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Review

Balancing proportions of competing omega-3 and omega-6 highly unsaturated fatty acids (HUFA) in tissue lipids

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ABSTRACT

People eating different balances of omega-3 and omega-6 nutrients develop predictably different proportions of competing highly unsaturated fatty acids (HUFA) in their tissue lipids. While epidemiological studies have associated wide differences in HUFA balance with disease severity, some clinical studies that did not examine wide differences failed to confirm the association. We examined the degree to which the relative amount of arachidonic acid, the major precursor of omega-6 eicosanoids, differs among people who have widely different dietary intakes of omega-3 and omega-6 nutrients. Gas chromatographic analyses of human blood samples describe the balance among n-3 and n-6 HUFA for different individuals. The proportion of the omega-6 arachidonic acid, from which potent eicosanoids are formed, is not constant. It ranges from 30% to 70% of HUFA while the competing n-3 HUFA range from 60% to 10% of HUFA. Significant differences in clinical outcomes between control and intervention groups have been seen when using dietary interventions that shift the balance of n-3 and n-6 nutrients far enough to create a biologically significant difference in the HUFA balance.

1. Introduction

Clinical decisions about the efficacy of omega-3 nutrients in moderating risks for health disorders related to omega-6 mediators may be improved by viewing the accumulated tissue levels in the context of the omega-3 acids competing with the omega-6 acids that form potent eicosanoid mediators. Many aspects of fatty acid metabolism involve an individual acid acting in the presence of competing closely-related acids. Reports of acyl chain turnover and phospholipid retailoring began in 1958 and led to a series of papers on selective placement of competing acyl chains by lysophospholipid acyltransferases and how beneficial effects come from balancing omega-6 (n-6) fatty acids with omega-3 (n-3) fatty acids [1]. Erythrocytes have a simple system for lecithin synthesis [2] in which unesterified fatty acids and lysophosphatidyl choline from plasma combine to form phosphatidylcholines that are retained in the membrane or released in facile exchanges with circulating lipoproteins. Relative abundances of competing substrate acids combine with enzymatic selectivities to predict closely the composition of fatty acids accumulated at the 2-position of phospholipids in human, rat and cow erythrocytes [2].

When dose-response studies with high affinity acyl-CoA substrates required unmanageably low concentrations, competitive effectiveness of acyl-CoA substrates was measured against arachidonoyl-CoA [3]. Competitive studies help interpret the relative ability of various polyunsaturated acyl-CoAs to accumulate in phospholipids from the mixture of competing substrates usually present in cells [4]. Many different lysophospholipid acyltransferases are now recognized [5,6], and the set of lysophospholipid acyltransferases in liver microsomes has an overall selective affinity for more highly unsaturated substrates relative to less unsaturated ones. However, that set does not discriminate appreciably between the omega-6 (n-6) or omega-3 (n-3) structure of HUFA that eventually serve as precursors of bioactive prostaglandins and leukotrienes. Docosahexaenoyl-CoA competed effectively with arachidonoyl-CoA, but it was transferred slowly and inhibited the overall rate of transferase action [3].

Linoleic and linolenic acids have similar competing hyperbolic dose-response actions in the elongation and desaturation steps that form the HUFA which accumulate in rat liver lipids [7,8]. This non-linear metabolic relationship was confirmed quantitatively with an empirical predictive equation that describes how the balance of n-3 and n-6 nutrients expressed as a percent of daily food energy (en%) leads to the accumulated proportions of n-3 and n-6 HUFA [9]. The empirical competitive hyperbolic equation also fit data from studies with humans [10], and it was extended to fit studies with added

Abbreviations: AA, arachidonic acid, 20:4n-6; ALA, alpha-linolenic acid, 18:3n-3; CoA, coenzyme A; cPLA2, cytosolic phospholipase A2; EPA, eicosapentaenoic acid, 20:5n-3; DPA, docosapentaenoic acid, 22:5n-3; DHA, docosahexaenoic acid, 22:6n-3; HUFA, 20- and 22-carbon highly unsaturated fatty acids; LA, linoleic acid, 18:2n-6; LTB4, leukotriene B4; LTB5, leukotriene B5

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dietary n-3 HUFA for humans, rats and mice [10]. More quantitative dietary data from humans led to slight revisions of three constants to better fit all of the combined results [11]. A subsequent literature search and analysis [12] showed that the revised empirical equation estimated well the observed balance of tissue n-3 and n-6 HUFA that developed from known daily intakes of n-3 and n-6 nutrients for nearly 4000 people in 92 subject groups in 34 different studies from 11 different countries.

