significant. Figure 1 shows differences among key fatty acids as bars proportional to their ratios.

Table 2 presents the totals for various groups of fatty acids and Table 3 the ratios for various groups of fatty acids. The term PUFA refers all to the *cis*-EFAs plus their derivatives. Significantly higher percentages of SFAs and ω7 and ω9 fatty acids (111%, 115%, and 127% of control values) and significantly lower ($P < .01$) values of ω6 and ω3 fatty acids (88% and 77%) were noted in patients as compared with control subjects. The patients had lower percentages of derivatives of linolenic acid and higher percentages of derivatives of oleic acid in plasma, with no

Table 1. Percentages (mean ± SE) of *Cis*–Fatty Acids in Plasma of Reference Subjects and Patients

Fatty Acid	Reference Subjects (n = 56)	GI Patients (n = 47)	*P*
16:0	18.36 ± 0.17	21.36 ± 0.28	.0001
18:0	6.98 ± 0.08	7.35 ± 0.15	.05
20:0	0.17 ± 0.01	0.10 ± 0.01	.002
22:0	0.42 ± 0.02	0.27 ± 0.02	.002
24:0	0.39 ± 0.02	0.22 ± 0.01	.001
16:1ω7	1.55 ± 0.07	2.20 ± 0.17	.0001
18:1ω7	1.61 ± 0.03	1.81 ± 0.05	.0001
18:1ω9	18.11 ± 0.34	21.17 ± 0.45	.0001
20:1ω9	0.13 ± 0.01	0.09 ± 0.01	.3
20:3ω9	0.11 ± 0.01	0.16 ± 0.01	.05
22:1ω9	0.04 ± 0.01	0.03 ± 0.00	.9
24:1ω9	0.46 ± 0.03	0.29 ± 0.02	.01
18:2ω6	35.24 ± 0.51	30.11 ± 0.80	.0001
18:3ω6	0.49 ± 0.02	0.46 ± 0.04	.7
20:2ω6	0.22 ± 0.01	0.18 ± 0.01	.3
20:3ω6	1.70 ± 0.05	1.65 ± 0.06	.1
20:4ω6	8.48 ± 0.21	7.94 ± 0.24	.8
18:3ω3	0.47 ± 0.02	0.49 ± 0.04	.6
20:5ω3	0.62 ± 0.04	0.50 ± 0.03	.04
22:5ω3	0.57 ± 0.02	0.51 ± 0.02	.07
22:6ω3	2.29 ± 0.12	1.57 ± 0.09	.06
Trans = 16:1ω7T	0.31 ± 0.01	0.41 ± 0.02	.01

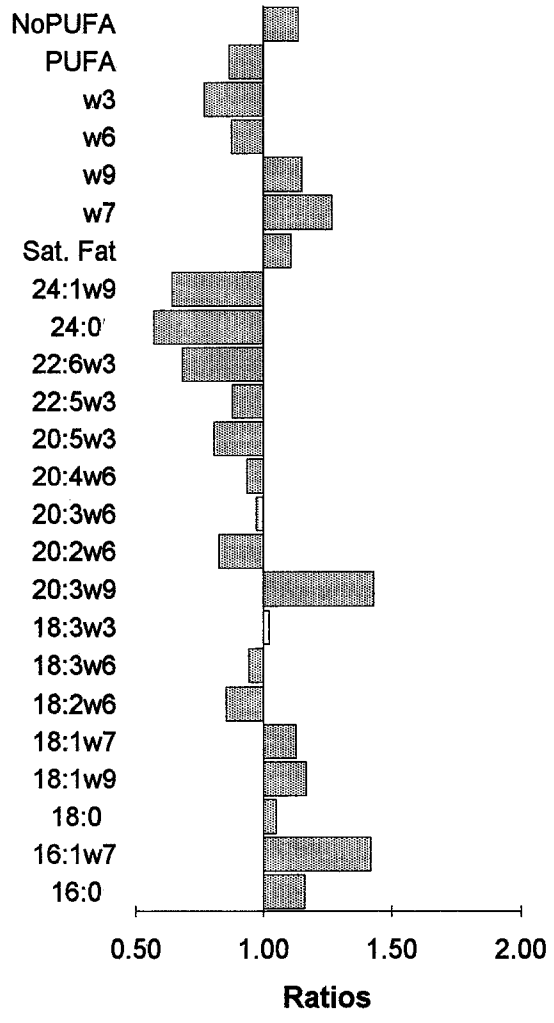

Fig 1. Ratios between means of major types of fatty acids for 2 groups of subjects, GI and reference. GI are patients with intestinal disease, Reference is a group of reference subjects; PUFA, polyunsaturated fatty acids; NoPUFA, saturated and monounsaturated fatty acids.

