J. Membrane Biol. 206, 75–83 (2005)
DOI: 10.1007/s00232-005-0785-0

The Journal of
Membrane
Biology

Learning how Membrane Fatty Acids Affect Cardiovascular Integrity

William E.M. Lands
College Park, MD 20740, USA

Received: 8 September 2005

Overview

The proportion of n-3 acids among the 20- and 22-carbon highly unsaturated fatty acids (HUFA) of tissues can vary from 20 to 80 percent depending on voluntary food choices of individuals. Such food choices are the origin of essential n-3 and n-6 HUFA in the tissues of humans. This review describes how my research on membrane lipids led me to believe that Americans should eat more n-3 (omega-3) and less n-6 (omega-6) fats. The belief has roots from my early studies in graduate school, which included the organic chemistry of many different fatty acids and made me wonder whether each fatty acid might have a special action in health. Combining concepts of enzyme kinetics with those of acyl chain structures helped me appreciate that our health results from a paradoxical combination of:

1-selective chain interactions with protein enzymes and receptors

2-which often are promiscuous in responding to available substrates and ligands.

This perspective grew gradually during my 50-year study of membrane lipids as I tried to learn whether the chemical property of acyl chains used by the enzymes synthesizing membrane lipids is the same as that mediating the biological success or failure of resulting membrane lipids.

Recognizing Saturated and Unsaturated Acids

In 1955, while others started raising concerns about the health consequences of eating saturated and unsaturated fatty acids, I began academic research by studying the saturated lung surfactant (dipalmitoyl lecithin) and the enigmatic plasmalogens (abundant in brain and heart tissue). The studies with lung tissue gave us early clues to enzymes that catalyze independent turnover of acyl groups on the glycerol "backbone" (Lands, 1958), and new knowledge of plasmalogen biochemistry let us make specific 2-acyl substrates for study of selective acyl chain transfer. Combining that knowledge with studies of venom phospholipase let us prove that typical phospholipids had saturated acids selectively placed at the 1-position and unsaturated acids at the 2-position (Robertson & Lands, 1962). This finding indicated that biosynthetic enzymes (still unknown at that time) do discriminate between saturated and unsaturated structures when forming membrane phospholipids (Lands, 1960; Lands & Merkl, 1963; Merkl & Lands, 1963).

I wanted to know what chemical properties of saturated and unsaturated acyl chains were involved during enzyme-catalyzed "decisions" and whether those properties matched the ones that we attributed to their role in membranes. Our measurements of selective esterification rates were eased by developing a rapid spectrophotometric assay to quantitate details of the selective rates of acyl transfer from CoA thiol esters to glycerolipids (Lands & Hart, 1965). We were happy that some esterification rates observed in vitro related, in part, to the known contents of fatty acids in membrane lipids, and we were intrigued to see that retailoring acyl transfers were more selective than those in the de novo synthesis pathway. Somehow, what we called "saturated" or "unsaturated" was being recognized differently by the different enzymes making membrane lipids.

In addition, changed abundances of phospholipid molecular species in tissues at different times indicated that no single pattern of composition is rigidly maintained. Much of the plasticity in lipid composition is likely due to promiscuity of the synthetic enzymes acting on different substrate abundances. We showed that although acyltransferases

Keywords: Acyltransferases — Highly unsaturated fatty acids — Membrane phospholipids — Omega-3 — Omega-6 — Saturated fats — Transfats

Correspondence to: W. E.M. Lands; email: wemlands@att.net

recognize acyl chain structures when making membrane phospholipids, the acyl compositions in membranes differ with changed substrate availability. For example, feeding a sugar-rich meal after fasting greatly induces fatty acid synthases and desaturases in the liver, and the molecular species of lecithins shift drastically to include the abundant oleate that is synthesized from the sugar (Lands & Hart, 1966). The resulting abnormal lecithin pattern with much oleate at the 1-position and abundant dioleoyl species then gradually shifts back to a more "normal" pattern with saturates at the 1-position and unsaturates at the 2-position as the super-induced enzymes return to lower levels and the supply of oleate is less. The typical pattern seen in 1966 for rat liver lecithins had most molecules with one n-6 essential fatty acid (either 18:2n-6 or 20:4n-6) and nearly all with one saturated fatty acid. Only many years later did we begin to question whether that "normal" pattern is the best for optimal health. The optimistic belief that typically observed patterns are likely to be optimal ones for health needs careful testing.

