Biochemistry and physiology of n-3 fatty acids

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ABSTRACT **Considering the n-3 fatty acids to be partial agonists relative to n-6 fatty acids helps consolidate into a unified interpretation the many diverse reports and controversies on the actions of these two types of essential fatty acids. Some research reports illustrate the similarities between these two types and some emphasize the differences, leaving readers to evaluate the status of n-3 fatty acids from a viewpoint that is conceptually similar to regarding a glass of water as half empty or half full. Both n-3 and n-6 types of fatty acids must be obtained through the diet because they are not synthesized de novo by vertebrates. Both types can support important physiological and developmental processes, can form eicosanoids (prostaglandins, leukotrienes, lipoxins, etc.), can be esterified to and hydrolyzed from tissue glycerolipids, and can be metabolically elongated and desaturated to a variety of highly unsaturated fatty acids. However, some nonesterified n-6 acids are vigorously converted to potent n-6 eicosanoids that exert intense agonist actions at eicosanoid receptors, whereas the n-3 acids less vigorously form n-3 eicosanoids that often produce less intense (partial) actions. Because both types owe their presence in vertebrate tissues to dietary intake, important physiological consequences follow the inadvertent selection of different average daily dietary supplies of these two types of polyunsaturated fatty acids.—Lands, W. E. M. Biochemistry and physiology of n-3 fatty acids. *FASEB J.* 6: 2530–2536; 1992.**

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LIFE AND DEATH ARE A SUMMATION of processes too complicated to be dependent solely on a single event, but there are situations in which a particular rate-limiting process can affect markedly the overall outcome. Research experiments are designed to limit enough variables so that factors that influence a specific process under consideration can be better observed and defined. Such experiments permit a clearer understanding of the complex reactions that are otherwise difficult to recognize and interpret. Sometimes nature provides examples in which a process may become critically rate-limiting even though it is not always apparent. Thrombosis is one of a variety of eicosanoid-mediated processes that can occur physiologically in ways that may give no evidence of pathophysiology. Nevertheless, thrombosis is frequently a rate-limiting event in life (or death) in the U.S. (1, 2). This review examines our understanding of how n-3 fatty acids can diminish competitively the intensity of n-6 eicosanoid formation (3) and diminish the rate-limiting events in thrombosis. These competitive actions link the inadvertent dietary choices of n-3 and n-6 polyunsaturated fatty acids to the frequency of thrombotic deaths, and they illustrate the vital importance of the n-3 and n-6 fatty acids in human physiology. A better understanding of that importance has come from quantitative interpretations of the similarities and differences in the interactions of n-3 and n-6 acids with specific enzymes and receptors. This growing understanding has been reviewed in extensive detail at several recent international conferences in which the biochemistry and physiology of n-3 and n-6 fatty acids were linked to a wide range of health effects in addition to thrombosis (4–6). This review, while describing actions of n-3 derivatives as partial agonists, also places importance on actions that the n-3 derivatives do *not* have.

Early clues to the possible importance of n-3 fatty acids came from studies showing that young humans (7) and experimental animals (8) experienced impaired growth when all fatty acids were removed from the diet. Small amounts of n-3 fatty acids prevented that impaired growth, and thus these acids were designated as essential fatty acids (8). In that protocol, increased growth accompanied increased dietary amounts of n-3 fatty acids in the range of 0–1% of daily food calories (0–1 en%), above which the supply was no longer rate-limiting for growth and its importance was less evident (9). The n-6 fatty acid 18:2n-6 showed a similar dietary range in which its supply limited growth (0–0.3 en%), and the highly unsaturated fatty acid (HUFA)[1] 20:4n-6 was several-fold more effective than 18:2n-6 (10). Rate of growth is a vital dimension for young, developing animals and humans, but it has little significance for adults. Other rate-limiting aspects of n-3 fatty acid biochemistry and physiology have importance in the chronic disorders that become more significant in adults.

