

Symposium: Evolution of Ideas about the Nutritional Value of Dietary Fat

The Slow Discovery of the Importance of ω 3 Essential Fatty Acids in Human Health¹

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ABSTRACT Although linoleic and linolenic acids have been known to be necessary for normal growth and dermal function since 1930, the ω 3 essential fatty acids (EFA) have not received much attention until recently. The two families of acids are metabolized by the same enzymes, making them competitive. Gross deficiencies of ω 6 plus ω 3 EFA have been observed in humans, induced by attempts at total parenteral nutrition (TPN) with preparations devoid of lipids. Deficiency of ω 3 acids has been induced by TPN containing high ω 6 and low ω 3 fatty acids. In natural human populations, a wide range of ω 3 and ω 6 proportions have been found, ranging from high ω 3 and low ω 6 content to low ω 3 and high ω 6 content, showing inverse correlation between $\Sigma\omega$ 6 and $\Sigma\omega$ 3. In humans with neuropathy or impairment of the immune system, significant deficits of ω 3 EFA have been measured. J. Nutr. 128: 427S–433S, 1998.

KEY WORDS: • *essential fatty acids* • (*ω 6 and ω 3*) *polyunsaturated fatty acids* • *metabolism* • *requirements* • *human deficiencies*

The discovery of the essentiality of the long-chain polyunsaturated fatty acids was made by Burr and Burr (1929) at the University of Minnesota Medical School. George and his wife, Mildred, completed their work in another publication a year later (Burr and Burr 1930). At that time, essentiality meant promotion of growth and prevention of the dermatitis observed when a fat-free diet was fed to rats. Both linoleic and linolenic acids provided these functions. The writer has written an extensive review of the early work on essential fatty acids (EFA)² (Holman 1968), and a brief biography of George O. Burr has been published in this journal (Holman 1988). The relationship of polyunsaturated fatty acids to human health and to disease has been reviewed recently in greater detail than allowed here (Holman 1992 and 1997).

In the mid-1940s, when I began research with Professor Burr on the chemistry of EFA, there were no methods to measure individual fatty acids in mixtures. Using Burr's very new Beckman DU Spectrometer (instrument #6, Beckman Instruments, Fullerton, CA), we exploited alkaline isomerization at high temperature to induce conjugation of the double bonds of the common EFA. We found that fully conjugated polyenoic acids had distinctive absorption bands, making it possible to distinguish and measure nonconjugated, methylene-interrupted diene-, triene-, tetraene-, pentaene- and hexaene-acids present in biological lipids (Holman and Burr

1948). We learned that a fat-free diet lowered the amount of tetraenoic acid (arachidonic acid), and that a triene acid, now known as Mead's acid (20:3 ω 9), was not present in animals fed EFA, but appeared in tissue lipids of EFA-deficient animals. Supplementing with corn oil elevated the amount of tetraenoic acid. Supplementation with cod liver oil elevated the tetraenoic, pentaenoic and hexaenoic acids in all tissues analyzed. The dietary fat influenced strongly the lipid composition of the animal! The metabolic effects of dietary EFA were measured in many tissues, and we found that blood lipids were a good indicator of the EFA status of the animal.

At Texas A&M University, Widmer and Holman (1950), using *pure* single EFA, were the first to discover that linoleic acid, fed to EFA-deficient rats, was the precursor of arachidonic acid in tissues, and that α -linolenic acid was the precursor of the pentaene and hexaene acids of their tissues. In rats fed a fat-free diet, the trienoic acid (20:3 ω 9) derived from oleic acid appeared. Both linoleic and linolenic acids stimulated growth and corrected the dermatitis of EFA deficiency. Before the days of known desaturases, we pondered about the mechanism by which the double bonds of the precursors fed were increased in the products. Later, Holman (1960) proposed the triene/tetraene ratio as an index of EFA deficiency and status.

At the Hormel Institute, Mohrhauer and Holman (1963a) used the new, more discriminating gas chromatographic method of analysis to study the effects of dose level of single pure EFA on the liver content of their metabolic products. At first, samples were analyzed by alkaline isomerization as well as by gas chromatography, and the results were found to be the same by the two methods, confirming the validity of our earlier studies using alkaline isomerization. **Figure 1** is the dose-response curve for dietary linolenic acid and its metabolites in rat liver lipids. It also shows that the level of liver

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² Abbreviations used: EFA, essential fatty acids; FA, fatty acids; PL, phospholipid; PUFA, polyunsaturated fatty acid; TPN, total parenteral nutrition.

