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BIOSYNTHESIS OF PROSTAGLANDINS

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CONTENTS

ESSENTIAL ACTIONS OF POLYUNSATURATED FATTY ACIDS

In lipid metabolism the enzymes that esterify unsaturated fatty acids into cellular lipids exhibit a broad range of selectivity rather than acting only with a single fatty acid. Research has not clarified whether this phenomenon is due to a large set of very specific enzymes acting in parallel or to a few enzymes

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of broad selectivity. Thus tissues maintain a general average pattern of fatty acid composition while exhibiting considerable capacity to shift that average composition as a variety of fatty acids are made available (reviewed in 57). This flexible situation does not provide a basis for naming either n-6 or n-3 fatty acids as essential irreplaceable components of membranes, and it contrasts sharply with the evidence for highly selective actions of certain n-3 and n-6 fatty acids in the formation and function of eicosanoids.

Animals cannot synthesize de novo either the n-3 type or the n-6 type of polyunsaturated fatty acids that are formed by plants. However, animals elongate and desaturate the 18-carbon dietary polyunsaturated fatty acids to obtain a range of 20- and 22-carbon highly unsaturated fatty acids (HUFA). Dietary linolenic acid, 18:3n-3, provides 20:5n-3, 22:5n-3, and 22:6n-3, whereas dietary linoleic acid, 18:2n-6, provides 20:3n-6, 20:4n-6, 22:4n-6, and 22:5n-6. These HUFA are esterified into the lipids of cellular membranes, and they also influence the rate of synthesis of physiologically important eicosanoids. Because the n-3 or n-6 HUFA can only be present in tissues if the acid or its precursor is provided in the diet, lipid nutrition is inescapably linked with the biochemistry, physiology, and pharmacology of eicosanoids in health and disease.

Interpretations of the nutritional essentiality of n-6 fatty acids easily include the formation and function of active n-6 eicosanoids. However, interpretations of the benefits from the n-3 fatty acids are less certain because of the inability of n-3 fatty acids to support all of the physiological functions that are suppported by the n-6 fatty acids (32, 68, 108). The n-3 type of acids are abundant in many membrane lipids (especially in retinal and neuronal tissues; 2), and a challenge remains to find and intrepret those physiological processes in which the n-3 type of fatty acids provide a benefit that is not obtained with an n-6 acid (68). This review considers this challenge while examining how n-3 and n-6 acids meet two different dynamic needs of animals:

1. A need for sufficiently rapid bursts of eicosanoid acitivity to support important physiologic events.
2. A need for a sufficiently rapid supply of 22-carbon highly unsaturated fatty acids to support rapid biogenesis of new membranes.

The need for rapidity in carrying out essential actions is balanced by a general need to prevent overreactions in living systems. Thus an action that is necessary at one specific moment may at another time be more vigorous than desired. In fact, many controlled physiological systems act to desensitize a previously vigorous response. In this situation, the less dynamic behavior of partial agonists may provide benefits.

Eicosanoids act in a transient and episodic manner---either in a series of intermittent pulses or at irregular intervals (22, 94, 99). Thus, pharmacodynamic descriptions of nutritionally essential fatty acids must include the episodic nature and the intensity of eicosanoid actions. However, traditional descriptions of essential nutrients (e.g. vitamins or minerals) contrast with this dynamic concept, and employ terms that suggest filling a continuous need for some constant (constitutive, unceasing) structural component or process that can be characterized by a quantitative daily requirement. Conflict between these two concepts of action (rates) and inaction (constitutive components) continues to confound interpretations of essential fatty acid nutrition.

Physiological Features of Essential Fatty Acids

The n-6 fatty acids were recognized as essential for maintaining a wide range of mammalian physiological processes (6, 7) long before their conversion to active eicosanoids [prostaglandins (3; e.g. PGE_2), thromboxane (25), prostacyclin (PGI_2; 110), leukotrienes (82), epoxides (8; reviewed in 19), hepoxylins (95)] was known. The action of eicosanoids in regulating the pulsatile or irregular release of hypothalamic and pituitary hormones probably underlies many "essential" roles of the n-6 fatty acid 20:4n-6. This relationship seems evident in the early report (23) that the abnormally low weights of testes, prostate, and seminal vesicles of animals deficient in essential fatty acids could be restored to normal values by either injected gonadotropin or dietary 18:2n-6. Further support for mediation by pituitary hormones was developed when signs characteristic of an essential fatty acid deficiency occurred in hypophysectomized rats even though they had normal tissue levels of n-6 fatty acids (24, 42). More recent reports have noted that eicosanoids derived from essential fatty acids modulate hypothalamic function (reviewed by 74, 92, 93) by stimulating growth hormone (GH) release from pituitary, by stimulating growth hormone–releasing factor (GRF) release from hypothalamus, by mediating the release of adrenocorticotropic hormone (ACTH) from the pituitary [perhaps by increasing corticotropin-releasing factor (CRF) from the hypothalamus], by enhancing the response of thyroid tissue to thyroid-stimulating hormone (TSH), by promoting prolactin (PRL) release by decreasing the prolactin release-inhibiting factor (PIF) and increasing the stimulatory prolactin-releasing factor (PRF) from the hypothalamus, by stimulating release of antidiuretic hormone (ADH or AVP) and moderating its action peripherally, and by stimulating gonadotropin (luteinizing hormone, LH, and follicle-stimulating hormone, FSH) release by stimulating luteinizing hormone-releasing hormone (LHRH) release from the hypothalamus [although PGI_2 decreased LHRH and PGE_2 increased LHRH (94)].