When developing the 1992 quantitative empirical equation relating dietary essential fatty acid intakes to the balance of accumulated tissue n-3 and n-6 HUFA [10], the authors noted that the proportion of n-6 acids in the HUFA of different populations was related to the incidence of heart attacks. If atherogenesis and thrombosis were mediated by n-6 eicosanoids, their severity might depend in part on the relative amounts of n-6 arachidonate and n-3 HUFA that competitively interact with cytosolic phospholipase A2 (cPLA2) as it initiates eicosanoid formation.

The evidence cited above suggests that the balance of competing n-3 and n-6 nutrients in foods eaten by each individual predictably affects the balance of n-3 and n-6 HUFA. Nevertheless, some researchers have suggested that arachidonic acid levels are constant in blood lipids and poorly related to health conditions. To see the degree to which the proportions of arachidonate and competing individual HUFA actually differ among people who have widely different dietary intakes of n-3 and n-6 nutrients, we examined analytical results from over a thousand Americans eating diverse food combinations.

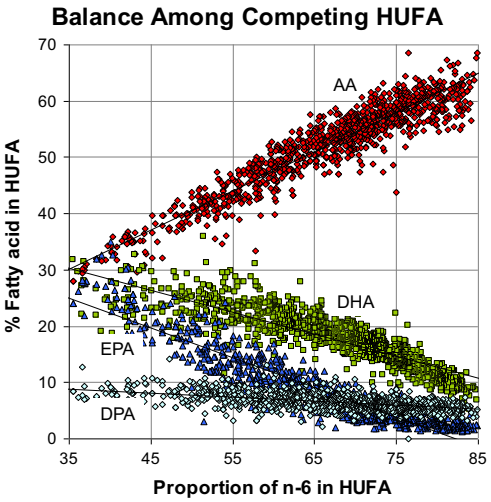

Fig. 1. Balance among competing n-3 and n-6 HUFA. Analysis of the proportions of individual HUFA among total HUFA in 1015 whole blood samples show lower proportions of 20:4n-6 (AA) when proportions of 20:5n-3 (EPA), 22:5n-3 (DPA) and 22:6n-3 (DHA) are higher. The proportions of 20:3n-6, 22:4n-6, and 22:5n-6 were all less than 10% of HUFA and are not shown. The dotted lines represent approximate HUFA balances for people in the indicated regional groups with different traditional food habits that cause different %n-6 in HUFA (34).

2. Methods

Gas chromatographic data on HUFA balance in finger-tip blood-spot samples were obtained from archived historic de-identified records from 1015 Americans during the past two years. More records were not used after 500 random samples added to the first 500 did not alter the pattern of results. Each anonymous electronic record describes the wt% amount of 35 different fatty acids, including seven major n-3 and n-6 20- to 22-carbon highly unsaturated fatty acids (HUFA). The samples of blood had been collected on paper [13], and the gas chromatographic values were expressed as proportions of competing n-3 and n-6 in the total HUFA as proposed at the 2004 ISSFAL Congress in Brighton, UK, [14]. The %n-6 in HUFA relates closely to the average daily balance of n-3 and n-6 nutrients ingested by individuals [10]. To avoid introducing any bias, the analytical values were not adjusted or modified by any further mathematical transformations.

3. Results

Fig. 1 shows the relative proportions of selected HUFA among the total HUFA in each blood sample. The mean and median values of %n-6 in HUFA (66% and 68%, respectively) are less than the 76–80% often reported for Americans [15]. The wide range of %n-6 in HUFA in this study reflects inclusion of many samples from individuals interested in eating more omega-3 nutrients in their daily diets. The proportion of n-3 and n-6 acids in blood HUFA has been reported to be a useful predictor of competing n-3 and n-6 biological roles [14,16]. Once formed, the n-3 and n-6 HUFA move in and out of diverse lipid pools with similar turnovers. Metherel et al [17] showed that the relative proportion of n-6 in HUFA was near 78% for samples of plasma, erythrocytes, whole blood and finger-tip blood spot samples even when the n-6 HUFA content differed from 7.7 to 17 wt% (and n-3 HUFA from 2 to 5 wt%).