PHYSIOLOGY MEDIATED BY n-3 AND n-6 FATTY ACIDS

Excellent reviews of the physiological actions of the essential fatty acids were provided by Aaes-Jorgensen (11) and Holman (12). Unfortunately, these reviews were based on studies that preceded the discovery and understanding of the many important actions of eicosanoids (reviewed in ref. 2). As a result, extensive descriptions of physiological phenomena were made without the insights now possible. Both n-3 and n-6 fatty acids facilitated growth and development, but two important concepts in those early studies had a powerful effect on the interpretations. One concept was the use of restricted water supplies when assessing essential fatty acid efficacy, and the other was the substitution of fatty acid compositional data for actual physiological measurements (reviewed in ref 13). The former approach exacerbated the

[1]Abbreviations: CoA, coenzyme A; HUFA, 20- and 22-carbon highly unsaturated fatty acids; SFA, saturated fatty acids; UFA, 16- and 18-carbon unsaturated fatty acids; PIF, prolactin inhibitory factors; PRF, prolactin stimulatory factors; PRL, prolactin; ADH or AVP, antidiuretic hormone. Number notations used for fatty acids include the n-3 designation of positional isomers rather than "omega" terminology. Thus n-3, n-6, n-7, and n-9 represent omega 3, omega 6, omega 7, and omega 9, respectively

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lesser ability of the n-3 partial agonist or agonists to support dermal and renal integrity (11), and the latter approach replaced actual physiological measurements with an indirect estimate of nutrient supply that was presumed to indicate physiological status. Thus, many scientists regarded only the n-6 fatty acids as essential (14), and few investigators accumulated further quantitative evidence of the physiological efficacy and action of the n-3 fatty acids. As a result, scientists now know much more about (n-6)-mediated events than (n-3)-mediated events. A major review evaluating the actions of n-3 fatty acids (15) noted little evidence for any unique action of the n-3 fatty acids that could not be met by n-6 fatty acids. However, a recent review examined a possible requirement for n-3 acids in terms of supporting visual and neural functions (16). Much more research is needed to define the situations in which altered supplies of n-3 and n-6 HUFA have a physiological effect and to define the threshold amounts that mark transitions between adequate and inadequate or adequate and excessive levels of these HUFA in the diet (13, 17). A major conceptual reconciliation is needed to clarify the status of n-3 nutrients supporting some, but not all, functions supported by their n-6 homologs.

Three physiological functions appear to be maintained less well with n-3 fatty acids than with n-6 fatty acids: dermal integrity, renal function, and parturition (reviewed in refs 11 and 17). Excellent insight into the role of n-3 fatty acids as partial agonists was provided by Leat and Northrup (18) when they obtained successful breeding of a second generation of rats by employing caesarian operations. The successful delivery of pups in this manner demonstrated that 18:3n-3 satisfied requirements for successful fertilization, implantation, growth, gestation, and development of the fetus, but it limited parturition. Apparently the latter process requires an intensity of eicosanoid formation and function that only n-6 agonists can provide (see actions of eicosanoids below).

The lack of sufficient attention to physiological measurements for evaluating the efficacy of essential fatty acids has permitted some extrapolations beyond controlled evidence to remain unchallenged. This problem is most evident in the assignment of an apparent minimum amount of essential fatty acid needed to maintain apparently normal physiology. Early studies maintained rats free of any sign of deficiency by supplying the highly effective 18:2n-6 at about 0.3 en% (9, reviewed in ref 13). Similarly, results with infants ingesting a diet very low in fat (7) were interpreted to indicate that 0.5 en% or less (reviewed in refs 17, 19) may be needed for human infants. Nevertheless, many nutritionists still think it is important to include at least 2 en% 18:2n-6, and the typical diet in the U.S. now averages about 7 en%. Because rapidly growing infants may need only 1/20th of this amount (19), and no clear evidence shows a dietary requirement of 18:2n-6 for adults, we should examine carefully the biochemical and physiological consequences of adults ingesting such amounts.

The search for physiological events that are limited by the biochemistry of n-3 fatty acids has involved consideration of membrane biogenesis (16, 20) and eicosanoid signaling (2, 21). The latter process has been intensively studied under many controlled conditions, whereas the former remains an area of promise in regard to rapid perinatal development of membranes in the retina and nervous system (16) when supplies of n-3 HUFA may be limiting (reviewed in refs 13, 20). The greater certainty of the rate-limiting roles for eicosanoids leads to a principal focus of this review on (n-3)-related factors that limit eicosanoid-mediated physiology.

FORMATION OF EICOSANOIDS

After the discovery in 1963 that the n-6 HUFA 20:4n-6 is converted to prostaglandins (22), more prostaglandins were discovered, and the 5 years including 1975 through 1979 introduced the biomedical community to an amazing set of eicosanoids: thromboxanes (23); prostacyclins (24); leukotrienes (25) (see **Fig. 1**). The total number of biologically active eicosanoids is now more than 20 and includes both n-3 (Fig. 1) and n-6 types of compounds. Upon stimulation of a tissue, the formation of the n-6 eicosanoids from 20:4n-6 is often associated with an explosive, but transient, burst of synthesis (21). This burst provides active eicosanoids that can activate specific receptors briefly before selective catabolic enzymes rapidly convert the eicosanoids to inactive metabolites. If the rate of synthesis is too slow, there will be insufficient active eicosanoid to occupy receptors. If the rate of synthesis is too fast, excess active eicosanoids can cause pathophysiology. Thus, biochemical factors that regulate the intensity of eicosanoid biosynthesis become major factors in the expression of eicosanoid-mediated physiology.