Cells that synthesize leukotrienes C_4, D_4, and E_4 have significant LHRH contents, and anterior pituitary cells in culture release LH in response to added leukotriene C_4 (37, 104).

The less active synthesis (61) and functioning (70, 83, 84, 113) of n-3 eicosanoids apparently make their precursor, 20:5n-3, a partial agonist that can support some (5, 38), but not all, of the physiological functions supported by 20:4n-6 (reviewed by 1, 31, 33). Either n-3 or n-6 dietary acids can support growth (80, 81), development, and gestation (69), but dermal and renal integrity and parturition require the more active n-6 fatty acids. Similarly, platelet aggregation is supported much more vigorously by 20:4n-6 than by 20:5n-3 (58), and this difference may be the basis for a greater mortality from acute thrombosis for individuals ingesting high dietary ratios of n-6/n-3 polyunsaturated fatty acids (17). The intensity with which n-6 eicosanoids enhance platelet aggregation and release of platelet-derived growth factor(s) while also enhancing local inflammatory processes is a probable factor in the greater mortality from atherosclerosis in chronic heart disease and cardiovascular disease (53). In addition to amplifying the behavior of platelets and leukocytes, the n-6 eicosanoids appear to enhance α-adrenergic-mediated vasospasms (16, 72) and ventricular fibrillation (14, 79a, 101) in a manner that is diminished by including n-3 HUFA in the diet.

A fatal hypersensitivity to environmental stimuli ("fainting shock" syndrome) is exhibited by rainbow trout that are fed diets containing n-6 fatty acids without sufficient counterbalancing amounts of n-3 fatty acids (10). As a result, the n-3 fatty acids are considered to be essential for adequate growth and behavior of this species, and excessive supplies of n-6 fatty acids are avoided. The full size of some fish raised on linoleate-rich diets indicates that 18:2n-6 can be as effective as 18:3n-3 in supporting growth of individual fish. However, research is needed to determine whether the dramatic heterogeneity in growth phenotype manifested by fish with diets rich in 18:2n-6 reflects an unusual genetic heterogeneity or an undesirable response to random environmental stimuli that generated episodes of excessive n-6 eicosanoids. Clearly, a wide range of undesired n-6 mediated processes can be moderated by including n-3 fatty acids in the diet.

Membrane Biogenesis and Membrane Integrity

Isolated cultures of cells can proliferate adequately as long as some unsaturated fatty acid is available, and there is no evidence that such cultures require either n-3 or n-6 fatty acids for constitutive structural or vegetative functions (103). In contrast, the coordinated physiological interactions among cells in tissues of a developing animal cannot proceed adequately without essential (n-3 or n-6) fatty acids, and dynamic transient signals among cells

are an excellent basis for interpreting the nutritional essentiality of these fatty acids. Perhaps the kinetic dynamics of developing cell membranes may also provide a basis for selective benefits from certain dietary fatty acids. Studies with unicellular systems (30, 47) showed that cells fail to develop properly when unsaturated fatty acids are not available at times of membrane genesis during growth and development. In these models, the cells made no unsaturated fatty acids, and the only acids capable of meeting the structural need of the cell membranes were from external sources. The prokaryote models provided an excellent system to quantitate the degree to which an exogenous unsaturated acid contributes to membrane fluidity (63, 64), and growth in the presence and absence of cAMP indicated differences among these acids in meeting the dynamic requirements for membrane assembly (90, 109). In quantitating the need for an exogenous fatty acid, the models are relevant to the formation of mammalian membranes for which n-3 and n-6 fatty acids are only available from external sources.

