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Review

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

Doug Bibus [a], Bill Lands [b,*]

[a] Community Faculty, University of Minnesota Center for Spirituality and Healing, and Lipid Technologies LLC, P.O. Box 216, Austin, MN 55912, United States
[b] Fellow, ASN, AAAS, SFRBM, 6100 Westchester Park Drive, College Park, MD 20740, United States

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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

* Corresponding author. Tel.: +1 301 345 4061.
E-mail address: wemlands@att.net (B. Lands).

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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.

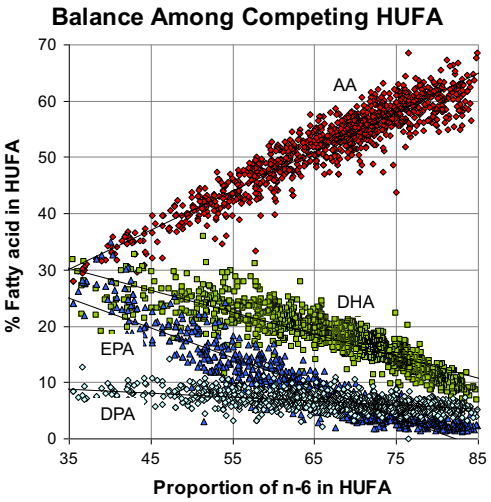

Fig. 1. Balance among competing n-3 and n-6 HUFA. Analysis of the proportions of individual HUFA among total HUFA in 1015 whole blood samples show lower proportions of 20:4n-6 (AA) when proportions of 20:5n-3 (EPA), 22:5n-3 (DPA) and 22:6n-3 (DHA) are higher. The proportions of 20:3n-6, 22:4n-6, and 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