Expressing HUFA abundance in the context of other competing HUFA differs from the technical analytical laboratory report of fatty acid composition which describes relative amounts of fatty acids as a percent by weight (wt%) of all acids analyzed in a sample. The wt% value has a simple "housekeeping" rationale, but it has no clear metabolic or biological significance. One strength of the HUFA-oriented context for using the %n-6 in HUFA is that it avoids including noise from saturated, monoenoic and dienoic acids which have metabolic selectivities different than the HUFA that interact strongly with cPLA2.

Fig. 2A shows that wt% values for linoleic acid (LA), the major precursor of tissue arachidonate, varied from 10.9% to 38.3% with a mean of 25.9% and a median of 25.9%. The wt% values for linoleate varied little in relationship to the diet-related biomarker, %n-6 in HUFA. The means for linoleic quintiles ranged from 21% to 31% (Table 1A). Paradoxically, the quintiles of progressively higher wt% linoleic acid in this study had progressively lower wt% levels of its major HUFA metabolite, arachidonate (AA). It is likely that none of the samples studied were from people eating linoleate at levels in the linear dose-response range of 0 to 1 en% for dietary linoleic acid conversion to tissue HUFA [7,10]. The quintiles of wt% LA also had no significant association with other variables (%AA in HUFA, %n-3 in HUFA and %n-6 in HUFA) which are related to eicosanoid biosynthesis. Arachidonate (AA) levels expressed as wt% of all acids in the sample had values that varied from 3.9% to 22.3% (mean=10.0%) and was higher when the %n-6 in HUFA was higher (Fig. 2), although the wt% values scarcely escaped the noise envelope for people maintaining typical American levels of 70% to 80%n-6 in HUFA. More importantly, the relative proportion of arachidonate within the HUFA differs from 39% to 61%, and it clearly relates to the quintiles of the diet-related biomarker, %n-6 in HUFA (Table 1B). The diet-related biomarker ranged from 25.3% to 93.9% (mean=66.1; median=68.2), and the quintiles had mean values of 48, 61, 68, 73 and 80.

Fig. 2B shows that wt% values for the most abundant dietary precursor of tissue n-3 HUFA, alpha-linolenic acid (ALA), had a very low with a mean value of 0.7%, and the values varied little in relationship to the diet-related biomarker, %n-6 in HUFA. For most people studied, the wt% of DHA was below 5% while the %DHA in HUFA ranged from 7% to 30%. A sharper focus on the competing HUFA derivatives is further illustrated in Fig. 3 where the relative abundance of arachidonate in HUFA is compared with the sum of competing n-3 HUFA in the blood sample. If the balance between competing n-3 HUFA and arachidonate influences the substrates made available for