Experimental yeast systems illustrated how a required exogenous fatty acid might manifest its dynamic role only during a brief, transient period of intense adaptation during which the membrane assembly is either successful or unsuccessful. Introduction of the needed fatty acids after this time of adaptation may not necessarily repair the effects of the prior deficit. For example, during active formation of new functional mitochondria, the acids existing in membrane lipids were not adequate to support the formation of new functional respiratory membranes (111, 112). Rather, an influx of fresh supplies of appropriate nonesterified unsaturated fatty acids was needed to form new lipoprotein complexes at the time of assembly to obtain adequate respiratory membrane units. Another example is that certain unsaturated fatty acids are required to support the replication of mitochondria during cell division (21). In this model, the replication of nuclei and the division of the cell were maintained adequately with fatty acid supplies that were inadequate for replication and assembly of mitochondria. As a result, an abortive assembly of mitochondria led to daughter cells that were missing mitochondrial DNA, and the accumulated "petite" mutants were no longer able to form functioning mitochondria.

Interpretations of experiments indicating a nutritional requirement of n-3 fatty acids for development of brain (115) and retinal function (reviewed by 87) need to keep in mind that fatty acids can be replaced (107, 108) rather quickly (13) without major apparent alteration in function. The de novo synthesis of phosphatidylethanolamines for rapid perinatal membrane biogenesis is catalyzed by ethanolamine phosphotransferase that works most rapidly with diacylglycerols containing 22-carbon HUFA (43, 44, 78). During times of slow membrane formation and turnover, the supply of either n-3

or n-6 HUFA may be sufficient, but too slow a formation of new 22-carbon HUFA may fail to provide adequate proportions of new phosphatidylethanolamines for membrane lipoprotein complexes. The rapid assembly of neural and retinal membranes, which occurs during the fetal, neonatal, and perinatal stages, may be more successful with dietary n-3 fatty acids that appear to have a greater ability than n-6 acids to provide rapidly the elongated 22-carbon HUFA (81).

KINETICS OF EICOSANOID FORMATION AND INACTIVATION

A transient regulation of cell function by eicosanoids is achieved by having a negligible basal level of active eicosanoid in tissues and by rapidly inactivating any active eicosanoid that is formed. Thus, typical eicosanoid-modulated regulations require that

1. eicosanoids must be synthesized at speeds that overcome inactivation;
2. eicosanoids must occupy a sufficient number of specific receptors;
3. eicosanoid receptors must signal further events to achieve the regulation.

An inadequate action in any of these three processes will impair eicosanoid-mediated phenomena even though eicosanoid synthesis occurs. Paradoxically, the system can often work so vigorously that there may be a risk of an over-response when mobilizing a sufficiently vigorous adaptive response. Overproduction of n-6 eicosanoids has been recognized in association with thrombosis, arrythmia, hypertension, asthma, arthritis, immune-inflammatory disorders, and metastatic processes (53), as well as headaches and dysmenorrhea.

The first committed step of biosynthesis for all active eicosanoids is an enzymatic oxygenation that converts the nonesterified HUFA substrate into either a prostaglandin (cyclooxygenase action), an epoxide (epoxygenase action), or a leukotriene (lipoxygenase action). A rapid burst of synthesis requires sufficient supplies of nonesterified precursor fatty acid (65) and activator hydroperoxide (29). Removing either of these needed reactants can slow the rate of eicosanoid synthesis (76) and thereby prevent eicosanoid action. Cells increase the rate of synthesis of active eicosanoids and promote eicosanoid actions by increasing the concentration of hydroperoxides (77) and nonesterified acids. These increases are evident during inflammatory events for which hydroperoxides act both as markers and mediators (46).

Precursor Nonesterified Fatty Acids

The episodic nature of eicosanoid formation is initiated by stimulant ligands binding to receptors that transmit secondary signals through mediators to shift

the distribution of HUFA from esterified forms toward the nonesterified form that is the substrate for eicosanoid synthesis. In this way, the stimulus for eicosanoid formation depends upon the intensity and intermittent nature of activating events that are primarily independent of nutritional status. These nutritionally independent events influence the frequency of eicosanoid synthetic events. Nevertheless, the nutritional supplies of essential fatty acids influence the relative amounts of different types of HUFA (n-3, n-6, n-7, n-9) that will be in the substrate pool for eicosanoid biosynthesis, and this way diet exerts an important effect on the intensity of the formation and function of n-6 eicosanoids once their formation is stimulated. Progressively greater proportions of dietary 18:2n-6 allowed tissues to maintain a greater proportion of 20:4n-6 in HUFA, which appeared to provide more intense pulses of eicosanoids as indicated by greater growth (80) and greater thrombotic tendency (35). A subsequent section of this review describes the quantitative competitive interactions by which dietary fatty acids influence the proportion of 20:4n-6 in the HUFA of tissue phospholipids that serve as a tissue reservoir of eicosanoid precursors. More vigorous physiological actions were quantitatively related to a greater proportion of 20:4n-6 in the phospholipid HUFA of a cell.