The distribution of common fatty acids (and some uncommon isomers; Lands et al., 1966) between the 1- and 2-positions was predicted well by acyltransferase selectivities, especially with the simple erythrocyte system (Waku & Lands, 1968a). In the latter case, combining relative acyltransferase rates with relative abundances of available non-esterified acids predicted the erythrocyte membrane phosphatidyl choline species. Acyl transfer seemed independent of the acyl structure at the adjacent 1-position (Brandt & Lands, 1967), although it differed when adjacent to an ether or alkenyl ether structure (Waku & Lands, 1968b).

Around this time, we also developed a method to examine acyl chain compositions at the three different positions of triglycerides and found different biosynthetic selectivities for each position (Lands et al., 1966; Slakey & Lands, 1968; Hill et al., 1968b). Again, the results reaffirmed that the abundances of different substrates strongly affect the outcome of biosynthetic enzymes acting with their inherent selectivities. We finally had methods to test what property of saturated acids put them at the 1-position and what property of unsaturated acids put them at the 2-position.

Saturated Fats Are Really not like *Trans*-Fats

Stories about cells shifting their acyl chain composition toward more unsaturated acids "in order to accommodate" lower environmental temperatures deserve careful examination for validity and for the actual mechanism that increased the proportions of unsaturated acids. A hypothesis that biosynthetic enzymes would "recognize" the high melting *trans*-acyl chains like saturated acids and preferentially place them at the 1-position seemed supported by finding that 9-*trans*-18:1 was esterified more rapidly at the 1- than at the 2-position, whereas 9-*cis*-18:1 had an opposite fate (Lands, 1965a). However, the fitness of replacing a saturated acid with a *trans*-unsaturated acid was still not clear. The 1-position sometimes has low-melting unsaturated acids (Lands & Hart, 1966), and we don't know the biological consequences of having *trans*-unsaturated acids there. Later studies showed that *trans*-acids have important effects on metabolic regulation that may be more significant in cell health than their effect on membrane fluidity.

Overall, the combined evidence does not support the hopeful teleological view that cellular acyltransferases "intelligently" select acyl chains to give a "correct" composition of molecular species (Brandt & Lands, 1967) or adapt to a changed environment (Jezyk & Lands, 1968; Ellingson & Lands, 1968; Ellingson Hills & Lands, 1970). Rather, the biosynthetic enzymes using acyl-CoA esters appear to be responding to abundance of substrate and to some acyl chain features that many had not previously regarded as significant. The enzyme is likely unconcerned with human-based rationales of fitness, and the outcome of a selective enzyme action may not always be survival. This author knows no way for an acyltransferase enzyme to alter its genetically coded selectivity and adapt to a changed environment (Lands, 1980a). The enzyme selectivities for esterification seem to depend on features of solvated acyl-CoA independent of the bulk physical properties of the resultant esterified product.

Later work showed that cells encountering marginally inadequate substrate acids died rather than adapting, as suggested by hopeful teleology. When adaptations in composition do occur, they seem to be a simple response to changed substrate abundance rather than fitting Panglossian ideas of the "best of all possible outcomes". Teleological rationales based on the presumed bulk melting properties of resultant membranes were poor predictors of the selective interactions of acyltransferases with soluble acyl-CoA substrates. Perhaps the most important outcome of our studies of membrane lipid biosynthesis was in recognizing that the properties enzymes used in mediating biosynthesis are not properties humans regard as markers of "fitness".

Subtle Properties of the "Right" Acid

Collaborations with Frank Gunstone and his students at St. Andrews provided us positional isomers of four sets of 18-carbon acids that contained either *cis*-ethylenic (Reitz et al., 1969), *cis*-cyclopropyl (Okuyama et al., 1969), *trans*-ethylenic (Okuyama

et al., 1972) or acetylenic (Tamai et al., 1973) bonds at different positions along the acyl chain. The spectrophotometric assay with CoA thiol esters showed unexpected subtle discriminations among acyl chains that went far beyond the information provided by common naturally-occurring acyl chains. We soon saw that generalizations about enzyme preferences that had been made from results with a limited set of customary saturated and unsaturated fatty acids were not based on a sufficient awareness of the ability of cell-free acyltransferases (and living cells also) to respond to details in acyl chain structures.