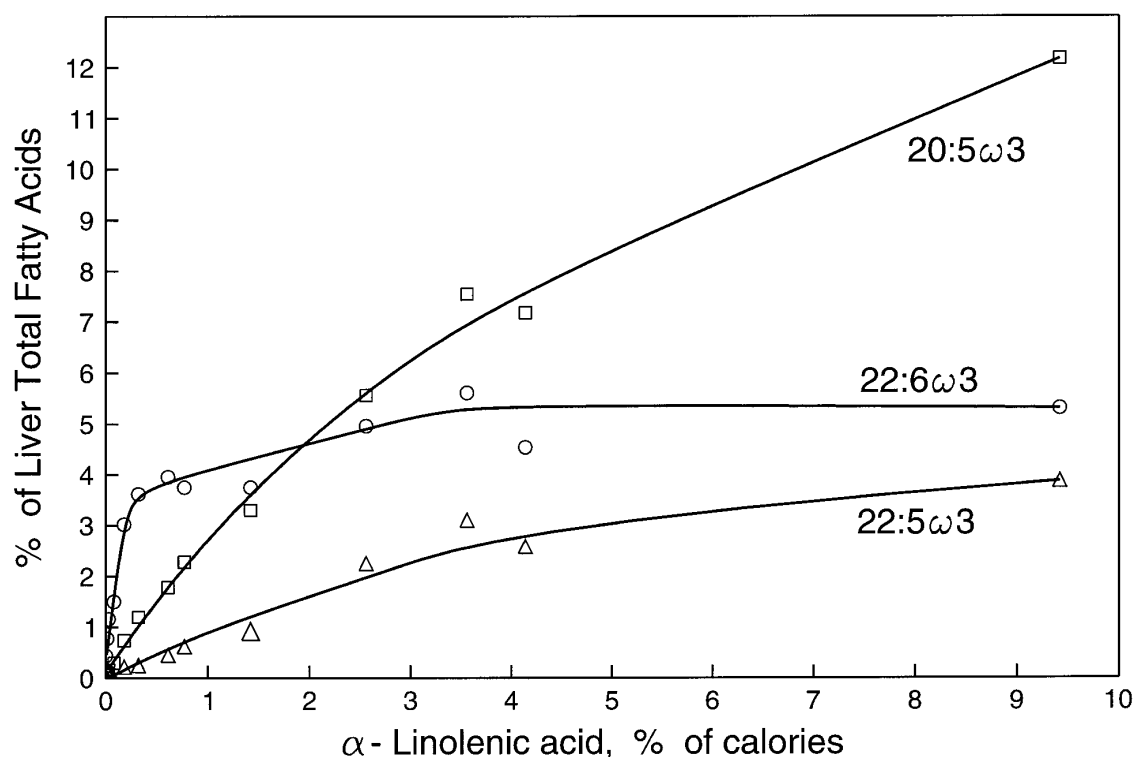


FIGURE 1 The effect of dietary level of α -linolenic acid (18:3 ω 3) on the content of its metabolites in liver lipids.

22:6 ω 3 rose much more abruptly than did the levels of intermediates 20:5 ω 3 and 22:5 ω 3, suggesting that 22:6 ω 3 is the more important structural acid of the three metabolic products.

These phenomena were also measured in erythrocytes and depot fat (Mohrhauer and Holman 1963b), brain (Mohrhauer and Holman 1963c) and heart tissue (Mohrhauer and Holman 1963d). These results, together with the report by Rieckehoff et al. (1949), in which chemical evidence of EFA deficiency was found in liver, kidney, heart, brain, muscle, skin and depot fat of the rat, indicated that EFA deficiency affects all tissues.

Because we were dealing with two cascades of fatty acids (FA) in which the locations of double bonds changed at each step of the cascade, use of the abbreviated Geneva chemical terminology became very confusing. We found it necessary to invent a new numbering system for the unsaturation, counting from the terminal methyl group, because in the biochemistry of these fatty acids, the terminal structure of the fatty acid remains unaltered. Omega (ω) is the terminal letter of the Greek alphabet, and it is used to indicate a reversal in the direction of counting. The number which follows ω is the length of the terminal saturated structure. Omega 6 (or ω 6) indicates that the double bond nearest the terminal methyl group lies after a 6 carbon saturated terminal structure. Omega 3 (or ω 3) indicates a 3 carbon saturated terminal structure. These terminal structures influence the physical properties of the membranes in which they occur. The use of the omega nomenclature was proposed by Holman (1964).

Linoleic acid, 18:2 ω 6, and α -linolenic acid, 18:3 ω 3, are metabolized by the same microsomal enzyme system, by alternating desaturation and elongation, to make two cascades of metabolic products up to 22 or more carbons long, which occur in tissue structural lipids in animals and in man. Linoleic acid and linolenic acid are the precursors of the two main families of essential polyunsaturated fatty acids (PUFA), namely, the ω 6 and the ω 3 families. For linoleic acid, Marcel et al. (1968) found the preferred metabolic cascade to be as follows:

18:2 ω 6 $\rightarrow$ 18:3 ω 6 $\rightarrow$ 20:3 ω 6 $\rightarrow$ 20:4 ω 6 $\rightarrow$ 22:4 ω 6 $\rightarrow$ 22:5 ω 6

The metabolic cascade for the metabolism of linolenic acid, found by Klenk and Mohrhauer (1960), is as follows:

18:3 ω 3 $\rightarrow$ 18:4 ω 3 $\rightarrow$ 20:4 ω 3 $\rightarrow$ 20:5 ω 3 $\rightarrow$ 22:5 ω 3 $\rightarrow$ 22:6 ω 3