Table 2. Percentages (mean ± SE) of *Cis*–Fatty Acids in Plasma of Reference Subjects and Patients

Fatty Acid	Reference Subjects (n = 56)	GI Patients (n = 47)	P
SFA	26.51 ± 0.18	29.48 ± 0.27	.001
ω9	18.85 ± 0.35	21.75 ± 0.46	.0001
ω7	3.16 ± 0.08	4.01 ± 0.20	.0001
ω6	46.34 ± 0.48	40.58 ± 0.77	.0001
ω3	4.03 ± 0.16	3.10 ± 0.12	.02
DFA9	0.11 ± 0.01	0.16 ± 0.01	.05
DFA6	11.10 ± 0.21	10.47 ± 0.27	.5
DFA3	3.55 ± 0.16	2.61 ± 0.12	.02
MUFA	22.01 ± 0.41	25.76 ± 0.61	.0001
PUFA	50.37 ± 0.51	43.68 ± 0.79	.0001
NoPUFA	48.52 ± 0.52	55.24 ± 0.78	.0001

Abbreviations: PUFA, all fatty acids of families ω3 and ω6 = ω3 + ω6; NoPUFA, all SFAs and fatty acids of families ω7 and ω9 = SFA + MUFA.

difference in derivatives of linoleic acid, as compared with the control population. Figure 2 shows that linoleic derivatives (mostly 20:4ω6) versus linoleic acid are relatively constant over a wide range of 18:2ω6 values. These data are consistent with active conversion of linoleic to arachidonic acid. Remarkably, the sum of all the derivatives (ω3, ω6, and ω9; Table 2) was similar in both groups (14.8% in control group and 13.3% in patients), a finding previously reported in severe EFAD patients[5] and patients with heart disease.[46] Figure 2 assists in the identification of subjects with low arachidonic acid levels, given their linoleic acid levels, possibly due to defective metabolic conversion. The alternative, increased utilization, is less likely, although we have seen one patient with terminal illness due to metastatic intestinal cancer who had severe depletion of EFA derivatives. In accordance with theoretical predictions,[5] the relative pathway activity measured by the ratio of derivatives to precursors follows the order, ω3 > ω6 > ω9. The ratio of ω6 derivatives to precursors is increased in patients

Table 3. Ratios of *Cis*–Fatty Acids in Plasma of Reference Subjects and Patients

Fatty Acid	Reference Subjects (n = 56)	GI Patients (n = 47)	P
20:3ω6/20:4ω6	0.210 ± 0.010	0.215 ± 0.009	.3
20:5ω3/20:4ω6	0.077 ± 0.006	0.065 ± 0.004	.04
20:4ω6/18:2ω6	0.24 ± 0.01	0.27 ± 0.01	.0005
20:5ω3/18:3ω3	1.40 ± 0.10	1.34 ± 0.14	.2
18:1ω7/16:1ω7	1.12 ± 0.04	0.98 ± 0.05	.03
ω3/ω6	0.087 ± 0.004	0.077 ± 0.003	.9
18:3ω3/18:2ω6	0.014 ± 0.001	0.016 ± 0.001	.04
DFA9/DFA6 + DFA3	0.008 ± 0.000	0.012 ± 0.001	.03
DFA9/PFA9	0.006 ± 0.000	0.007 ± 0.001	.5
DFA6/PFA6	0.32 ± 0.01	0.36 ± 0.02	.005
DFA3/PFA3	8.08 ± 0.47	7.52 ± 1.13	.3
DFA3/DFA6	0.33 ± 0.02	0.25 ± 0.01	.01
PUFA/MUFA	2.35 ± 0.07	1.77 ± 0.07	.0001
PUFA-*trans*/noPUFA	1.03 ± 0.02	0.79 ± 0.03	.0001
PUFA/noPUFA	1.05 ± 0.02	0.81 ± 0.03	.0001
20:3ω9/20ω46	0.013 ± 0.001	0.020 ± 0.002	.04
16:1ω7/18:2ω6	0.046 ± 0.003	0.082 ± 0.009	.0001

NOTE. Abbreviations as in Table 2.

as compared with reference subjects (0.36 *v* 0.32, $P < .01$), indicating EFAD. Because ω3 levels are usually low in the US population and can be changed by small dietary changes, there is high individual variability in ω3 levels. Under these circumstances, group averages are not likely to be significant, and we did not find any consistent group differences. Our results do not preclude a finding that some patients may have decreased ω3 levels and increased DFA3/PFA3, indicating ω3 EFAD.[5]

The ratio of EFAs and derivatives to nonessential fatty acids (PUFA/noPUFA) was 0.81 among patients, as compared with 1.05 among controls ($P < .0001$; Table 3). The T/T ratio has been used to measure EFAD for more than 30 years, but the upper limit previously used was 0.2, whereas our upper limit is 0.025 due to improved methodology.[7] The mean value for the T/T ratio was 0.020 among patients and 0.013 among controls ($P < .04$; Table 3). Thirteen patients had values of palmitoleic acid (16:1ω7) greater than 2.6%, 16 patients had a percentage of 18:2ω6 less than 27% of total fatty acids, and 10 patients had T/T ratios greater than 0.025. Any of these parameters indicate the presence of EFAD (primarily ω6 deficiency).[7] Other ratios that are significantly increased in the patients as compared with the control subjects include the ratios of 18:2ω6 and 18:3ω3 to the sum of other 18-carbon fatty acids (18:0 + 18:1ω7 + 18:1ω9) and the ratio of 16:1ω7 to 18:2ω6.

Table 4 shows striking inverse correlations between percentages of plasma EFAs and various SFAs and MUFAs computed using the patient data. Significant ($P < .01$) inverse linear correlations of linoleic acid, 18:2ω6, were noted with 16:0 ($r = -.74$) and the MUFAs, 16:1ω7 ($r = -.80$), 18:1ω7 ($r = -.33$), and 18:1ω9 ($r = -.80$). Negative linear correlations were also found between ratios that serve as indicators of EFAD (20:3ω9/20:4ω6, PUFA/noPUFA, and 16:1ω7/18:2ω6) and the major plasma ω3 and ω6 fatty acids. The inverse relationship between 16:1ω7 and 18:2ω6 is shown in Fig 3. A similar inverse relationship (not shown) exists between T/T and PUFA/noPUFA. We incorporated the group, severe EFAD, in Fig 3 to illustrate the extent of possible linoleic acid deficiency in humans.