As expected, we saw that some *cis*-ethylenic chains were esterified more rapidly at the 2- than the 1-position, but some (especially the 8-, 10- and 12-isomers) were esterified faster at the 1-position where saturated acids typically reside. Clearly, even though the common 9- and 11-*cis*-isomers are not favored, esterification at the 1-position was NOT selecting only saturated or high-melting acids. The pattern of preference for esterifying the low-melting *cis*-isomers to the 2-position also had no relationship to the melting points of the isomers. The rat liver acyltransferases acting on soluble acyl-CoA esters likely have no "awareness" of the physicochemical nature of the membrane product or of the human-assigned designation "saturated" or "unsaturated".

Esterifications of *cis*-cyclopropyl chains at the 1-position had rates that very closely fit a sawtooth pattern of selectivity seen for *cis*-ethylenic analogs, but esterifIcation rates at the 2-position had little agreement. Three *trans*-isomers (that we expected to be rejected from the 2-position) were transferred to the 2-position as fast as the *cis*-isomers. It was as if the enzyme detecting unsaturated acids responded to ethylenic *pi*-bonds at certain locations independent of melting point or *cis*-/*trans*-configuration. Alternatively, a sawtooth pattern of transferring *trans*-isomers to the 1-position (where saturated acids reside) resembled the preference for *cis*-isomers, but with a 1-carbon frameshift that favored the 9-, 11- and 13-*trans* isomers as it did the 8-, 10- and 12-*cis* isomers.

The 1-carbon offset is visible when superimposing space-filling models of *cis*- and *trans*-isomers, indicating that the transferase system likely was responding to a common spatial conformation irrespective of its *cis*- or *trans*-bond configuration. Melting points of the acyl chains were not related to esterification selectivity, making unlikely the teleological hypotheses about synthetic enzymes using fitness for membrane function as a criterion for incorporating acids into membrane lipids. The new insights puzzled many colleagues who preferred to retain the old familiar stories about saturated acids and fluidity.

One unexpected problem occurred when measuring esterification selectivities of the de novo pathway in rat liver preparations. Although biochemists prefer to study purified enzymes and substrates, we found less selectivity for the de novo pathway when measured with partially purified systems (Lands & Hart, 1964; Hill & Lands, 1968; Hill, Husbands & Lands, 1968a) compared to that observed for intact liver. The discrepancy led us to explore the degree to which competing substrates or other incubation factors cause promiscuity or lack of specificity. Placement of acyl chains seemed less selective as the enzyme system became more purified and greater amounts of substrate were used with cell-free systems (Husbands & Lands, 1970; Okuyama, Eibl & Lands, 1971). Acylation of 1-acyl-GP is inhibited by higher concentrations of acyl-CoA esters (Lands & Hart, 1965), and the enzyme has a very low apparent *K*m value for acyl-GP and acyl-CoA substrates (Okuyama & Lands, 1972). Selective esterification was seen with low concentrations, but not when high concentrations were used.

A final explanation for high selectivity in vivo remains to be clarified, but it likely reflects the relatively low availability of competing substrates and intermediates in healthy intact cells where lipids bind to cytosolic proteins that may be as abundant as 100 mg/ml. We learned that many in vitro studies overloaded the enzymes with excessive amounts of lipid substrates and obscured important selective competitions that occur in vivo. A later study used competing pairs of acyl-CoA esters to produce clearer evidence for relative selectivity during esterification (Lands et al., 1982). Eventually, better answers will come from purified cloned enzymes and from systems formed by specific knock-out or knock-down methods of molecular biology.