Mohrhauer and Holman (1963e) then found that when dietary 18:2 ω 6 was held constant, increasing the level of dietary 18:3 ω 3 acid suppressed the content of ω 6 products. These data are shown in **Figure 2, left panel**. Conversely, when 18:3 ω 3 was held constant, Rahm and Holman (1964) found that increasing levels of dietary 18:2 ω 6 suppressed the ω 3 products as is shown in **Figure 2, right panel**. These studies led to a general hypothesis by Holman and Mohrhauer (1963) that *all fatty acids compete with EFA at all steps of the above cascades for metabolism of the essential fatty acids*. The ω 6 and ω 3 acids compete for the same enzyme sites involved in these reactions. As intake of 18:3 ω 3 increases, metabolic products of linoleic acid are suppressed, and linoleic acid itself is increased in the liver lipids. Conversely, with constant dietary 18:3 ω 3 and increasing dietary 18:2 ω 6, ω 3 products are suppressed, but 18:3 ω 3 itself increased in liver lipids. Strong suppression of ω 6 metabolism was accomplished by <2% of calories of 18:3 ω 3, whereas an equal suppression of ω 3 metabolism required nearly 10 times as much dietary linoleate. Omega 3 PUFA are more strongly conserved than are the ω 6 PUFA. Suppression of 20:4 ω 6 by dietary 18:3 ω 3 of 20:4 ω 6 to 50% of its maximum value occurred at ~0.5% of calories of 18:3 ω 3, whereas suppression of 22:6 ω 3 to 50% of its maximum level by dietary 18:2 ω 6 occurred at 7% of calories of 18:2 ω 6. To be equally competitive, these precursors, 18:2 ω 6 and 18:3 ω 3, should be in the ratio of 14:1. Equality of competition, however, may not be the criterion for optimal function. Yehuda and Carasso (1993), in studies of cognition in rats, found the optimum functional ratio of ω 6/ ω 3 to be 4:1.

Mohrhauer et al. (1967) found that in elongation of 18:2 ω 6

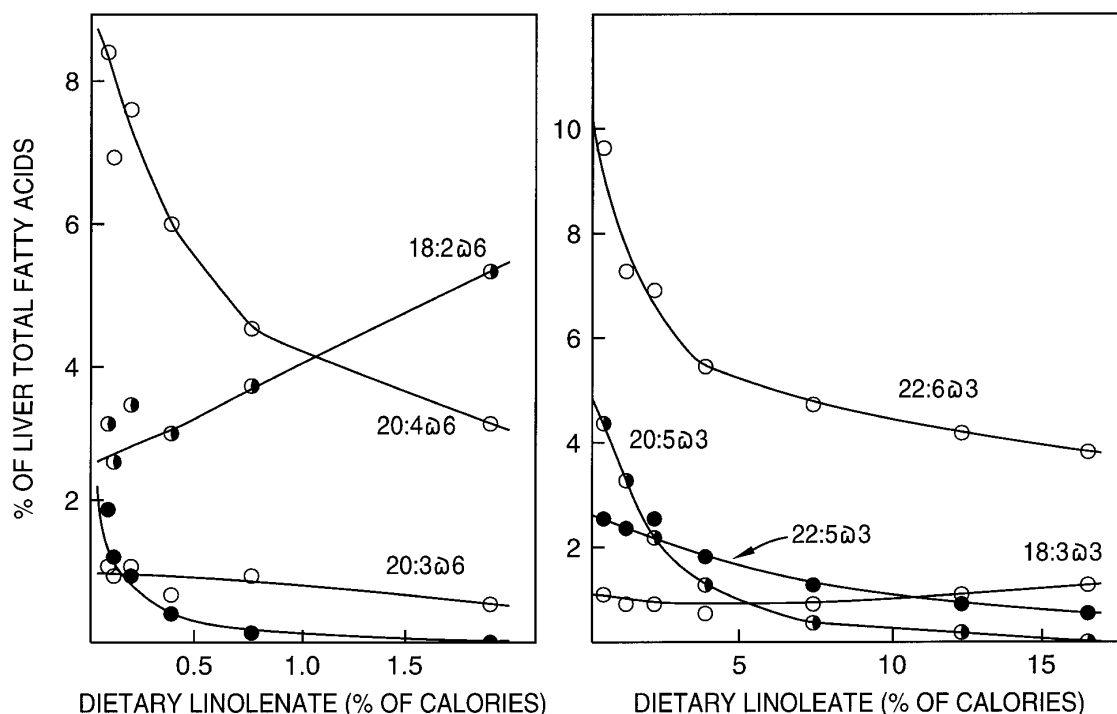


FIGURE 2 Left panel: Suppression of the metabolism of 0.6% of dietary calories of 18:2 ω 6 by increasing levels of dietary 18:3 ω 3, as measured in liver lipids. Right panel: Suppression of the metabolism of 1% of calories of 18:3 ω 3 by increasing levels of dietary 18:2 ω 6 as indicated by changes in liver fatty acids.

to 20:2 ω 6 by liver microsomes, other fatty acids in the medium act as competitive substrates and inhibit the reaction (see Fig. 3). Brenner and Peluffo (1966) made similar studies of the desaturation reactions involved in the EFA cascade and found that substrate competition occurred also for the desaturation steps.