Recognition that the rate of biosynthesis of prostaglandins was much slower with 20:5n-3 than with 20:4n-6 (3) led immediately to a demonstration that n-3 fatty acids competitively attenuate the rate of n-6 eicosanoid formation (3). This discovery was discussed (3) in terms of the possible therapeutic benefits in diminishing excessive n-6 eicosanoid-mediated events. This concept of competition acquired greater significance 2 years later with the discovery that biosynthesis of the n-6 eicosanoid, thromboxane A2, was a rate-limiting step in the aggregation of platelets, which leads to thrombosis (23). Agents slowing that rate (e.g., aspirin or the n-3 HUFA in fish oil) became subjects of intense interest (26, 27; reviewed in ref 2). Support for the concept of competition

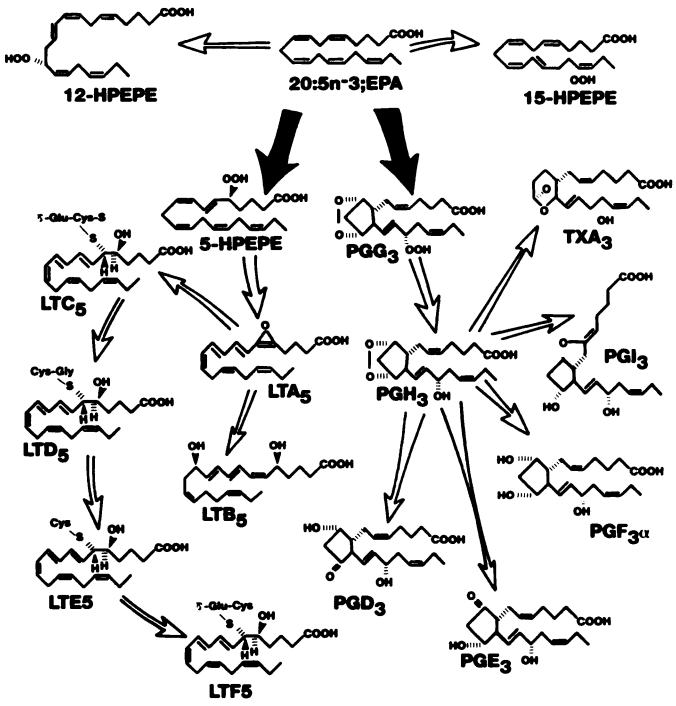

Figure 1. n-3 Eicosanoids formed from eicosapentaenoic acid (20:5n-3). The filled arrows indicate the fatty acid oxygenases that convert the nonesterified precursor to eicosanoids. The left arrow indicates 5-lipoxygenase, and the right arrow, prostaglandin H synthase.

during eicosanoid formation intensified after the 1979 discovery (25) of the biosynthesis of the leukotrienes (see Fig. 1), which are major rate-limiting mediators of immune-inflammatory events. By this time, a major biomedical research effort had been mobilized to interpret mechanisms for diminishing excessive formation and function of n-6 eicosanoids (2, 28).

The first irreversible, committed step in the synthesis of prostaglandins and leukotrienes is an hydroperoxide-activated fatty acid oxygenase action on the nonesterified HUFA, 20:4n-6 and 20:5n-3 (indicated as bold arrows in Fig. 1). An explosive, positive feedback of product hydroperoxide upon prostaglandin synthase (reviewed in ref 21) is less intense with 20:5n-3 than with 20:4n-6 (29), making the intensity of formation of n-3 prostaglandins less than that for n-6 prostaglandins under physiological conditions of limited hydroperoxide availability. Furthermore, the active n-3 eicosanoids synthesized from 20:5n-3 have been associated with less vigorous responses than n-6 eicosanoids when bound to the specific receptors (30, 31). Thus, in two different ways 20:5n-3 appears to be a partial agonist relative to 20:4n-6: intensity of biosynthesis and intensity of receptor activation.