The amount of excreted urinary eicosanoid metabolites provides a minimal estimate of the overall eicosanoid produced by all tissues during an extended time period, but it fails to indicate the intensity of local pulses of synthesis of eicosanoids occurring in selected tissues or their physiological efficiency. Severe chronic stimulation of arterial tissues can elevate the overall amount of eicosanoid synthesized and excreted per day (18), although a majority of the prostaglandin metabolites in the urine of healthy individuals may originate in the gastrointestinal tract (48). Evidence from urinary metabolites (48) confirms the enzyme kinetic data showing low rates of synthesis of n-3 prostaglandins in conditions of low peroxide tone as occurs normally in vivo (76). The successful reduction of excreted thromboxane metabolites from patients with atherosclerosis (50) by dietary n-3 HUFA illustrates the benefits of reducing the proportion of 20:4n-6 in tissue HUFA.

The intensity of a given pulse of prostaglandin synthesis can be diminished by the presence of competing analogs in the cellular pool of nonesterified fatty acid. Ibuprofen, a commercially successful competitive inhibitor, has a Ki value (ca 2 μM; 102) near that of the naturally occurring competitor, 22:6n-3 (2 μM; 61) and the partial agonist, 20:5n-3 (61). Familiarity with the effectiveness of ibuprofen makes it easy to appreciate the possible benefits of dietary n-3 fatty acids. These acids diminish the intensity of synthesis of n-6 eicosanoids by forming n-3 HUFA that compete with 20:4n-6 for esterification into the cellular reservoirs of eicosanoid precursors as well as compete for binding to the active sites of the oxygenases that form the eicosanoids.

Activator Lipid Peroxides

Fatty acid oxygenases require a hydroperoxide activator that initiates the subsequent free radical chain reaction inserting oxygen into the polyunsaturated substrate (29) and forming more hydroperoxide. This explosive positive feedback provides a mechanism for rapidly converting an inactive synthetic system to an active one, and the class of antiinflammatory drugs that act on radical intermediates (e.g. acetamidophenol; 52) is only effective in hyperalgesic conditions of low peroxide tone with its low rate of chain initiation (26, 60). The n-3 partial agonist, 20:5n-3, forms hydroperoxide more slowly than does the n-6 substrate, 20:4n-6 (96). As a result, when the amount of hydroperoxide activator is kept low in tissues by cellular peroxidases, 20:4n-6 remains active whereas 20:5n-3 is an ineffective substrate for prostaglandin synthesis.

KINETICS OF INTERACTIONS OF n-3, n-6, n-7, AND n-9 FATTY ACIDS

The enzymes that incorporate fatty acids into glycerolipids place saturated fatty acids (SFA) at the *sn*-1 position. This general selectivity occurs widely in many different animal tissues, and it leads to diacylglycerophospholipids that have an average content of about one-half (40 to 48%) SFA and to triacylglycerols that have about one-third (25 to 35%) SFA. Enzymes catalyzing the de novo synthesis of glycerolipids esterify the 16- and 18-carbon unsaturated fatty acids (UFA) at the *sn*-2 position, whereas the retailoring acyltransferases tend to esterify 20- and 22-carbon highly unsaturated fatty acids (HUFA) at the *sn*-2 position (reviewed by 57). These general esterification selectivities are similar in rats and humans, and they produce phospholipids with approximately 41–46% SFA, 32–35% UFA, and 20–25% HUFA (Table 1) and triglycerides with about 30% SFA, 60% UFA, and 2–5% HUFA (Table 1).

The avidity with which some cells maintain HUFA in the phospholipids is aided by a distinct acid:CoA ligase (114) that activates most naturally occurring HUFA (86). This HUFA-selective activity is greater than the nonselective ligase activity in platelets and smooth muscle, whereas it is less than the nonselective activty in adipose and liver (66). Alternatively, the avidity for 22:6n-3 in glycerophospholipids may reflect the reversible action of the ethanolamine phosphotransferase, which can form diglycerides (43) and can selectively incorporate the 22-carbon diglycerides into phosphatidylethanolamine (44, 78).

Endogenous and Exogenous Acids

Most components of foods are converted to useful energy and carbon dioxide. Some are used to replace and repair needed cellular constituents, and if the

dietary influx is more rapid than can be accommodated by these processes, excess carbohydrate and protein produce acetyl-coenzyme A that can lead to the biosynthesis of fatty acids and cholesterol. Fatty acids formed endogenously [SFA (16:0, 18:0), UFA (16:1n-7, 18:1n-7, 18:1n-9)] represent the major type of fatty acids present in tissue lipids of animals and humans (Table 1). Abundant n-7 fatty acids during conditions of low-fat, high-carbohydrate ingestion (62) indicated that synthesis of palmitoleate (16:1n-7) was a major route for the conversion of foodstuff into lipids, and nearly 40–50% of the UFA in phospholipid were endogenous n-7 fatty acids (62). When diets contain no n-3 or n-6 polyunsaturated fatty acids, the HUFA maintained in tissue lipids are predominantly the n-7 and n-9 type (20:3n-7, 20:4n-7, 20:3n-9), which are formed by elongation and desaturation of endogenous UFA (20, 49). However, even small amounts [0.2 to 0.5% of total calories (0.2–0.5 en%)] of dietary 18:3n-3 or 18:2n-6 permit tissues to maintain significant amounts of n-3 or n-6 HUFA that displace the endogenous n-7 or n-9 HUFA from the glycerophospholipids (80, 81).