EFA DEFICIENCY IN HUMANS

Burr and Burr (1929) had fed weanling rats a fat-free diet throughout life. Despite gaining many times their original weight, they failed to grow at the rate of fat-fed controls. Burr's group applied this new knowledge to medical problems in humans. His student, Arild Hansen (1933) found infant eczema to respond to supplements of lard, which contains both linoleic and arachidonic acids. Hansen made a career studying EFA deficiency in infants, for it was then common to give skim milk and sugar as a substitute for mother's milk. Infant eczema, due to EFA deficiency, was a real medical problem in the 1940s and 50s. Hilda Wiese learned our alkaline isomerization procedure, and with Hansen, made a masterful study of EFA deficiency in infants given skim milk and sucrose (Hansen et al. 1958).

Brown et al. (1938), in Burr's laboratory, made an unsuccessful attempt to induce overt dermatitis in a man fed a "low fat" diet for 6 mo. Although this study began with a normal adult, the diet was not fat free and was continued only 1% of a lifetime. The failure to induce dermatitis was taken by some in the medical community to mean that essential fatty acids are needed by rats, but not by humans. It took three decades before total parenteral nutrition (TPN) was developed as a relatively safe life-saving procedure in emergencies, but the first preparations were fat free, and they rapidly induced severe EFA deficiencies, ultimately convincing the medical community of the essentiality of EFA for mankind.

EFA deficient infant. In 1970, an infant experienced a volvulus at birth, underwent duodenal-colon anastomosis and was maintained by a fat-free TPN preparation. After 3 mo, skin lesions developed, and we were asked to provide analytical evidence of EFA status. Analyses of the phospholipid (PL) fatty acids of plasma were made by the new technique of gas chromatography by Paulsrud et al. (1972). The PL FA profile, shown in Figure 3, indicates that all of the ω 6 and ω 3 acids had normalcy ratios (equivalent to z-scores) <1, indicating low values. Most of the PUFA were <10% of control values. Only 20:3 ω 9, derived endogenously from oleic acid (18:1 ω 9), was elevated as an endogenous replacement, produced in the absence of the EFA. After 3 mo of EFA-free TPN, the $\Sigma\omega$ 6 acids in the plasma PL were 3.31%, $\Sigma\omega$ 3 acids were 0.01% and 20:3 ω 9 was 27.5% of the total FA of plasma PL. The infant was deficient in ω 3 and ω 6 EFA. This was our first case in an extensive study of EFA deficiency in infants induced by long-term TPN without lipids. At autopsy, specimens of many tissues were subjected to analysis of PL FA, and the same EFA deficiencies were found as in plasma PL, confirming in a human that the profile of plasma PL is a measure of EFA status of tissue PL.

Adult EFA deficiency. In late 1969, a female aged 78 y experienced a mesenteric infarction, underwent a duodenal-colon anastomosis and was maintained for 7 mo on fat-free TPN at the University of Minnesota Hospitals (Holman 1981). A dermatitis of EFA deficiency was expected, and it appeared at about 1 mo. The plasma PL triene/tetraene ratio was elevated. Attempts were made to apply corn oil to the skin as a means of administering EFA, but this had only a minimal effect on the triene/tetraene ratio. Samples of plasma were taken at 1, 2 and 3 mo; their mean profile is shown in Figure 4, indicating severe deficiencies of both ω 6 and ω 3 PUFA. The third sample from this woman showed that $\Sigma\omega$ 6 acids in plasma PL had reached a low of 21.8%, and that $\Sigma\omega$ 3