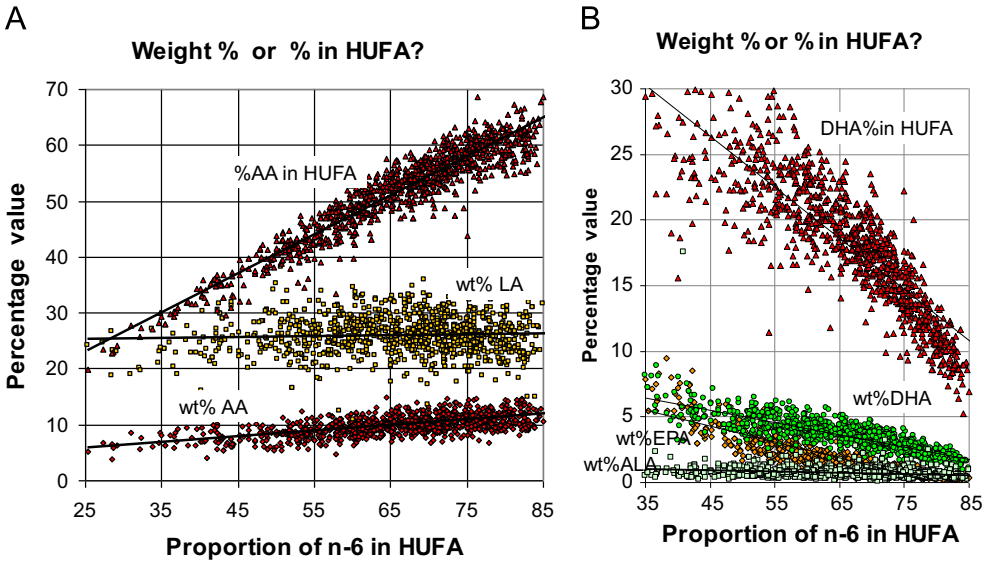

Fig. 2. Comparing two modes of describing abundances. 2A.The wt% of linoleate (LA) and arachidonate (AA) are less associated with the proportion of n-6 in HUFA than is the percent of arachidonate in HUFA. 2B. The wt% of alpha-linolenate (ALA), EPA and DHA are less clearly associated with the proportion of n-6 in HUFA than is the percent of DHA in HUFA.

Table 1
Comparison of biomarkers in quintiles of wt% of linoleic acid and quintiles of %n-6 in HUFA.

A. Quintiles of wt% of linoleic acid in total acids

Wt%18:2n-6	Q1	Q2	Q3	Q4	Q5
10.9 to 38.3	21.2 (2.1)	24.2 (0.5)	25.9 (0.5)	27.6 (0.6)	30.8 (1.6)
Wt%20:4n-6	10.0 (2.1)	10.2 (2.1)	10.0 (1.9)	9.9 (1.7)	9.7 (1.6)
%AAinHUFA	50.3 (10.3)	51.6 (9.3)	51.9 (8.0)	52.4 (7.0)	52.8 (7.4)
%n-3inHUFA	35.2 (14.3)	34.1 (12.3)	33.5 (10.5)	33.2 (9.9)	33.3 (10.1)
%n-6inHUFA	64.8 (14.3)	65.9 (12.3)	66.5 (10.5)	66.8 (9.9)	66.7 (10.1)

B. Quintiles of percent of n-6 HUFA in total HUFA

%n-6 in HUFA	Q1	Q2	Q3	Q4	Q5
25.3 to 93.9	48.2 (7.1)	60.9 (2.3)	68.1 (1.6)	73.4 (1.6)	80.1(2.9)
Wt%18:2n-6	25.2 (3.8)	26.5 (3.3)	26.4 (3.6)	26.1 (3.1)	25.5 (3.3)
Wt%20:4n-6	8.1 (1.5)	9.5 (1.4)	10.3 (1.4)	10.8 (1.4)	11.2 (2.0)
%AAinHUFA	38.8 (5.7)	48.8 (3.3)	53.7 (2.6)	57.1 (2.9)	60.6 (4.2)
%n-3inHUFA	51.8 (7.1)	39.1 (2.3)	31.9 (1.6)	26.6 (1.6)	19.9 (2.9)

Each quintile ($n=203$) has values for the biomarkers as means with standard deviations in parentheses. The range of 18:2n-6 values in A is 10.9 to 38.3 wt%. The range of values in B for % n-6 in HUFA is 25.3% to 93.9%.

eicosanoid formation, it likely has important biological consequences that will affect decision making in medical practice, policy, education, and research.

4. Discussion

The cPLA2 hydrolase action that initiates eicosanoid-mediated responses favors highly unsaturated substrates (20:4 > 18:3 > 18:2 > 18:1 > 16:0), but it does not discriminate appreciably between the n-6 and n-3 HUFA that serve as precursors of eicosanoids [18]. The indiscriminate action allows the diet-determined HUFA balance in tissues to be reflected in the released substrates. The cPLA2 resembles the acyltransferase in being slowed by competing DHA substrates [19], perhaps due to steric hindrance from the carbon 4 double bond being near the ester bond. In this way, the presence of DHA may slow the release of both EPA and AA. Overall, the cPLA2 preference for HUFA combines with the relative abundance of arachidonate within the HUFA available (Fig. 1) to provide diet-dependent competing n-3 and n-6-mediators that have different biological outcomes.