The process of converting dietary 18:2n-6 to 20:4n-6 inevitably supplies also 20:3n-6, 22:4n-6, and 22:5n-6. Competition among these n-6 HUFA creates an upper limit for the proportion of 20:4n-6 that can be maintained in the phospholipid HUFA (ca 85%). Competition against other types of HUFA (n-3, n-7, or n-9) is the only way that the proporton of n-6 HUFA in the total HUFA of a glycerolipid can be diminished. Thus, as dietary n-3 increases, the amount of n-6 HUFA maintained in tissues decreases (39, 40, 62, 75, 81). The average ratio of n-3/n-6 HUFA that is maintained in tissue phospholipids tends to approximate that of the dietary polyunsaturated fatty acids (54, 62).

Triglyceride Formation

In the absence of exogenous polyunsaturated fatty acids, tissue triglycerides maintained by rat tissues contain predominantly the fatty acids that are synthesized endogenously from carbohydrate and protein [16:0 (22–26%), 18:0 (3%), 16:1n-7 (8–14%), 18:1n-7 (5–9%), 18:1n-9 (25–43%); 62, 98]. Increasing the dietary level of 18:2n-6 or 18:3n-3 tends to increase the amount of these acids maintained in tissue triglycerides. Over a range 0 en% to 10 en% of these acids in the diet, rat tissues maintained a consistent linear relationship between the percentages of these fatty acids in plasma, liver, and adipose triglycerides and the dietary level (62). Tissues maintained a ratio of 18:3n-3/18:2n-6 in plasma triglycerides, which averaged about 0.6 of that provided in the diet. Thus, the content of these exogenous fatty acids in plasma triglycerides may be a useful index of the average proportion of the acid in the ingested calories. For example, the higher ratio of 18:3n-3/18:2n-6 in triglycerides in Finland (Table 1) could reflect a greater use of rapeseed oil in the diet.

Table 1 Fatty acid composition of lipids in rat and human plasma

Plasma Lipid	Rat +flax (62)	Rat +corn (62)	Human Eskimo (17a)	Human Dane (17a)	Human Japan (105)	Human Sweden (4)	Human France (67)	Human Finland (88)	Human USA (91)	Human USA (34)	Human USA (97)	Average human values
Phospholipid												
14:0	0.4	0.4	0.1	0.2	0.4	—	0.4	—	0.4	0.2	—	0.2
16:0	16.1	25.4	34.9	30.6	31.5	31.2	30.4	26.7	33.7	26.8	24.0	30.0
18:0	32.6	18.9	19.6	17.2	17.1	14.3	16.0	13.5	13.7	13.6	12.5	15.3
16:1n-7	1.1	0.7	2.7	0.8	0.3	0.9	0.7	1.5	1.3	1.7	1.0	1.2
18:1n-7,9	8.6	8.1	15.9	15.4	8.3	11.5	10.2	12.2	11.2	13.4	8.9	11.9
18:2n-6	16.3	19.6	6.6	21.0	20.0	24.8	16.3	19.6	21.1	21.2	23.9	19.4
18:3n-3	1.8	—	0.0	0.5	0.1	0.1	0.2	0.7	0.6	0.3	0.2	0.3
20:3n-6	0.9	0.7	2.3	2.0	1.7	2.4	4.0	3.5	2.7	3.6	3.4	2.8
20:4n-6	6.0	20.8	0.8	8.0	9.4	7.5	12.2	9.6	9.1	10.6	12.8	8.9
22:5n-6	0.0	0.9	0.0	0.0	0.0	0.1	0.0	0.0	1.0	1.3	1.0	0.4
20:5n-3	8.3	—	7.1	0.2	3.4	1.4	2.1	1.7	1.1	1.1	0.6	2.1
22:5n-3	1.7	0.5	0.2	0.0	0.6	0.9	0.8	—	1.1	0.8	1.0	0.6
22:6n-3	5.7	2.3	3.9	3.0	7.1	4.9	5.6	5.3	3.3	1.6	3.6	4.3
SFA	49.1	44.7	54.5	48.0	49.0	45.5	46.8	40.2	47.8	40.7	36.5	45.4
UFA	27.8	28.3	25.2	37.6	28.7	37.3	27.4	33.9	34.2	36.5	34.0	32.7
HUFA	22.5	25.2	14.3	13.2	22.2	17.2	24.7	20.1	18.3	19.0	22.4	19.0
n-6 HUFA as % HUFA	30.4	88.8	22.0	75.7	50.0	58.1	65.7	65.2	69.9	81.9	76.9	62.8