The slope of a line connecting each point with the origin in a plot of 20:3ω9 versus 20:4ω6 (not shown) equals the T/T ratio. T/T ratios for patients are greater than those for controls mainly because of differences in 20:3ω9, not 20:4ω6, which is fairly constant for all subjects as seen in Fig 2. Figure 4 shows the highly significant inverse linear relationship ($r = -.93, P < .001$) between MUFA and PUFA.

Ratios of derivatives to precursors for the respective fatty acid families were positively correlated with each other (DFA6/PFA6 *v* DFA3/PFA3, $r = .68$, $P < .001$; DFA6/PFA6 *v* DFA9/PFA9, $r = .57$, $P < .001$) and negatively correlated with linoleic acid (DFA6/PFA6 *v* 18:2ω6 [PFA6], $r = -.79$, $P < .001$; the correlation would be zero if DFA levels were proportional to PFA levels). These data support the concept that conversion of precursors to derivatives increases for all unsaturated fatty acids when levels of EFAs decrease. Correlations remain significant whether they were made using only the patients or the aggregate of

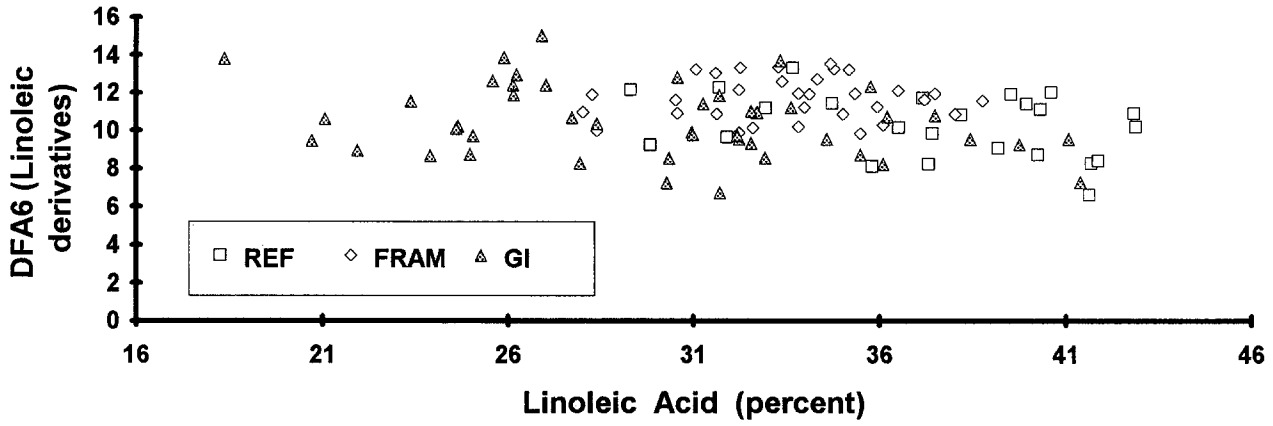

Fig 2. Derivatives *v* precursors of 18:2ω6 (%). FRAM, random sample from the Framingham Cardiovascular Study, cycle 3; DFA6, derivatives of linoleic acid.

Table 4. Correlations of Fatty Acids of Patients (n = 47)

Fatty Acids	16:1ω7	18:2ω6	18:3ω3	SFA	MUFA	PUFA
16:0	.70	−.74	−.19	.84	.64	−.78
18:0	−.30	.08	−.16	.23	−.19	.08
24:0	−.42	.26	.02	−.33	−.30	.34
16:1ω7	1.00	−.80	−.29	.54	.80	−.80
20:3ω9	.66	−.58	−.28	.45	.44	−.50
24:1ω9	−.16	−.13	−.19	−.07	.01	.03
18:2ω6	−.80	1.00	.35	−.72	−.87	.91
18:3ω6	.55	−.51	−.07	.45	.40	−.47
20:3ω6	.34	−.36	−.03	.15	.19	−.20
20:4ω6	−.07	−.13	−.14	−.17	−.23	.24
18:3ω3	−.29	.35	1.00	−.29	−.34	.36
20:5ω3	.14	−.08	.13	.01	−1.00	.08
22:6ω3	−.20	.01	−.08	−.04	−.29	.25
SFA	.54	−.71	−.29	1.00	.55	−.76
ω9	.63	−.81	−.35	.53	.97	−.93
ω7	.91	−.78	−.23	.44	.81	−.78
ω6	−.80	.94	.31	−.77	−.94	.98
ω3	−.19	.06	.37	−.12	−.34	.31
DFA9	.66	−.58	−.28	.45	.44	−.51
DFA6	.10	−.29	−.14	−.05	−.09	.09
DFA3	−.11	−.05	.06	−.03	−.25	.21
MUFA	.80	−.87	−.07	.55	1.00	−.96
PUFA	−.80	.91	.36	−.76	−.96	1.00
20:3ω6/20:4ω6	.30	−.16	.08	.23	.23	−.27
20:5ω3/20:4ω6	.15	.00	.20	.12	−.03	−.01
20:4ω6/18:2ω6	.48	−.71	−.36	.33	.38	−.40
20:5ω3/18:3ω3	.60	−.53	−.61	.43	.38	−.43
18:1ω7/16:1ω7	−.89	.80	.29	−.55	−.76	.76
18:3ω3/18:2ω6	−.10	.08	.95	−.11	−.11	.12
DFA9/DFA6 + DFA3	.70	−.52	−.27	.47	.50	−.56
DFA6/PFA6	.59	−.79	−.35	.44	.48	−.52
DFA3/PFA3	.42	−.47	−.52	.40	.31	−.38
DFA3/DFA6	.26	.13	.16	.03	−.21	.15
PUFA/MUFA	−.75	.89	.32	−.63	−.98	.98
PUFA/noPUFA	−.76	.92	.34	−.75	−.96	.99
20:3ω9/20:ω46	.71	−.52	−.23	.50	.51	−.57
16:1ω7/18:2ω6	.98	−.83	−.35	.60	.80	−.82