Subtle Effects on Membrane Physiology

Once appreciable evidence showed that enzymes mediating biosynthesis can make highly selective transfers, we tested whether subtle structural differences could affect cell physiology. To do this, we used cells unable to synthesize unsaturated acids. These cells continue to synthesize saturated acids and require an external supply of some acid to maintain minimal membrane fluidity and function. The benefit of such added acids can be easily measured in terms of cell yields and gas chromatographic measures of esterified nutrient per cell. The latter relates to molal volume and excess molal volume of acyl chains in membrane lipids, additive properties that relate linearly to measures of fluidity, compressibility and viscosity (Barber & Lands, 1973; Holub & Lands, 1975; Ohlrogge et al., 1976).

These additive properties allowed us to assign quantitative biological functionality factors to various nutrient acids supporting membrane function in the growing cells (Lands, 1980b; Lands & Davis,

1983). More effective acids permit forming more cells per femtomole during their subsequent dilution by palmitate in the membrane lipids of daughter cells. When growth efficiencies paralleled values for the gas-chromatographically determined functionality factors (as they did for most *cis*-ethylenic isomers), we had a confirmation of an expected outcome, but when growth efficiencies diverged from functionality factors (as they did for acetylenic and cyclopropyl isomers), we had a discovery of a previously unknown phenomenon.

The large set of structurally varied positional isomers showed very different abilities to support cell proliferation. However, poor growth was not always associated with a higher melting point of the fatty acid and its presumed lower contribution to membrane fluidity. The lack of growth with *trans*-isomers (Vandenhoff et al., 1975) fit expectations that these higher-melting acids would be handled like saturated acids, but some acetylenic isomers with even higher melting points were more effective than the corresponding *cis*-isomer in supporting growth (Lands, et al., 1977). The 9-*yne*-isomer was the most effective in supporting yeast cell growth, whereas it was very much less effective than the similar 7-, 8- or 10-*yne*-isomers with *E. coli*. In many cases of low cell yields, much of the nutrient isomer remained unesterified. Evidently, cell function was influenced by some property other than the melting point of the isomeric acid.

We were surprised to find that *cis*-cyclopropyl isomers (that closely matched *cis*-ethylenic isomers in acyltransferase interactions) had only three isomers (8-, 9- and 11-) that supported *E. coli* growth on glucose (Lands et al., 1978), but when glycerol was the source of energy, nearly all supported growth as effectively as the *cis*-ethylenic isomers in a pattern resembling aspects of fluidity. Somehow, growth conditions with glycerol (or glucose with added cAMP) allowed *cis*-cyclopropyl and acetylenic isomers to attain growth predicted by fluidity considerations. The usually discussed physicochemical properties of the acyl chains were not the only aspect controlling the actions of an acid on *E. coli* membrane function.

Another surprising feature was a dramatic saw-tooth pattern of growth effectiveness that resembled the earlier-mentioned selectivity pattern for rat liver microsomal acyltransferase action at the 1-position. Again the shift by 1-carbon atom between *yne*-and *cis*-isomers (Lands et al., 1978) is visible with molecular models, indicating that some unknown gene in *E. coli* codes for a protein that dramatically discriminates detailed conformational features of acyl chains. Identifying the gene and its protein will likely give useful new tools for understanding how fatty acid structures affect health.

Recognizing that cells growing with glycerol (or in glucose with added cAMP) give cell yields in accord with functionalities predicted from fluidity considerations led us to re-examine the quantitative contribution of *trans*-isomers to membrane function (Tsao, & Lands, 1979). The 9- and 11-t*rans*-isomers can provide sufficient membrane fluidity to support cell growth in the presence of added cAMP. Thus, results with *trans*-acids alert us to some toxic action on metabolic regulatory system(s) that we need to know better.

In this regard, a highly efficient mutagenic action occurs in *S. cerevisiae* when certain fatty acids support nuclear DNA replication but fail to support replication of mitochondrial DNA. With these acids, petite daughter cells are formed with none of the genes of mtDNA, and their failure at respiration leaves them dependent on glycolysis for metabolic energy (Graff, Sauter & Lands, 1983; Lands & Graff, 1981). In addition, the effectiveness of different acids in supporting growth and induction of respiration of *S. cerevisiae* requires synthesizing new phospholipids to form functional respiratory complexes. The existing membrane lipids apparently cannot move to the new locations being assembled (Walenga & Lands, 1975 a,b). Cellular enzymes respond to substrate abundances, but they cannot change their selectivity to correct for unhealthy abundances.