Triglyceride												
14:0	0.3	2.7	2.1	1.5	1.9	—	2.7	2.4	2.9	2.0	—	1.7
16:0	11.9	23.6	25.1	28.8	26.7	27.0	29.4	26.5	34.2	24.6	24.0	27.4
18:0	5.8	4.3	5.9	5.8	3.1	4.3	5.0	3.6	4.8	6.2	3.6	4.7
16:1n-7	1.7	1.7	9.5	5.2	5.1	5.0	4.8	6.1	4.3	5.1	1.0	5.1
18:1n-7,9	36.8	26.0	35.1	40.1	33.1	40.6	40.1	38.8	33.9	43.7	37.8	38.1
18:2n-6	14.1	35.2	6.2	12.5	20.8	18.5	12.9	13.2	16.4	14.4	21.0	15.1
18:3n-3	24.1	0.9	0.1	0.8	1.4	1.3	0.8	1.9	1.0	0.8	0.9	1.0
20:3n-6	—	—	1.7	0.3	—	0.1	0.2	—	1.2	0.2	0.4	0.5
20:4n-6	0.4	2.6	0.6	0.2	1.7	1.2	1.7	—	1.4	1.1	1.6	1.1
22:5n-6	—	0.7	0.2	0.0	—	0.1	—	—	—	0.1	1.0	0.2
20:5n-3	2.7	—	4.2	0.0	1.5	0.3	0.5	—	0.6	0.2	0.2	0.8
22:5n-3	1.2	—	0.3	0.0	0.9	0.4	0.0	—	0.5	0.1	1.0	0.4
22:6n-3	1.1	0.7	2.4	3.3	3.4	1.3	0.0	—	0.8	0.1	0.4	1.3
SFA	17.9	30.6	33.1	36.1	31.7	31.3	37.1	32.5	41.9	32.8	27.6	33.8
UFA	76.7	63.8	50.8	58.6	60.4	65.4	58.6	60.0	55.6	63.9	60.8	59.3
HUFA	5.4	4.0	9.3	3.8	7.5	3.4	2.4	0.0	4.5	1.7	4.6	4.1

Glycerophospholipid Formation

Increasing dietary levels of n-3 and n-6 fatty acids from 0 en% to 0.5 en% increases the corresponding HUFA derivatives in tissue phospholipids of rats. However, above 0.5 en% the composition of tissue HUFA tends to approach an upper limit (62, 80, 81). The competitive interactions of the various HUFA for the limited number of esterification sites can be described quantitatively by hyperbolic equations (62):

$$\text{n} - 3 \text{ as \%HUFA} = \frac{100}{1 + C_3/\text{en\%3}\ [1 + \text{en\%6}/C_6 + \text{en\%0}/C_0 + \text{en\%3}/K_S]},$$

$$\text{n} - 6 \text{ as \%HUFA} = \frac{100}{1 + C_6/\text{en\%6}\ [1 + \text{en\%3}/C_3 + \text{en\%0}/C_0 + \text{en\%6}/K_S]},$$

$$\text{n} - 9 \text{ as \%HUFA} = \frac{100}{1 + K\ [1 + \text{en\%3}/C_3 + \text{en\%6}/C_6]}.$$

At this time, the equations and their associated constants provide approximate quantitative predictions of the effect of dietary fatty acids upon n-3/n-6 compositions in tissue glycerolipids and on the n-6 eicosanoid synthetic capacity in rats (56, 62). Small differences in the constants permit the equations to fit many different tissues, most likely representing different relative contributions of the nonspecific and HUFA-specific ligases and the de novo and retailoring pathways. Preliminary data suggest that the equations and constants for rats may be similar to those for humans, so that parallel calculations with the amounts of dietary n-3/n-6 acids may help estimate the capacity for n-6 eicosanoid synthetic intensity in human tissues. If these approximations can be confirmed, then much of the insight on lipids and eicosanoids that was obtained from studies of rats (56) may be conveniently applied to interpreting human studies.