ACTIONS OF EICOSANOIDS

The action of eicosanoids in modulating the pulsatile or irregular release of hypothalamic and pituitary hormones has a major effect on many major conditions, and it probably underlies the role of the n-3 and n-6 essential fatty acids in supporting growth and development. This role seems evident from the report that the abnormally low weights of testes, prostate, and seminal vesicles of animals deficient in essential fatty acids could be restored to normal values either by injected gonadotropin or dietary 18:2n-6 (32). Mediation of the signs characteristic of an essential fatty acid deficiency by an inadequate release of pituitary hormones was recognized when hypophysectomized rats showed the signs even though they had normal tissue levels of n-6 fatty acids (33). Eicosanoids derived from essential fatty acids are now known to modulate hypothalamic function (reviewed in ref 34) in stimulating growth hormone (GH) release from pituitary, by stimulating GRF release from hypothalamus, in mediating the release of ACTH from the pituitary (perhaps by increasing CRF from the hypothalamus), in enhancing the response of thyroid tissue to TSH, in promoting prolactin (PRL) release by decreasing the inhibitory (PIF) and increasing the stimulatory factors (PRF) from the hypothalamus, in stimulating release of antidiuretic hormone (ADH or AVP) and moderating its action peripherally, and in stimulating gonadotropin (LH and FSH) release by stimulating LHRH release from the hypothalamus. [Anterior pituitary cells in culture release LH in response to added leukotriene C4 (35).] Researchers often neglect studies of the n-3 eicosanoids, even though indirect evidence (9, 18) indicates that they may serve effectively in supporting many essential pituitary functions.

The typical n-3 eicosanoids in Fig. 1 (derived from 20:5n-3) have homologs derived from 20:4n-6 with which they compete, moderating the intensity of formation (3) and function (31) of n-6 eicosanoids and thereby modifying physiological responses in human tissues. Because excessive signaling by n-6 eicosanoids is associated with pathology such as thrombosis (heart attacks and ischemic strokes), immune-inflammatory disease (arthritis, lupus, asthma, etc.), dysmenorrhea, and other disorders affecting the quality of life, the benefits of ingesting the competing and moderating n-3 fatty acids merit further study (2, 3).

Thrombotic events include platelet activation and an explosive biosynthesis of the n-6 eicosanoid, thromboxane. Any chronically ingested substance that reduces the intensity of that biosynthesis (e.g., aspirin or n-3 HUFA) can be expected to save lives in the industrial nations that have high rates of cardiovascular mortality. A controlled feeding study showed a wide range of responses by platelets from different individuals in terms of both aggregation and biosynthesis of thromboxane (36). After ingesting dietary n-3 HUFA for several weeks, most subjects in the study showed a significant decrease in thromboxane formation, aggregation, or both. The platelet responses to collagen followed a hyperbolic pattern, with maximum aggregation associated with about 40 ng thromboxane/ml, making previous linear comparisons of results somewhat limited in interpretability. Once the non-linear hyperbolic nature of the response was evident, previous comparisons were recognized to be merely incomplete and not contradictory (36). That work has been extended to include a successful British intervention trial (37), showing that increased intake of n-3 fatty acids led to decreased mortality. Further confirmation of the benefit of n-3 fatty acids was evident in a long-term study in the U.S. (38) in which 0.66 g/day was associated with 40% lower cardiovascular mortality.

The participation of n-6 eicosanoids in mediating major disease processes (2) makes it important to understand the ways that dietary n-3 fatty acids can mix in intermediate pools of eicosanoid precursors (**Fig. 2**) and moderate the intensity of n-6 eicosanoid formation and function (2-6). Although the frequency and intensity of the hydrolytic formation of nonesterified HUFA in stimulated tissues is independent of dietary influence, the intensity of n-6 eicosanoid signaling from the released HUFA will inevitably be greater as 20:4n-6 becomes a greater proportion of the HUFA. In this way, when all other variables remain unchanged the relative proportion of n-6 HUFA in the HUFA of tissue phospholipids (values on the ordinate axis of **Fig. 3**) reflects the probable intensity of n-6 eicosanoid response (13). In this context, the ordinate values reported (20) for the U.S., Japan, and Greenland (75, 50, 22%) reflect the relative incidence of thromboxane-mediated thrombotic death in the