A comprehensive study of the relative intensity of eicosanoid formation and action [20] showed that many steps after phospholipase release are less vigorous for n-3 than competing n-6 analogs. For example, prostaglandin synthase forms prostaglandins [20] and leukotriene C synthase forms cysteinyl leukotrienes [21] with less intensity when using the omega-3 rather than omega-6 substrate. Blood platelet aggregation, which mediates thrombotic heart attacks, was less when the HUFA balance shifted toward less n-6 HUFA [22]. Also, the chemotaxis that mediates many immune-inflammatory disorders may be 50-fold less with the omega-3 LTB5 than for the omega-6 LTB4 [23]. These examples illustrate important processes in which the context of balance among competing tissue HUFA has a vital role in interpreting the subsequent clinical outcomes.

Common health problems made worse by excessive omega-6 eicosanoid actions include atherosclerosis, arthritis, asthma, bone loss, cancer growth, heart attacks, length of hospital stays, depression, suicide, classroom disruptions, oppositional behavior and unproductive workplace behaviors [24]. Importantly, the proportion of HUFA that is arachidonate (AA), from which potent n-6 eicosanoids are

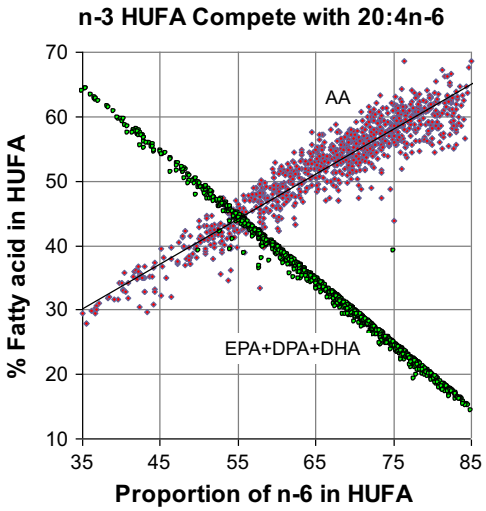

Fig. 3. Three n-3 HUFA combined compete with arachidonate for accumulation in tissue lipids and for subsequent downstream actions.

formed, is not constant. Fig. 1 shows that AA can range from 30% to 70% of HUFA while the competing EPA proportion ranges from 25% to 5% and the DHA proportion from 30% to 10% of HUFA. Long-recognized differences in heart attack mortality rates for people in Greenland, Japan and Mediterranean countries, all of which were lower than that for Americans and Northern Europeans [25] were closely associated ($r^2=0.99$) with observed proportions of n-6 in HUFA for these groups [26]. Thus, the balance of n-3 and n-6 acids in blood HUFA is a useful health risk assessment biomarker in addition to being a useful measure of daily average intakes of the competing n-3 and n-6 nutrients in foods. The following examples about disease risk compare reports that either did or did not examine a wide rangfe of HUFA balance values. They illustrate how a transparent display of the measured HUFA balance gives a clear context for developing constructive clinical interpretations of the efficacy of dietary omega-3 interventions for lowering excessive n-6 eicosanoid actions in several health conditions.

A paradox occurs in the apparent dissonance between a cross-national association of depression with low intakes of n-3 HUFA [27] that contrasts with a large longitudinal study of American women which found no statistically significant association [28]. However, estimates of the likely HUFA balance in the different groups (using the empirical relationship described in the introduction) suggest the estimated HUFA balance ranged from 30% to 80%n-6 in HUFA (Fig. 3, Ref. [27]) for the cross-national report, and it ranged from only 69% to 73%n-6 in HUFA for the quintiles of 54,632 women in the longitudinal study (Fig. 2, Ref. [28]). The latter report used quintiles of ALA intake to define statistically significant numerically different subsets of the group, but those subsets may not define biologically significant differences (for example, see ALA wt% values in Fig. 2B). It seems likely that a clinical study with a wider range of the biologically significant HUFA balance might have quintiles with significant differences in depression.