The hyperbolic relationship between the amount of dietary 18:2n-6 and the amount of tissue n-6 HUFA predicts that very small amounts of dietary 18:2n-6 (ca 0.2 en%) will permit n-6 HUFA to be maintained at nearly maximal levels in tissues if there is no competition from n-3 fatty acids. The equation indicates that changing the dietary fatty acid n-3/n-6 ratio from 0.03 to 3.0 can decrease the proportion of 20:3 + 20:4n-6 in phospholipid HUFA from about 80% to about 20%, thereby decreasing fourfold the probable intensity of eicosanoid biosynthesis. In this regard, standard laboratory rat chow is actually an extreme diet in terms of permitting tissues to maintain a high percentage of phospholipid HUFA as 20:4n-6 (ca 80%; 98). This proportion is comparable to that maintained with a purified diet that contained high amounts of corn oil (74%; 79), and this proportion was not increased further by adding corn oil to the rat chow (98). The hyperbolic equations

provide a fresh opportunity to reexamine the concept of what is a "normal" standard dietary level of n-6 fatty acids (and a "normal" probable intensity of synthesizing n-6 eicosanoids) in terms of what is desired rather than what is typical. For example, 20:3 + 20:4n-6 were about 75% of the plasma phospholipid HUFA in USA samples, whereas they were only 50% of the HUFA in Japanese samples (Table 1).

The in vitro ability of stimulated platelets to form the n-6 eicosanoid, thromboxane (40), quantitatively fits the hyperbolic relationship to nutrient 18:2n-6 (discussed in 56) as does the in vivo tendency for thrombosis (35 discussed in 56). Thus the capacity for synthesizing n-6 eicosanoids is reduced by dietary n-3 acids (39, 58) and seems to be predicted by the proportion of 20:3n-6+20:4n-6 in the HUFA of glycerophospholipids. In support of this hypothesis, the proportions of n-6 HUFA in the HUFA plasma phospholipids (see Table 1), which reflect the probable intensity of thromboxane biosynthesis, are related to the 10-fold lower mortality rates from myocardial thrombosis in Greenland Eskimos (Ref. 53, Chap. 1) and 5-fold lower rates in Japan (59) relative to the rate in the United States and Denmark (22% HUFA vs 50% HUFA vs 76% HUFA, respectively). The rationale for this mechanism-based relationship with cardiovascular mortality seems to be at least as compelling as the widely discussed relationship for plasma cholesterol, which still has no clear mechanism of action.

Proliferation of breast tumors is enhanced by dietary 18:2n-6 and seems to be facilitated by n-6 eicosanoids (9, 51). Careful dietary studies relating tumor proliferation to the dietary abundance of 18:2n-6 (41) showed a hyperbolic relationship with a midpoint at a higher en% of 18:2n-6 (56) than the midpoint observed for 20:3n-6 and 20:4n-6 in phospholipid HUFA (0.1 en%; 62). A lower activity of the HUFA-specific ligase in cells involved in tumor proliferation might cause the higher midpoint for the amount of dietary 18:2n-6 that saturates eicosanoid synthetic capacity. Fatty acid compositions of total tumor lipids (36, 51) were difficult to interpret because they reflected a combination of the linear increase of 18:2n-6 in triglyceride accompanied by a "saturated" level of 20:4n-6 in tissue HUFA. Nevertheless, many studies indicate diminished tumor proliferation as increased amounts of dietary n-3 fatty acids decreased the proportion of n-6 HUFA in the tissue HUFA (45, 85, 89, 100).

ESTIMATING NUTRITIONAL INADEQUACY, ADEQUACY, AND OVERSUPPLY

Clinical, Physiological, and Biochemical Measurements

Dermal signs have been regarded as the most sensitive physiological indicator of a deficiency of essential fatty acid (106). Signs of an n-6 deficiency were evident in rats ingesting diets with less than 0.1% of total calories (<0.1 en%)

as linoleate, 18:2n-6 (81). All of the animals ingesting 0.07 en% 18:2n-6 showed some dermal signs (but little growth deficit), and none of the animals receiving 0.3 en% 18:2n-6 showed signs of deficiency (81). Studies with 428 human infants produced similar results (27). All of the infants ingesting 0.04 en% 18:2n-6 had signs of impaired skin function, whereas only 40% of those receiving 0.07 en% did (28). Such signs were not evident in infants who ingested as little as 0.5 en% 18:2n-6 (15).

What minimum level of 20:4n-6 in the HUFA tissue phospholipids is necessary to maintain adequate physiology? Ratios of 20:3n-9/20:4n-6 in liver lipids of rats on marginal diets were approximately 0.8 to 1.9 (81), and values ranging from 0.18 to 0.94 occurred in animals free of deficiency signs. Similarly, infants showing signs of deficiency had triene/tetraene ratios of 1.4 in plasma lipids (12, 28), whereas infants free of physiological deficiency signs had ratios of 0.3. A surgical patient exhibited dermal signs of a deficiency of essential fatty acids only after 60 or 70 days of fat-free intravenous feeding, when the 20:3n-9/20:4n-6 ratio in plasma phospholipids exceeded 1.0 (11). Dermal signs disappeared when the ratio was lowered below 1.0 by infusing triglycerides containing 18:2n-6. A combined deficiency of n-3 and n-6 acids (with large amounts of n-9 HUFA) decreases macrophage-mediated inflammatory events more effectively than a deficiency of n-6 acids (with large amounts of n-3 partial agonist HUFA) (71).