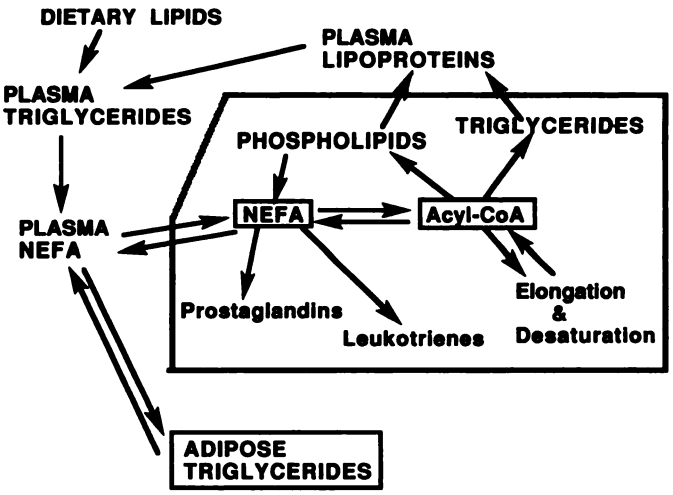

Figure 2. Availability of nonesterified precursors of eicosanoids. The metabolic relationships among tissue lipid pools in converting dietary n-3 and n-6 fatty acids into eicosanoids. Nonesterified fatty acids are indicated as NEFA.

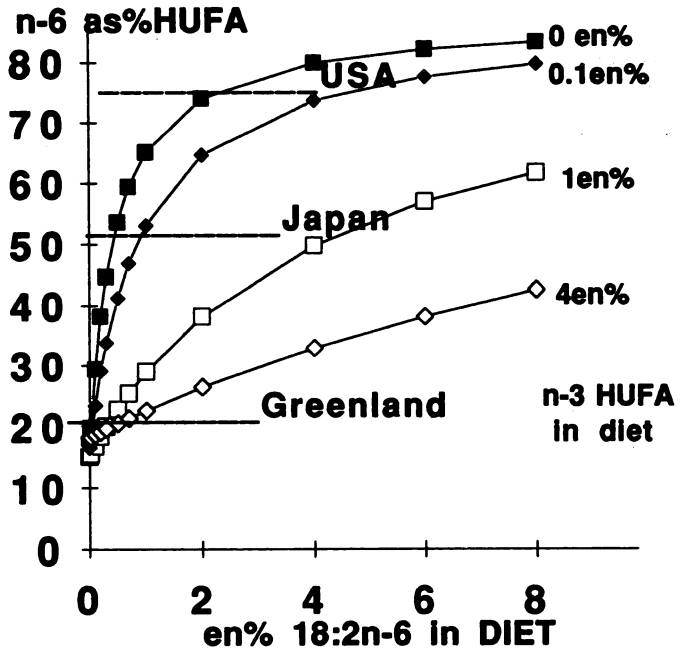

Figure 3. The proportion of n-6 eicosanoid precursors maintained in phospholipids. Ingestion of n-3 HUFA decreases the relative proportion of n-6 HUFA (ordinate axis), which reflects the probable intensity of n-6 eicosanoid response when stimulated. Reported values for U.S., Japan, and Greenland are indicated on the ordinate axis.

U.S., Japan, and Greenland (approximately 100, 20, and 10%, respectively) (20, 39). Another eicosanoid-related example is the several-fold lower clinical incidence of rheumatoid arthritis in Japan compared with the U.S. in spite of a greater genetic vulnerability based on HLA genotypes (40). The lower incidence in Japan has been considered to result from nutritional factors similar to those that cause a lower thromboembolic mortality in Japan (40). The abundance of dietary n-3 HUFA in the typical Japanese diet (39) predicts (Fig. 3) a lower capacity for forming n-6 eicosanoids that mediate arthritis and thrombosis. Studies of mortality differences for native Japanese in Japan and emigrated Japanese in Hawaii and San Francisco (with considerable genetic similarity) support the hypothesis of an important role for dietary fat that overrides many genetic considerations. For both thrombosis and arthritis, the effect of dietary n-3 acids on the supply of precursors for active eicosanoids merits careful interpretation.

PRECURSOR POOLS FOR n-3 AND n-6 EICOSANOIDS

Figure 2 illustrates the biochemical steps that link the tissue lipid pools with the nonesterified fatty acids that are immediate precursors for the formation of eicosanoids. A greater relative abundance of dietary n-3/n-6 fatty acids flowing through these intermediates creates the physiological and clinical conditions that demonstrate the importance of the n-3 fatty acids.

Nonesterified fatty acids

The composition of the intracellular pool of nonesterified fatty acids accessible to the fatty acid oxygenases is influenced by influx of extracellular nonesterified fatty acids and by a stimulus-initiated, receptor-mediated hydrolysis of cellular lipids (phospholipids, glycerol esters, or cholesterol esters) (reviewed in refs 13, 21). An unexplained tendency for the ratio of n-3/n-6 HUFA to be greater in the nonesterified fatty acids than in the phospholipids (41–43) provides a relatively greater abundance of the n-3 partial agonist with a lower probable intensity of n-6 eicosanoid formation than would be predicted from phospholipid data alone (see below).