Another paradoxical example of an apparent dissonance involves the widely different prostate cancer mortality rates in five different countries [29] which is associated with estimates of likely HUFA balance that ranged from 35%n-6 in HUFA for "traditional" Japanese, 50%n-6 in HUFA for "modern" Japanese" [11] compared to 60%n-6 in HUFA for Italy and about 70–80%n-6 in HUFA for UK and USA [15]. This association of HUFA proportions with prostate cancer deaths contrasts with interpretations from two recent nested case–control comparisons: controls had 77%n-6 in HUFA compared to 77% in low-grade cases and 76% in high-grade cases in one study [30], and a second study had 72%n-6 in HUFA in controls compared to 71% in low-grade and high-grade cases [31]. In these two studies with a very narrow range of HUFA balance, the authors interpreted data by grouping individuals by quartiles of the n-3 HUFA weight percent in the total plasma phospholipid fatty acids. This parameter has a different biological significance than the balance of competing n-3 or n-6 in HUFA (Fig. 1), and it did not have the wide biologically significant range of %n-6 in HUFA seen in the cross-national study. Viewing a wider biological range of HUFA balance and including the context of competing n-3 and n-6 HUFA will likely give more valid interpretations of fatal prostate tumorigenesis.

A third example of apparent contradiction occurred with randomized controlled clinical trials of patients with chronic pain. A 2001 study [32] reported that increased intakes of n-3 HUFA failed to give a significant difference in the number of pain attacks in the last 4 weeks of the comparative treatment period. In contrast, a 2013 report [33] used concepts mentioned in the introduction to design sufficient lowering of the competing n-6 nutrients for three months to lower the %n-6 in HUFA of erythrocytes from 78% to 74%. This protocol led to a small, statistically significant lower self-administration of acute medications (vasoactive abortive medications, acute opioids, and non-steroidal anti-inflammatory drugs).

More importantly, another group of patients who raised intakes of omega-3 nutrients in the presence of lowered intakes of competing omega-6 nutrients lowered the %n-6 in HUFA from 77% to 61% in 3 months. These patients lowered their self-administration of acute medications further as they reduced headache hours per day by 44% [33]. This trial showed a clear benefit in changing food intakes to lower the HUFA balance below the level of 76–80%n-6 in HUFA that is often observed for Americans. Shifting from 77% to 61%n-6 in HUFA clearly has biological significance.

A 2014 historical perspective of the impact of n-3 and n-6 nutrients on health gave evidence for a very narrow therapeutic window for dietary linoleic acid that is reflected by the health risk assessment biomarker, the %n-6 in HUFA [34]. Fortunately, n-3 nutrients have no known upper limit of risk [35], and their competitive actions widen the therapeutic window for n-6 nutrients. Ramsden et al showed clearly that dietary linoleate can cause harm [36] and that lowering intakes of omega-6 nutrients while raising intakes of omega-3 nutrients [33] can be an effective form of preventive medicine. The context of an unwanted diet-determined balance in tissue HUFA which has an unwanted clinical outcome makes clear the need for explicit public information about food choices by which people can voluntarily balance their intakes of n-3 and n-6 nutrients and maintain a tissue HUFA balance linked to lower risk of unwanted health conditions.

Describing HUFA abundance in the context of competing eicosanoid precursors may be regarded by some to introduce a limiting bias. However, clinical reports that describe only wt% amounts of n-3 EPA and DHA and omit data on the amounts of competing n-6 HUFA fail to make accessible to the scientific community important evidence related to the balance between n-3 and n-6 HUFA that will be precursors of biologically significant eicosanoid actions. The competing n-3 and n-6 nutrients in each food item give an Omega 3–6 Balance Food Score [37] that summarizes data in the USDA Nutrient Database as a single value expressing the balance among eleven omega-3 and omega-6 essential fatty acids in the food. As the average daily food balance score for people with different food habits ranges from -8 to $+3$, the %n-6 in HUFA ranges from 88% to 28% [37]. Over 5000 different Omega 3–6 Balance Scores are in an "app" for personal mobile devices [38]. Using these food Scores along with semi-annual monitoring of personal HUFA balance values from finger-tip blood-spot assays gives a very simple corporate wellness intervention that may decrease annual health care claim costs [24,34].

Acknowledgments

BL developed the empirical diet-tissue equation with a Pfizer Biomedical Research Award. DB obtained analytical HUFA balance data from historic, de-identified assays of whole blood in his laboratory database. BL had primary responsibility for final content. Both authors read and approved the final manuscript. No authors declare a conflict of interest. No funding was obtained for this study.

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