NOTE. For significance values, general rules are as follows: correlation $R^2 > .25$ has $P < .1$ and $R^2 > .31$ has $P < .05$. Abbreviations as in Table 2. Only patients are included.

the patients and the control population (the correlations are even more significant when using nonlinear models optimized to fit the data). Figure 5 shows the ratio of 20:5ω3/20:4ω6 (ratio of series 3 to series 2 eicosanoid precursors) versus the total concentration of ω3 fatty acids, indicating that low levels of ω3 fatty acids are associated with a decrease in this ratio. This figure identifies subjects at risk for thrombosis (low 20:5ω3/20:4ω6).

The mean total fatty acid concentration was lower in patients than in the control group (214 *v* 284 mg/dL, $P < .01$), with lower mean absolute concentrations of almost every fatty acid among the patients. The exceptions were 14:0, 16:1ω7, and 20:3ω9, which were slightly higher in patients. Concentrations of SFAs and MUFAs were significantly lower ($P < .001$) in patients (63 and 56 mg/dL, respectively) than in the control population (75 and 63 mg/dL, respectively). Concentrations of PUFAs were also lower ($P < .04$) in patients (93 mg/dL) than in the control population (143 mg/dL). (Readers can compute approximate concentrations from the total fatty acid concentration and the percents shown in tables.)

We analyzed fatty acid profiles by disease type and found no significant differences (data not shown to save space). The PUFA/noPUFA ratio was lower in all patients as compared with the control group. As a group, the mean PUFA/noPUFA ratio for Crohn's disease was slightly lower than the mean for ulcerative colitis patients. Equivalent results were obtained using the T/T ratio (patients had higher levels than controls). The distribution of 20:5ω3/20:4ω6, DFA6/PFA6, DFA3/PFA3, and 16:12ω7/18:2ω6 was similar for all patients.

Multivariate regression with an independent dummy variable, GI (GI = 1 for GI disease patients and 0 for reference subjects), which is an indicator of the probability of GI disease (values of GI > 0.5 indicate GI disease), produced the equations shown in the appendix. The linear equations are equivalent to analyses of covariance. The high multiple correlation coefficient ($R^2 > .60$) and highly significant F ratio supports the validity of the linear models used. The models indicate that the concentration of PUFA

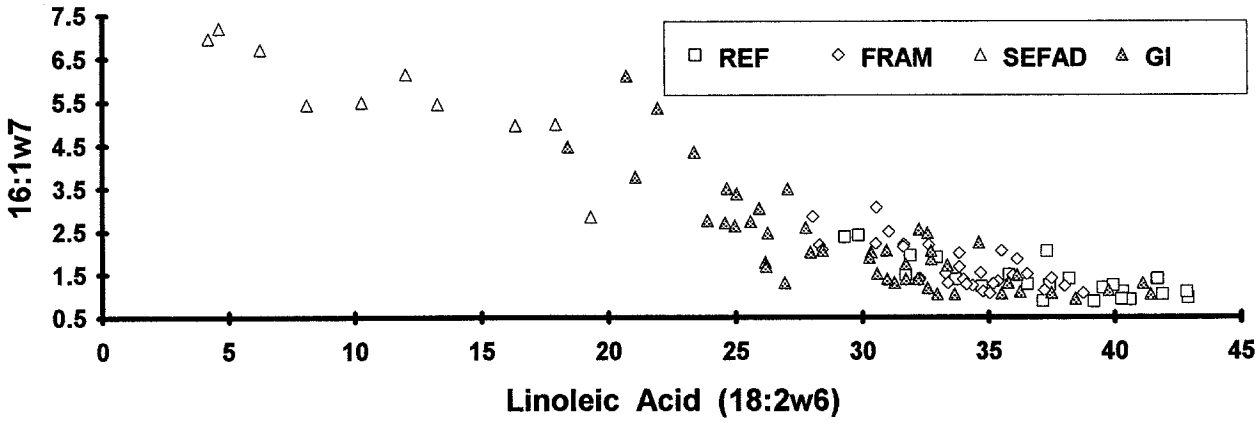

Fig 3. Linoleic acid *v* palmitoleic acid (%). SEFAD, patients with severe EFAD.

is the most significant variable that predicts chronic intestinal disease. Decreased PUFA and increased SFA are associated with intestinal disease. Other variables have far smaller coefficients and statistical significance. We repeated the analyses using logistic regression, and the results were similar: the concentration of PUFA was the single most significant predictor that distinguished patients from controls and predicted 90% of cases (MUFAs and SFAs had practically no additional predictive value). In these patients, the concentration of PUFA combines the effects of decreased plasma lipid concentrations and decreased PUFA percent levels associated with intestinal disease.