Highly unsaturated fatty acids in glycerolipids

Hydrolytic release from phospholipid esters appears to occur indiscriminantly with n-3 and/or n-6 types of HUFA (44) and to involve all major classes of glycerolipids (i.e., phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl inositol, etc.). In relation to providing eicosanoid agonists and partial agonists for tissue responses, the indiscriminate hydrolysis confers importance to selective factors that maintain the overall composition of esterified n-3/n-6 HUFA. Ultimately, the major factor is the relative supply of dietary n-3/n-6 fatty acids that affects the proportions of n-3/n-6 eicosanoid precursors in the HUFA pools in tissues.

When rats were fed standard rat chow, the patterns of n-3/n-6 HUFA maintained a high proportion of n-6 HUFA in plasma lipids, reflecting a relative lack of n-3 acids in the diets employed. Added corn oil failed to shift the ratio to a significantly higher level (ref 41, as reviewed in ref 45). Thus standard rat chow has an extreme status in terms of n-6 HUFA and does not provide an experimental model of intermediate responsiveness. In this regard, rat chow resembles the typical U.S. diet, which includes only small average amounts of HUFA (about 0.1 en% 20:4n-6 (ca. 0.22 g/day) and 0.08 en% n-3 HUFA (ca. 0.17 g/day)) and has about 6–8 en% 18:2n-6 (ca. 14.6 g/day) and about 0.6–1 en% 18:3n-3 (ca. 1.7 g/day) (data from ref 38), reflecting the large contribution of soy oils to the American diet (see **Fig. 4**). Shifting the food oils from soy oil to corn oil would produce only a slight increase in the proportion of n-6 eicosanoid precursors in the HUFA of phospholipids (see Fig. 4), whereas inclusion on n-3 fatty acids would decrease the proportion of n-6 eicosanoid precursors in HUFA. The current range of n-3/n-6 composition of typical dietary fats may cause the current biomedical research interpretations of normal responses to be biased because typical data are collected only from subjects whose tissues are at the extreme end of the natural range of n-6 HUFA.

Enzyme-catalyzed transfer of fatty acids from their acyl-CoA esters to form glycerolipid esters discriminates among saturated fatty acids (SFA); 14:0, 16:0, 18:0; unsaturated fatty acids (UFA), 16:1n-7, 18:1n-7, 18:1n-9, 18:2n-6, 18:3n-3; and HUFA, 20:3n-9, 20:4n-7, 20:3n-6, 20:4n-6, 22:4n-6, 22:5n-6, 20:5n-3, 22:5n-3, 22:6n-3. In the de novo synthesis of glycerolipids, SFA is placed at position 1 and UFA at position 2 (reviewed in ref 45). This leads to phospholipids with nearly 50% of the fatty acids as SFA and triglycerides with about 33% SFA. The subsequent action of phospholipid retailoring acyltransferases replaces UFA with HUFA at position 2 and the replacement probably occurs to a different extent in different tissues. The average general fatty acid composition of phospholipids of rat plasma includes about 43% SFA, 36% UFA, and 20% HUFA (43). A similar pattern maintained in human plasma phospholipids (42% SFA, 35% UFA, and 20% HUFA; summarized in ref 43) reflects similar general selectivities in forming glycerolipids in rats and humans. Because the retailoring acyltransferases act somewhat nonselectively with regard to the n-3, n-6, n-7,