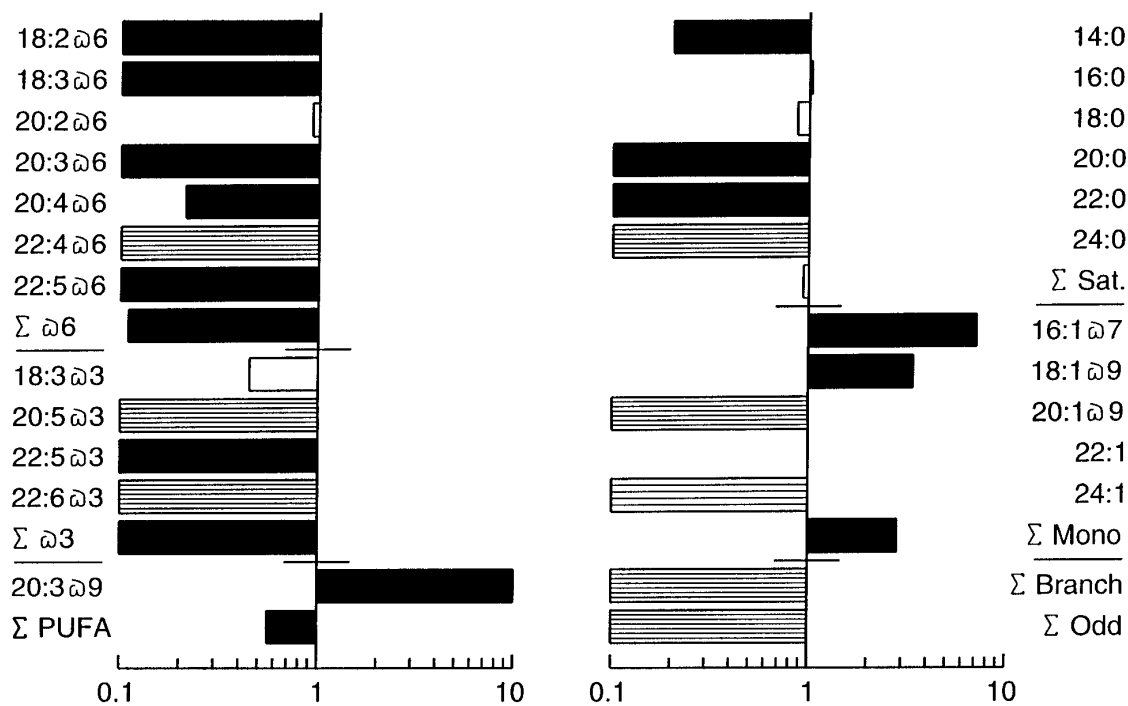


FIGURE 3 Changes in fatty acid composition of plasma phospholipid (PL) in a case of volvulus at birth after 48 d of fat-free total parenteral nutrition (TPN). Three samples from the patient are compared with 38 adult controls. The profiles are expressed in terms of normalcy ratios with polyunsaturated fatty acids (PUFA) at the left and saturated and monounsaturated fatty acids at the right. Bars to the left are decreases and bars to the right are increases, compared with controls. In these profiles, black bars indicate $P < 0.001$, heavy shading $P < 0.01$, light shading $P < 0.05$ and no shading nonsignificant.

acids were 0.01% of the fatty acids of plasma PL. This case was also an example of nearly complete ω 3 deficiency! After 7 mo of fat-free TPN, the patient died of a systemic infection radiating from the site of her indwelling catheter. These two cases illustrate that EFA deficiency can occur in the young

and in the old, and that it can be rapid and extreme. Figure 4 shows the plasma PL profile of the woman in comparison with 33 female Minnesota controls (Phinney et al. 1990).

Omega 3 deficiency. The first reported case of ω 3 deficiency (Holman et al. 1982), was induced by an ω 3-free TPN

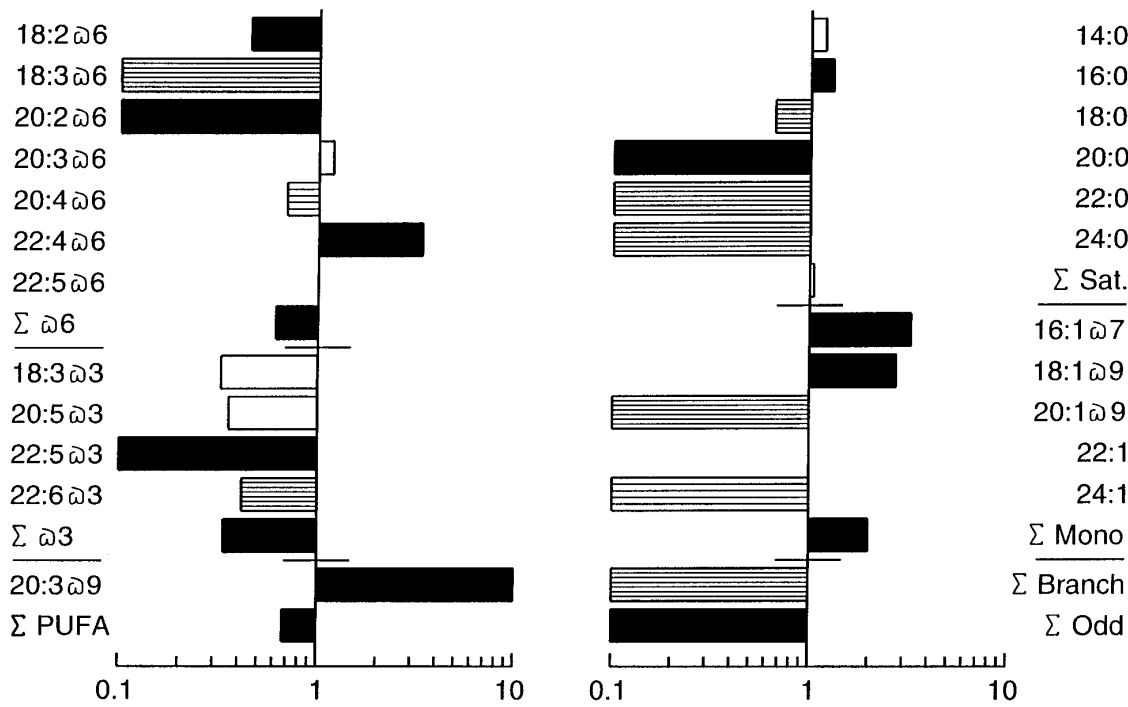


FIGURE 4 Fatty acid profile of an essential fatty acid (EFA)-deficient 78-yr-old woman maintained on fat-free total parenteral nutrition (TPN) for 3 mo. See legend of Figure 3 for description of the profiles.