DISCUSSION

Compared with the control population, patients with chronic intestinal disorders had (in percents) the plasma fatty acid pattern characteristic of absolute EFAD[46]: (1) increased ω7 and ω9 fatty acids, (2) higher 16:1ω7/18:2ω6, (3) higher 20:3ω9/20:4ω6, (4) higher 16:1ω7, (5) lower percent and concentration of ω6 and ω3 fatty acids, and (6) lower PUFA/noPUFA ratio. These interpretations are further supported by the finding that statistically significant negative correlations were observed between linoleic acid and various MUFAs. Previous studies used assays that could not detect 20:3ω9 when peaks were less than 10% of arachidonic acid because 20:3ω9 was superimposed on other peaks, often 20:2ω6.[7] Superimposition on 20:2ω6 required high 20:3ω9 levels before the sum of 20:2ω6 + 20:3ω9 was statistically significant. Subjects with normal levels of linoleic acid had high levels of 20:2ω6, whereas those with low levels of linoleic acid had higher 20:3ω9 and lower 20:2ω6. Therefore, T/T ratios less than 0.1 were effectively undetectable and regarded as normal. With improved methods of gas chromatography, we measured T/T ratios less than 0.01. The large gap between the upper limit of control (normal) values and values observed in severe EFAD with regard to the various indices of EFAD[7] suggests that there exist individuals with EFA insufficiency. Our data indicate that normal T/T ratios are approximately one tenth of the previous detection limit, and ratios in patients with chronic intestinal disorders are an average of 50% higher than in subjects without disease. Absolute EFA insufficiency may cause significant abnormalities of prostanoid metabolism,[47] which would be a contributory factor to the so-called "systemic" consequences of intestinal disease.[48] These abnormalities may also produce subtle clinical symptoms, such as increased platelet aggregation,[49] and suboptimal cell function, including reduced cell life. We report that, on average, patients with intestinal malabsorption have EFA insufficiency. In future studies, we will

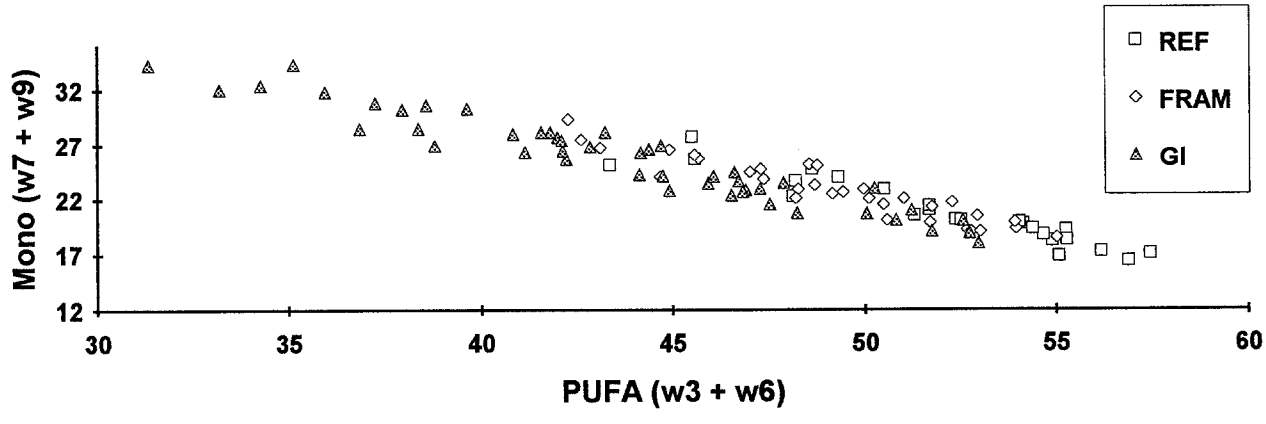

Fig 4. Monounsaturated fatty acids *v* polyunsaturated fatty acids (%). MONO, monounsaturated fatty acids.