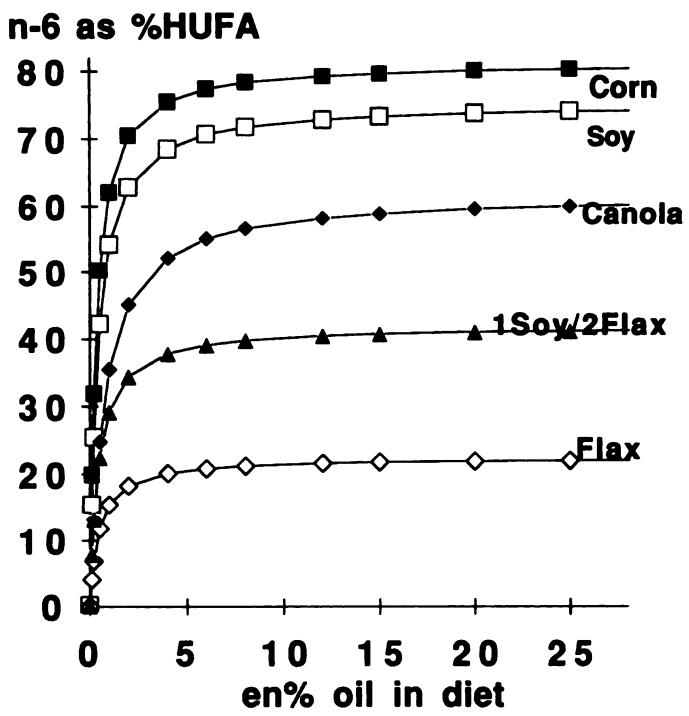

Figure 4. Effect of n-3 acids in food oil on the proportion of n-6 eicosanoid precursors that is maintained in phospholipid HUFA.

and n-9 chemistry of the fatty acids (44, 46), the relative abundance of the types of HUFA made available to tissues greatly influences the overall pattern of HUFA that is maintained in a tissue.

Another factor that enhances the accumulation of HUFA in tissue phospholipids is the fatty acid:CoA ligase that is selective for HUFA (47, 48). This enzyme provides an enrichment of HUFA-CoA esters to the retailoring acyltransferases whereas the nonselective ligase activates all nonesterified fatty acids to CoA esters. As a result, the phospholipids in tissues that have the HUFA-selective ligase can accumulate greater ratios of HUFA/UFA than those in other tissues. The dynamic balance between the HUFA-specific ligase and the nonspecific helps maintain about 30% HUFA in liver phospholipids (43). The HUFA-selective ligase, however, has shown little discrimination in vitro between the n-3 and n-6 types of HUFA, suggesting that simple competitive interactions influence the final overall composition of tissue HUFA. If further research showed selective incorporation of n-6 HUFA in vivo, this process may help explain the observed but unexplained greater tendency for n-3/n-6 ratios in the nonesterified fatty acids to be higher than in phospholipids.

In contrast to the relatively nonselective reactions noted previously, some highly selective reactions occur with still undetermined enzymes to diminish the amount of 18:3n-3 in glycerophospholipids and to combine 20:4n-6 with 18:0 in certain diacyl units. Work remains for future researchers to identify how those selective events occur, but relatively nonselective competitive interactions between n-3 and n-6 acids clearly influence the supply of n-6 agonists and n-3 partial agonists in the HUFA of cellular phospholipids.

Competitive elongation and desaturation

Enzymes catalyzing the conversion of 18-carbon UFA into 20- and 22- carbon HUFA act fairly nonselectively on the n-7 and n-9 types of acid (formed endogenously by 9-desaturase action on 16:0 and 18:0, respectively) as well as the n-3 and n-6 types of acid (provided exogenously), converting 18-carbon UFA into 20- and 22-carbon HUFA (reviewed in refs 49, 50). A result of the nonselective metabolism is that the relative availability of the n-3 and n-6 types of 18-carbon polyunsaturated fatty acids in the diet strongly influences the proportions of the types of HUFA that are accumulated in tissue lipids (51, 52). The resultant proportions influence, in turn, the proportions of eicosanoid precurso that can be mobilized by tissues upon stimulation, and thereby the intensity of the n-6 eicosanoid response.

After a preliminary report of competition between 18:2n-6 and 18:3n-3 in forming tissue HUFA (51), Mohrhauer and Holman (9, 52) provided a unique series of reports showing that, in response to the different diets, the total amount of HUFA in each tissue changed little in relation to the large changes in the proportions of n-9, n-6, and n-3 HUFA. The reciprocal relationships among the n-9, n-6, and n-3 acids and the percent of dietary energy (en%) in the form of n-3 and/or n-6 fatty acids followed hyperbolic patterns (see Fig. 1 in ref 51), indicative of the competitive interactions (13). The constants representing 50% effective doses that fit the competitive hyperbolic patterns for dietary 18:2n-6 and 18:3n-3 had values near 0.1 en% (13). This low value coincided with that for growth of young animals (13), suggesting that the limiting process for growth was the conversion of dietary 18:2n-6 into n-6 eicosanoids. Adding 18:2n-6 in amounts greater then 0.5 en% gave progressively more 18:2n-6 in tissue lipids, but it did not appreciably elevate further the proportion of n-6 eicosanoid precursors in phospholipid HUFA (9) or the rate of growth. More precise estimates of the competitive interactions of dietary 18:2n-6 and 18:3n-3 in forming tissue HUFA (43; B. Libelt, N. C. Kramer, A. Morris, T. E. Prewitt, P. Bowen, D. Schmeisser, M. H. Davidson, J. H. Burns, and W. E. M. Lands, unpublished results) in tissue phospholipids confirmed the low constant (< 0.1 en%) and its ability to help predict the intensity of thromboxane formation (13). Understanding the quantitative relationships among dietary fats, tissue phospholipids, and the nonesterified eicosanoid precursors (see Fig. 3) is a necessary part of interpreting the biochemistry and physiology of the n-3 fatty acids in their role as partial agonists. Once the nonlinear hyperbolic nature of dietary n-3/n-6 actions in the maintenance of eicosanoid precursors in tissues is recognized, previously linear comparisons can be recognized to be merely incomplete and not contradictory.