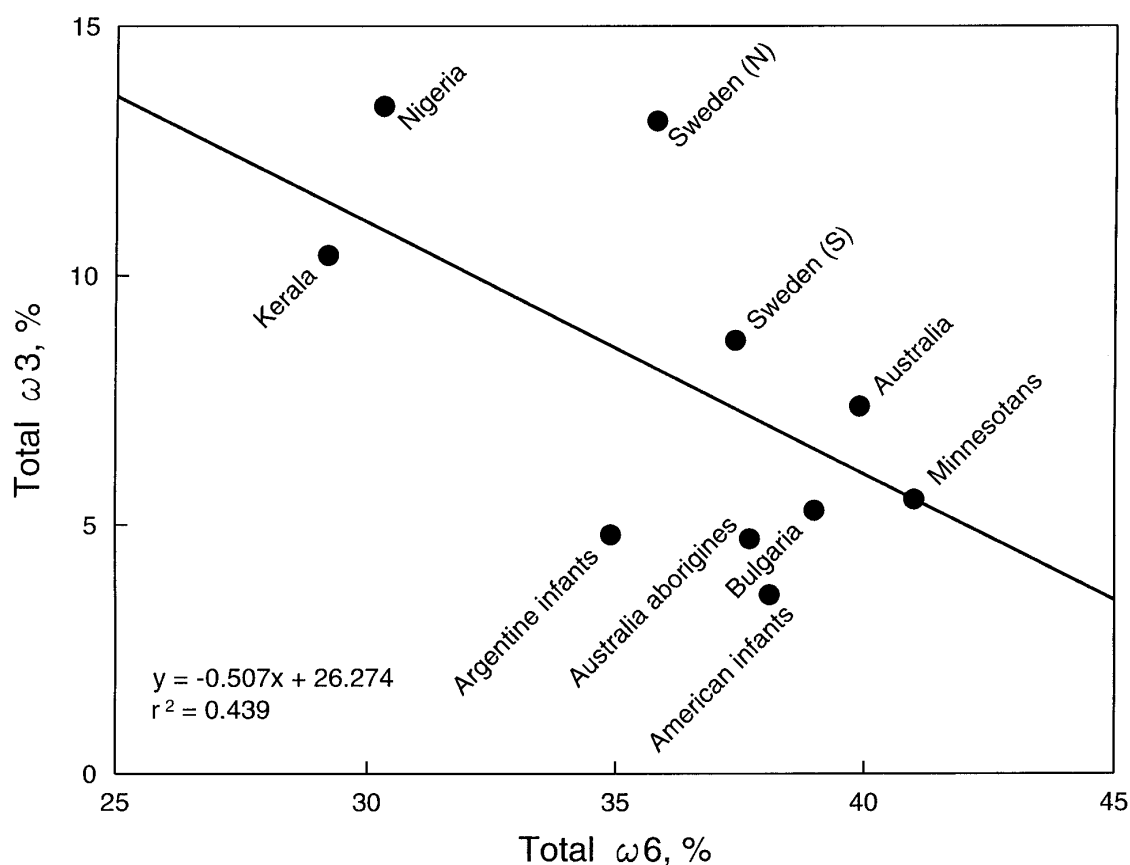


FIGURE 5 Total $\omega 3$ acids plotted vs. total $\omega 6$ acids for plasma phospholipid (PL) from 10 naturally occurring populations studied to date, indicating both $\omega 3$ status and $\omega 6$ nutritive status. The diagonal line is the line relating $\omega 3$ to $\omega 6$, determined by method of least squares, the formula for which is given in the lower left corner. The number of persons sampled in each study was as follows: Nigerians, 38; Northern Swedes, 17; Keralites, 39; Southern Swedes, 19; Australians, 33; Minnesota omnivores, 100; Bulgarians, 30; Australian aborigines, 93; Argentine infants, 48; and American infants, 38.

preparation. A girl, 6 y of age, experienced an accidental abdominal gunshot wound and underwent repeated surgical repairs. Recovery time increased with each surgery. By 1982, the FDA had approved the use of TPN with lipid emulsions to provide EFA, and two preparations were then available, one containing soybean oil, a source of $18:3\omega 3$. The other contained safflower oil, almost devoid of $18:3\omega 3$, but with a very high content of $18:2\omega 6$. After 5 mo of TPN with safflower oil, the girl exhibited episodes of numbness, tingling, weakness, inability to walk, leg pain, psychological disturbances and blurred vision. The clinicians suspected that her intravenous alimentation was inducing an $\omega 3$ deficiency and requested a fatty acid analysis of her plasma PL. Her profile showed $\omega 3$ deficiency, with a level of total $\omega 3$ EFA at 34% of control value and total $\omega 6$ at 75% of its control value. When TPN was changed to the soybean oil preparation, the neuropathy disappeared, and subsequent analysis showed that the $\omega 3$ deficiencies were restored toward normal.