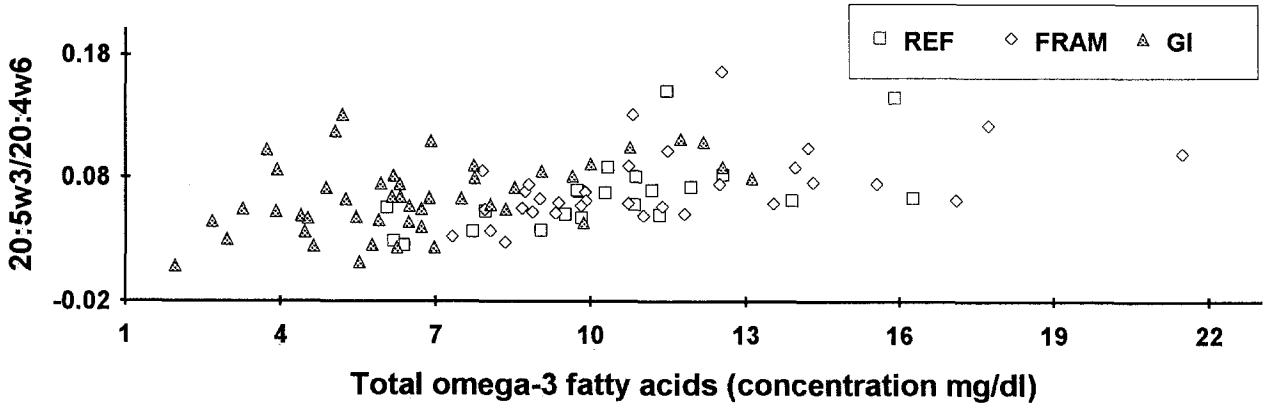

Fig 5. Ratio of prostaglandin precursors series 3/series 2 *v* total ω3 fatty acids (concentration).

correlate these abnormalities with other nutritional deficiencies, and will seek to establish separate fatty acid profiles for different intestinal disorders. However, our clinical experience indicates that the most significant factors accounting for EFA status are individual variability, treatment effects often due to unknown interactions of a multitude of drugs, exercise, disease activity (which fluctuates and is difficult to assess), and large variabilities in dietary intake (which are practically impossible to measure reliably because people substantially change their dietary intake of EFAs almost every week, and EFA composition of food depends on the actual oil used in the food, often unknown, and the mode of food preparation). For these reasons, it is practically impossible, in our clinical experience, to predict EFA status from clinical observations, and it is practically useless to assess intake of EFAs by dietary questionnaires in these patients.

The regression equations identify the amounts of PUFAs as significant discriminators between patients and healthy subjects. Other statistically significant variables had small coefficients and therefore are less reliable. Decreased 20:5ω3/20:4ω6 ratios are associated with decreased bleeding time and increased platelet aggregation.[50] Patients had decreased 20:5ω3/20:4ω6, suggesting that, on average, patients may have hypercoagulable platelets (due to decreased ratio of series 3 to series 2 eicosanoids). Figure 5 suggests that this problem would be most significant for subjects with low total ω3 concentration. Subjects with low ω3 levels may not be able to produce enough 20:5ω3 to prevent excessive production of series 2 over series 3 prostanoids and thromboxanes.[51] It may be years before the full significance of these findings is known. For subjects on intravenous feeding or at risk for hypercoagulability (ie, abnormal antithrombin III level, protein C deficiency, protein S deficiency, thrombocytosis, or history of thrombosis),[52] physicians should consider screening for low ω3 or 20:5ω3/20:4ω6 levels as an additional risk factor, and change diets accordingly.

The ratio of PUFA to nonessential fatty acids and derivatives (PUFA/noPUFA) provided one of the best discriminating variables based on *t* test analysis between patients and control subjects ($P < .0001$). A 15% decline in 18:2ω6 leads to only a 6% decline in linoleic acid derivatives, consistent with increased pathway activity (higher DFA6/PFA6 ratio). The small quantities of linolenic acid and the wide variability among patients make it difficult to draw conclusions about ω3 pathway activity. Because of the great variability in ω3 fatty acids and the fact that 20:3ω9 production is primarily determined by ω6 deficiency (there is ~10 times as much ω6 as ω3), the ratios 20:3ω9/20:5ω3 and 20:3ω9/22:5ω6 are variable, have limited value, and are therefore not included in this report. Instead, we propose that physicians use two or more concurrent variables, including ω3/ω6, 22:4ω6/22:5ω3, 22:5ω6/22:6ω3, or (DFA3/PFA3)/(DFA6/PFA6) as indicators of relative ω3 deficiency.

Our findings are consistent with the view that with increasing deficiency of EFAs, there is enhanced formation of derivatives of linoleic acid and oleic acid. EFAD subjects compensated by producing more MUFAs and actively converting ω6 EFAs to their derivatives to attempt to maintain plasma levels of ω6 EFA derivatives. A similar phenomenon was found in patients with coronary artery disease.[46] We propose that increased pathway activity helps to regulate the amount of EFA derivatives available for cell function, and substitutes EFAs with MUFAs in an attempt to maintain membrane fluidity (a MUFA is closer to a PUFA than is a SFA).

We know from theoretical reasons and our data (unpublished) that total plasma fatty acid concentration is highly correlated with the sum of the concentrations of cholesterol and triglycerides. The patients herein reported had low total plasma fatty acid concentrations. Under these circumstances, the plasma concentration of PUFA combines the reduced total plasma lipid concentration with reduced PUFA percent in patients. For well-fed patients with cholesterol and triglyceride levels within the average population ranges, plasma fatty acid concentrations will also be within average population ranges, and we must use percents for the diagnosis of EFA status. In contrast, patients with coronary artery disease have elevated plasma concentrations of total fatty acids and normal to elevated plasma concentrations of PUFA with decreased PUFA percent.[46]

Variations in concentrations of fatty acids are by themselves less significant indicators of EFAD than percents because the concentrations depend on total plasma lipid

levels and thus on total cholesterol and triglyceride levels.[46] A patient may have mildly elevated plasma lipids, that result in EFA concentrations within reference values, but the percent of EFA would be low and the percent or concentration of 20:3ω9 would be increased. The best diagnosis is achieved by concurrent analyses of concentrations, percents, and ratios of fatty acids using two-dimensional (or higher-dimensional) plots. Concurrent analyses of several variables also identify patients with a partial block in the conversion of EFAs to their derivatives (reduced derivatives in the presence of adequate precursors), impaired utilization (increased 16:1ω7 and 20:3ω9 in the presence of normal to elevated concentrations of EFAs), and other metabolic abnormalities, or a suboptimal proportion of eicosanoid precursors (ω3 *v* ω6 levels).[46]