The quantitative hyperbolic relationship of dietary intake of 18:2n-6 to the proportion of 20:3 + 20:4n-6 maintained in HUFA of tissue phospholipids was used to generate curves shown in Fig. 4. The figure illustrates the nonlinear manner in which dietary proportions of 18:2n-6 and 18:3n-3 in different food oils can be expected to affect competitively the proportion of n-6 HUFA in tissue phospholipids. The curves illustrate that increasing the dietary 18:3n-3 from 1 to 10 en% can be expected to markedly affect the proportion of n-6 HUFA maintained in tissues. In contrast, little change should be expected by varying dietary intake of 18:2n-6 from 1 to 10 en%. The composition of 18:3n-3 and 18:2n-6 in the food oils determines the ultimate proportion of n-6 HUFA that will be maintained in the tissue HUFA. The curves in Fig. 4 indicate that only a small change will occur in the composition of tissue HUFA when increasing the proportion of the food oil from 1 to 20% of the average daily calories. Also, they indicate that a significant effect may occur in shifting dietary oils from soy to canola, and they suggest the

amounts of flax oil that can be expected to alter the proportion of n-6 HUFA.

CHOICES AND CONSEQUENCES

The quantitative experiments of Mohrhauer and Holman (9, 52) clearly demonstrated the competitive hyperbolic relationship between 18:3n-3 and 18:2n-6, although the exact algebraic equations were developed only recently (43; B. Libelt et al., unpublished results, and W. E. M. Lands, B. Libelt, A. Morris, J. H. Burns, and M. H. Davidson, unpublished results). The equations and constants provide an approximate fit to results for experiments with either HUFA or 18-carbon polyunsaturated fatty acids fed to rats (53), mice (54), or humans (20). For the reasons noted previously, relatively few data sets for humans are available to permit refining the rough estimates for these constants. In experiments with negligible amounts of dietary n-3 and n-6 HUFA, the new equations (W. E. M. Lands et al., unpublished results) reduce to the simpler forms developed for dietary 18:2n-6 and 18:3n-3 (43; B. Libelt et al., unpublished results). For example, the simple equation predicts the approximate proportion of n-6 HUFA in plasma phospholipids of rats (43) and fits results for humans from Denmark, France, Sweden, Finland, and the U.S. (listed in ref 20), where HUFA are not a major dietary component (and about 75% HUFA in plasma phospholipids are the n-6 type). In contrast, the simple equation makes poor predictions for Japanese (ca. 50% n-6 HUFA) and Greenlanders (ca. 20% n-6 HUFA), whereas the full equation (W. E. M. Lands et al., unpublished results) fits closely to the observed results with all national averages (Fig. 3).

A careful longitudinal study in the U.S. (38) showed that people in the upper quintile of n-3 HUFA intake (ca. 0.66 g/day; approximately 0.3 en%) had 40% fewer cardiovascular deaths during the study. Because the typical American appears to maintain n-6 HUFA in phospholipids near the maximal capacity (Fig. 3), it seems useful to consider that the n-6-rich diet in the U.S. may have contributed to the incidence and severity of eicosanoid-mediated diseases (e.g., thrombosis, arthritis). The biomedical community has advanced far beyond the first suggestion (3) that dietary n-3 fatty acids might moderate n-6 eicosanoid-mediated pathology, and methods are now available for more interpretable controlled studies of physiological responses of individuals who differ widely in their average daily intake of n-3 fatty acids. For example, a definitive longitudinal study in Japan (39), where the average n-6 HUFA value is near the middle of the range (Fig. 3), could provide valuable information because large numbers of individuals will be above and below that level (in contrast to the limited range in the U.S. (38). Results from such a study are more likely to provide convincing evidence on the physiological and clinical effect of dietary n-3 fatty acids. Such studies can provide insights (2, 39, 42) that may lead us to shift the current typical proportions of dietary n-3 and n-6 fatty acids to one that is deliberately selected for a more desirable health status. FJ

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