The adult control population for studies involving Americans consists of 100 omnivorous students and staff of the University of Minnesota, gathered as controls for a study of vegetarianism (Phinney et al. 1990). Each subject was examined by a physician and found to be healthy. In **Figure 5**, naturally occurring control populations from five continents are compared with these Minnesota controls, plotting $\Sigma\omega 3$ value vs. $\Sigma\omega 6$ value. The data reveal a wide range of $\Sigma\omega 3$ EFA content in plasma and an inverse correlation in humans between $\Sigma\omega 3$ content and $\Sigma\omega 6$ content of plasma PL. This correlation was

predictable from our studies with rats and from the competition hypothesis of Holman and Mohrhauer (1963).

Several naturally occurring control populations have been studied. Of these, Nigerians, studied by Holman et al. (1996), were found to have the highest content of $\Sigma\omega 3$ EFA, 2.4-fold higher than the Minnesota controls sampled the same year. American newborn infants were found to be the lowest control group with respect to $\Sigma\omega 3$. Minnesota adults lie at the 20th percentile of the range between the low for American infant cord plasmas and the high for normal adult Nigerians. Keralites, from the Malabar coast of India, reputed to consume coconuts, coconut oil and fish, were also found to have high $\omega 3$ status (unpublished data). Keralites lie at the 64th percentile of the range of our natural controls. Nigerians, Keralites and Swedes eat fish frequently. The limited data shown in **Figure 5** indicate that "normal" populations from different cultures and nutritive environments differ widely, and that there are broad ranges of $\omega 6$ and $\omega 3$ PUFA that permit life. The data show that Minnesota controls are near the bottom of the range of $\omega 3$ acids found in naturally occurring control groups, and that American newborn infants have the lowest $\omega 3$ status of natural groups tested.

$\omega 3$ AND $\omega 6$ EFA STATUS OF DISEASED PATIENTS

The case of nutritionally induced $\omega 3$ deficiency prompted study of several neuropathies within a Peripheral Nerve Center