Our discrepancies with the findings of Esteve-Comas et al[36,37] are most likely due to the use of widely different methods. Esteve-Comas et al used over 1 mL plasma (~ 10 times as much as we did). In our experience, the macroesterification methods they used cause small but significant losses in EFAs as compared with our micromethods. They used a 30-m column and a fast (short) method for peak separation. In our experience, a short method is insufficient to separate the significant fatty acids from contamination due to BHT and cholesterol derivatives and other chemicals in plasma. Esteve-Comas et al did not report their integration methods, but integration systems that do not display baseline data on a monitor cause inaccurate peak integration (this is a frequent source of error, as indicated earlier). The combination of these factors increases the probability that Esteve-Comas et al improperly measured key fatty acid levels and thereby affected the profile (in percents) of all fatty acids reported. They reported elevated levels of 18:3ω3 because their control subjects had almost zero levels of 18:3ω3. This has to be an error—we have consistently found levels of approximately 0.5% in hundreds of samples we analyzed (similar to their patients). Published research by others using modern technology is consistent with our results.[40] A similar problem occurs with their measures of 18:3ω6. Esteve-Comas et al reported far larger amounts of 16:0 and lower amounts of 18:2ω6 in control subjects than the amounts found by ourselves and others who used modern capillary techniques similar to ours.[40] Their increased levels of 18:1ω9 are most likely due to superimposition with 18:1ω7, 18:1ω5, and numerous *trans* and isomers in the same chromatographic region. Our data show characteristic inverse relations between MUFAs and EFAD indicators and EFA levels. Their data are internally inconsistent. They do not find a clear inverse relation between 18:1ω9 and 18:2ω6, and they found increased plasma fatty acid concentrations in patients versus controls. In our experience, patients with chronic intestinal disease have reduced plasma lipids and therefore reduced plasma fatty acid concentrations.

We found reduced levels of 20:5ω3, 22:5ω3, and 22:6ω3—Esteve-Comas et al found increased levels. It is easy to superimpose the small peaks corresponding to these substances with a large number of other peaks in the same chromatographic region. They found increased conversion of precursors to derivatives, and attribute their findings to some unusual metabolic characteristic of inflammatory bowel disease. We have reported that increased conversion of precursors to derivatives is a characteristic of EFAD.[5] Because patients with inflammatory bowel disease have reduced levels of ω6 fatty acids (both in our data and their data), one would expect the compensatory increase in metabolic activity that we previously reported across subjects regardless of disease status. Thus, we submit that increased EFA metabolic activity is not a unique characteristic or genetic defect or pathogenic mechanism of inflammatory bowel disease (as Esteve-Comas et al propose), but rather a human metabolic adaptation to reduced levels of EFAs. This phenomenon is also found with coronary artery disease.[46]

Although, over a wide range of EFA levels, decreased amounts of precursors lead to increased conversion, a severe decline in EFA levels may cause a decline in conversion of precursors to derivatives. The elevated ratio of 20:3ω9/20:2ω6 (0.56 in control *v* 1.05 in patients) is indicative of ω6 EFAD; the elevated ratio of 22:6ω3/20:4ω6 (the two largest derivatives) is probably indicative of more ω3 than ω6 EFAD (0.28 in control *v* 0.20 in patients). In controls versus patients, respectively, ω3/ω6 was 0.09 versus 0.08 (nonsignificant), DFA3/DFA6 was 0.33 versus 0.25, and (DFA3/PFA3)/(DFA6/PFA6) was 26 versus 19. These data suggest that, on average, the patients had relatively more severe ω6 than ω3 deficiency (because the ω3 pathway is less active in patients than in controls).[5] We found that DFA3/PFA3 ≫ DFA6/PFA6 in healthy subjects[5] and patients with heart disease.[46] Our data indicate that in patients with chronic intestinal disease, conversion of linolenic acid to its derivatives is more active than conversion of linoleic acid to its derivatives.

We propose that EFA insufficiency is not only a consequence of chronic intestinal disease, but a contributing factor to its pathology. At least two physiological mechanisms explain the role of EFAs in the pathology of Crohn's disease. EFAs alter eicosanoid activity and the immune system. In addition, EFAs (and their derivatives) are key membrane components and are essential to form new cells in the gut, cells normally renewed every few days. EFA insufficiency may prevent formation of normal cells and therefore produce further pathology (ie, malabsorption, absorption of large protein molecules causing systemic immune alterations, ulceration, etc.). EFAs must be obtained from the diet. Dietary intake data cannot accurately measure fatty acid intake (due to conversions of glucose and protein to saturated fatty acids, or changes in fatty acid structure caused by food processing such as *trans* fatty acid formation).[53] It is practically impossible to determine in a given patient whether EFA abnormalities are caused by insufficient EFA intake, malabsorption, increased demand for fatty acids, excess SFAs, or an imbalance of ω3 versus ω6 fatty acids.[54] We propose that fatty acid profiles be incorporated into indices of intestinal disease activity.[55] In cystic fibrosis, the disease contributes to EFAD and EFAD is a primary contributor to many of the systemic consequences of the disease.[56]