These results suggest that tissue maintenance is marginal when the HUFA contain about 30% 20:4n-6 during a low-fat diet with 20:3n-9 as the competing HUFA or about 15% 20:4n-6 with 20:5n-3 as the competing HUFA.

Chronic Deficiencies versus Chronic Excesses

The ability of tissues to maintain adequate proportions of n-6 eicosanoid precursor in tissue HUFA (> 40% n-6 HUFA in the total HUFA) with dietary supplies of 18:2n-6 as low as 0.3 en% means that a chronic n-6 deficiency is nearly impossible for free-living humans since almost all foods that are generally available contain at least that much 18:2n-6. The capacity to convert dietary 18:3n-3 into n-3 HUFA apparently also protects against physiological deficits, but many processed foods have negligible amounts of the n-3 fatty acids. This fact, plus the current tendency to supplement diets with oils rich in 18:2n-6, places significant chronic competitive pressure against maintaining tissue levels of n-3 HUFA. Whether or not this imbalance causes some undesired physiological outcome (87) remains a serious issue in nutrition research.

Nearly equal proportions of 18:2n-6 and 18:3n-3 occur in vegetables (e.g. squash, 2.2/3.7; snap beans, 1.4/2.3; pinto beans, 0.6/1.0), whereas many seeds and grains have much greater amounts of n-6 (e.g. squash seeds, 34/0.3; corn, 5.7/0.2) (55). The average dietary level of 7 en% 18:2n-6 in the

USA (much more than is needed to prevent physiological signs of deficiency) provides a chronic high proportion of tissue HUFA in the form of 20:4n-6. Biomedical researchers interpreting the frequency and severity of disorders mediated by n-6 eicosanoids (54) may question whether chronic excesses of n-6 eicosanoids are a more serious problem at this time in the USA than are chronic deficiencies of n-6 fatty acids.

Tissues of individuals consuming diets that contain n-6 fatty acids with few competing n-3 fatty acids tend to maintain a maximal proportion of 20:4n-6 in phospholipid HUFA and exhibit a high capacity for rapid synthesis of n-6 eicosanoids. Increasing the amount of dietary n-3 fatty acids decreases the proportion of 20:4n-6 maintained in the HUFA and appears to decrease the intensity of eicosanoid-mediated events such as platelet aggregation (39, 40, 58) and its associated cardiovascular mortality (17). The boundary between inadequate and adequate proportions of 20:4n-6 in HUFA of plasma phospholipids may be at approximately 20 to 30%, and further tests of eicosanoid-mediated physiology are needed to confirm this estimate. The boundary between adequate and excessive levels of 20:4n-6 in tissue HUFA may be below the levels maintained with the present USA diet (with high incidence of heart attacks) and above those maintained with the traditional Japanese diet (with high incidence of hemorrhagic strokes) (59).

Every meal that we eat ensures that we are poised in a balance between n-3 and n-6 HUFA and in a balance between more and less intense eicosanoid-mediated physiology. The challenge is to perceive where we are in that balance and to estimate where we would like to be. The typical dietary ratio of n-3/n-6 fatty acids has little compelling reason for its continuation except the comfort of convenience and familiarity. The logic for using quantitative physiological endpoints to choose a potentially safer balance of n-3/n-6 HUFA merits careful appraisal.

CONCLUSIONS AND APPLICATIONS

Tissues maintain a relatively constant amount of glycerophospholipids as well as a relatively constant percent of 20- and 22-carbon highly unsaturated fatty acids (HUFA) in the glycerophospholipids. Most foods ingested by humans contain enough polyunsaturated fatty acid of the n-6 and n-3 types to form HUFA that displace the endogenous n-7 and n-9 HUFA. Thus, tissues maintain a relatively constant sum of n-3 plus n-6 HUFA. The intensity of n-6 eicosanoid-mediated events depends, in part, upon the proportion of 20:4n-6 in the tissue HUFA, which in turn reflects the proportions of n-3 and n-6 fatty acids in the diet. Increasing the proportion of n-3 HUFA in tissue HUFA is the only practical route to diminishing the proportion of n-6 HUFA in tissue HUFA. Many diets regarded as "normal" (such as laboratory rat chow and the

typical average diet in the USA) have low ratios of n-3/n-6 fatty acids, and they lead to proportions of 20:4n-6 in tissue HUFA that may support near maximal intensities of synthesis of n-6 eicosanoids. A possible benefit of dietary n-3 fatty acids may be moderation of the excessively vigorous actions of n-6 eicosanoids (53).

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