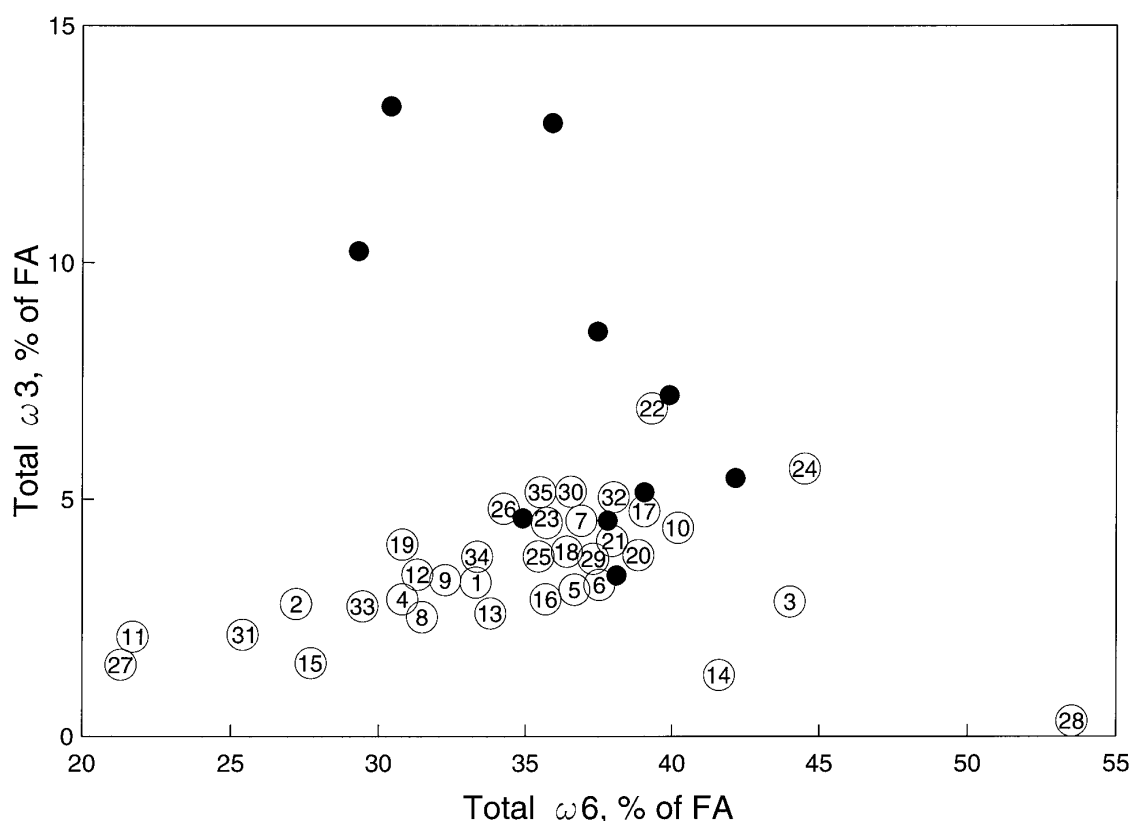


FIGURE 6 Mean values for $\Sigma\omega 3$ plotted against $\Sigma\omega 6$ for 35 groups of patients studied in this laboratory are indicated by open, numbered circles. These data are superimposed on the mean values of 10 natural control groups shown in Figure 4. For each study of a diseased group, the number, the diagnosis, the number of patients and the medical colleague for the study were as follows: 1- Adrenoleukodystrophy (10) W. Krivit; 2- Biotin deficiency (3) D. Mock; 3- Hypertrophic cardiomyopathy, carnitine deficiency, maintained on Vivonex (1) W. Hamilton; 4- Abetalipoproteinemia (6) B. Bernard; 5- Celiac disease (6) K. Bravo; 6- Crohn's disease (22) C. McClain; 7- Rheumatoid arthritis (37) R. Zurier; 8- Scleroderma (8) R. Zurier; 9- Lupus (9) R. Zurier; 10- IgA nephropathy (15) J. Donadio; 11- Renal transplants (17) J. Donadio; 12- Infant hepatitis (6) H. Sharp; 13- Non-alcoholic hepatitis (10) C. McClain; 14- Hereditary tyrosinemia (2) H. Sharp; 15- Wiscott-Aldrich Synd. (6) L. Spitler; 16- Osteoporosis (23) B. Riggs; 17- Alcoholic cirrhosis (8) J. Bloomer; 18- Alcoholic cirrhosis (16) S. Phinney; 19- Anorexia nervosa (8) R. Nelson; 20- Alzheimer's disease (13) E. Ahlskog; 21- Parkinson's disease (18) E. Ahlskog; 22- Multiple sclerosis (10) E. Kokmen; 23- Huntington's disease (28) M. Nance; 24- Prader-Willi obesity (6) R. Nelson; 25- Preeclampsia (11) P. Ogburn; 26- Obesity (10) S. Phinney; 27- Suspected $\omega 3$ deficiency (1) S. Phinney; 28- Hepatopancreato-renal syndrome (1) H. Sharp; 29- Retinitis pigmentosa (10) J. Crofts; 30- Macular degeneration (20) J. Crofts; 31- Multisystem neuronal degeneration (2) P. Dyck; 32- Reye's syndrome (12) P. Ogburn; 33- Achrodermatitis enteropathica (1) R. Cash; 34- Cystic fibrosis (41) J. Lloyd-Still; 35- Gay bowel disease (1) J. Turner.

with the Mayo Clinic; our subsequent involvement in a Liver Disease Center at the University of Minnesota facilitated cooperative study of several diseases involving the liver and the immune system. Without those cooperative efforts, our present understanding of $\omega 3$ deficiency could not have developed. The $\omega 6$ and $\omega 3$ EFA status associated with some of the diseases studied are shown in Figure 6.

In Figure 6, values for our control populations and for patients with diagnosed diseases are plotted together. The diagnosed diseased groups lie in the lower half of the range of $\omega 3$ values, as do the adult Minnesota controls and the infant American controls. I conclude that our adult Minnesota controls have less than a prudent reserve of the essential $\omega 3$ fatty acids needed for prevention of disease, repair of abnormal nerve structure or correction of abnormal immune functions.

The relatively low $\omega 3$ level of the American population is probably of nutritional origin, because $\omega 3$ EFA are not currently provided at adequate levels by many of our commonest foods, thus not supporting protective levels of $\omega 3$ in human tissue or providing sufficient $\omega 3$ EFA for resistance against disease processes. In modern time, our major food sources of $\omega 3$ EFA have been exchanged for $\omega 6$ -rich and $\omega 3$ -poor sources to increase shelf-life. Much of the 18:3 $\omega 3$ in our $\omega 3$ -rich oils

has been hydrogenated away for the past half century. The medical community has emphasized the desirability of linoleic acid oils as being the only source of "polyunsaturated fat" and has ignored the $\omega 3$ polyunsaturates, although data supporting their importance have been available for decades. Meanwhile, our meat industry has been increasingly based upon animals and birds fed corn, which is rich in 18:2 $\omega 6$ and poor in 18:3 $\omega 3$, producing $\omega 3$ -deficient meat, which our population prefers to fish. Our present day food shelves provide some sources of $\omega 3$ EFA, if the consumer selects nonhydrogenated soybean, canola and other $\omega 3$ -rich oils, and if saturated and partially hydrogenated fat products are avoided. Birds, fish and mammals become $\omega 3$ deficient when they consume diets low in $\omega 3$ EFA, and we become $\omega 3$ deficient when we eat their flesh. Our meats should again be derived from $\omega 3$ -fed animals. Molecular biologists are now able to modify the genetics of corn to allow it to synthesize 18:3 $\omega 3$ and 18:2 $\omega 6$ in equal proportions. To reach the major segment of the public, our popular manufactured, prepackaged and fast foods should be redesigned to become $\omega 3$ -rich rather than $\omega 3$ -poor. Food labels should be permitted to indicate $\omega 3$ content. As a first approximation, we should strive for a diet in which $\omega 6/\omega 3$ approaches 4:1 as suggested by Yehuda and Carasso (1993). It is time the public

becomes conscious of this group of vital nutrients required to maintain health.

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