EFAs and EFA derivatives have been proposed for treatment of a variety of diseases.[13] The extent of the

deficiency in individual fatty acids provides clues to determine whether to use foods high in ω3 or ω6 fatty acids. Correction of fatty acid imbalances may improve the disease; for example, dietary fish oil reduces progression of chronic inflammatory lesions in a rat model of granulomatous colitis.[57] A randomized double-blind placebo-controlled study of ω3 supplementation in patients with inflammatory bowel disease showed a reduction in inflammatory lipid mediators and morphological improvement.[58] To determine whether a patient with EFAD or EFA insufficiency needs intravenous lipids, one can supplement the diet with ω3 and ω6 fatty acids using 2 tablespoons of soybean or flax seed oil per day for several weeks (together with ~100 IU vitamin E/d per tablespoon of oil) and test again for plasma fatty acid composition in approximately 3 months. Patients who cannot tolerate the oils (ie, due to severely increased diarrhea even with Questran [Bristol-Myers Squibb, Princeton, NJ]) or do not show a consistent increase in PUFAs will require periodic infusions of intravenous lipids. The frequency and formula for such lipids is determined by monitoring the fatty acid profile.[8,46]

Although the active conversion of precursors to their derivatives (Table 2) indicates that patients could obtain adequate nutrition from the EFAs, because of fat malabsorption it may be more efficient to supplement these patients with EFA derivatives such as 18:3ω6 or 20:5ω3 commercially available (which could provide more of the "active" fatty acids with smaller absolute fat quantities). Further research is required to identify the optimal treatment mixture of derivatives versus precursors and ω3 versus ω6 fatty acids.

What interpretation should be given to a 50% elevation of the T/T ratio? It is currently regarded that a 50% elevation in cholesterol is a sign of pathology. A 50% elevation in blood pressure is serious. We hypothesize that a 50% elevation in the T/T ratio or 16:1ω7 is a risk factor and indicator of serious pathology. Due to the multifactorial abnormalities and deficiencies found in chronic intestinal disease, it will take many years to determine the full clinical significance of EFA abnormalities herein discussed. Whether EFAD leading to suboptimal cell function reduces life expectancy may be impossible to prove because studies depleting patients of EFAs for long periods are unethical. While the effects of normalization of fatty acid profiles on chronic intestinal disease are further studied, we propose that significant EFA abnormalities be diagnosed and treated because the benefits outweigh the risks. There is little doubt that significant EFAD should be treated. Whether milder deficiencies require treatment has to be left to the discretion of the physician and patient and involves a balance of risks and benefits.[46] In our clinical experience, we found that EFA abnormalities are probably the single most important nutritional deficiency overlooked in patients with intestinal disease. When treatment is recommended, we use a diet that will bring the plasma fatty acid profile of a patient closer to the profile of a healthy control population. Such a diet requires a mixture of oil supplements optimized for each patient. The optimal mixture can be formulated with safflower and sunflower oil (rich in ω6), soybean oil (rich in ω6 and ω3), and flax seed oil (mostly ω3). Sequential fatty acid profiles can assess whether oral EFA supplements are sufficient or intravenous lipids are needed.

APPENDIX

Multivariate Regressions Predicting GI Disease

$GI = 1.40\ (P < .1 \times 10^{-14}) - 0.02 \times CPUFA\ (P < .1 \times 10^{-14}) + 0.02 \times CSatFat\ (P < .001) - 0.007 \times CMONO\ (P < .11)$
$[F = 57, P < 1 \times 10^{-15}, R^2 = .80]$

$GI = 1.46\ (P < .1 \times 10^{-14}) - 0.02 \times CPUFA\ (P < .2 \times 10^{-12}) + 0.02 \times CSatFat\ (P < .006) - 0.03 \times C1617\ (P < .26) + 0.05 \times C1617t\ (P < .69)$
$[F = 42, P < 1 \times 10^{-15}, R^2 = .79]$

$GI = 1.38\ (P < .5 \times 10^{-5}) - 0.02 \times CPUFA\ (P < .1 \times 10^{-14}) + 0.01 \times CSatFat\ (P < .0009) - 0.57 \times C2039\ (P < .4) + 0.92 \times P2039\ (P < .6)$
$[F = 42, P < 1 \times 10^{-15}, R^2 = .79]$

$GI = 1.94\ (P < .2) - 0.02 \times PUFA\ (P < .1) + 0.13 \times SatFat\ (P < .0003) - 0.15 \times P1617\ (P < .03) + 0.27 \times P1617t\ (P < .46)$
$[F = 24, P < .6 \times 10^{-13}, R^2 = .70]$

The number in parentheses after each coefficient is the probability of statistical significance.
F = value of the test statistic for ANOVA, P = probability for such a value, and R^2 = multiple correlation coefficient.
GI = 0 for reference subjects and 1 for GI subjects.
C1617, C1617+ = 16:1ω7, 16:1ω7t (concentration, *cis,* and *trans,* respectively).
CSatFat, SatFat = total SFAs (concentration and percent, respectively).
CMONO, MONO = total MUFAs (concentration and percent, respectively).
CPUFA, PUFA = total EFAs plus derivatives (concentration and percent, respectively).
C2039, P2039 = concentration and percent of 20:3ω9, respectively.

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