Triglycerides accumulate in yeast cells provided with *trans*-acids (Vandenhoff et al, 1975; Graff & Lands, 1976), suggesting that impaired regulation of phospholipid formation is coupled to impaired expression or replication of DNA. A shift from phospholipid to triglyceride biosynthesis is a common metabolic response of cultured mammalian cells to toxic levels of added fatty acids. The eukaryotic yeast cells do not secrete accumulated excess fat in the form of lipoproteins as mammalian liver does. Recent study of the metabolic regulatory cofactor, PGC-1, may eventually lead us to the insight needed to interpret how dietary saturated and *trans*-fats promote elevated plasma triglycerides and greater synthesis of isoprenoid derivatives by altered regulation of gene expression (Lin et al., 2005). It may be useful to examine whether the PGC-1 gene product acts differently with the 8- or 9-*trans* isomers or whether acetylenic acids affect PGC-1 function. Our studies that began with simple concepts of saturated and unsaturated molecules led us to learn that factors other than fluidity influence the fitness of an acid for cell health. We have much more to learn about the genes that code for proteins that selectively discriminate among different acyl chain structures.

Prostaglandins, Eicosanoids and Physiology

By 1965, the general pathways esterifying different fatty acids into membrane lipids were recognized to have different general selectivities for the common

saturated (SFA), unsaturated (UFA), polyunsaturated (PUFA) and highly unsaturated (HUFA) fatty acids (reviewed by Lands, 1965b). The scientific community was aware that preferential accumulation at the 2-position of membrane lipids occurs with HUFA > PUFA > UFA > SFA. As a result, when Bergstrom and his colleagues in Stockholm showed that three HUFA (20:3n-6,20:4n-6,20:5n-3) form three types of hormone-like prostaglandins (Bergstrom et al., 1964), I suggested that a major location of tissue precursors for the hormones would be the 2-position of membrane phospholipids.

A brief stay in Stockholm showed that prostaglandin biosynthesis followed release of the HUFA esterified at that location (Lands & Samuelsson, 1968). Thus, the type of HUFA accumulated in membrane lipids can strongly influence what type of prostaglandin might be formed when HUFA are mobilized in tissue responses during inflammation and smooth muscle contraction. Past experience with biosynthesis of membrane lipids showed that the relative supply of n-3 and n-6 substrates would likely be an important factor in maintaining relative proportions in tissues. The broad biological importance of having HUFA in tissues was further supported when Unilever researchers showed that the relative activity of structurally different HUFA as essential fatty acids (EFA) matched their relative rate of prostaglandin formation (Beerthuis et al., 1968). As a result, during the next twenty five years (1968-1993), my research divided between events linked to maintaining compositions of membrane fatty acids and events linked to the hormone-like materials made from them.

The impact of tissue HUFA on health was soon seen to go far beyond membrane fluidity or nutritional essentiality, as many 20-carbon hormone-like derivatives of HUFA became recognized as powerful self-healing autacoids. The potent thrombotic agent, thromboxane, was discovered and identified as a derivative of the n-6 HUFA, arachidonate (Hamberg, Svensson & Samuelsson, 1975). Similarly, prostacyclin was discovered (Moncada et al., 1976) and identified (Whittaker et al., 1976) and leukotrienes were identified (Murphy, Hammarstrom & Samuelsson, 1979). All of these potent materials gave new roles for the HUFA stored in membrane lipids, and opened important new areas of biomedical knowledge, recognized by the Nobel Prize in 1982. Autacoids derived from HUFA have both beneficial and harmful actions, and many chronic diseases are now recognized as having excessive formation and function of the n-6 forms of these autacoids.

My curiosity about the quantitative dynamics of enzyme action led me to detailed studies of fatty acid oxygenase mechanisms to understand how biological and pharmacological controls over prostaglandin biosynthesis can affect inflammatory and thrombotic events. Because acyltransferases and phospholipases do not appreciably discriminate between n-3 and n-6 acyl chains, the proportions of these two types in tissue HUFA is controlled mostly by dietary abundances. However, mechanisms controlling fatty acid oxygenase actions gave us much more to explore (Lands, Lee & Smith, 1971; Smith & Lands, 1972) with unexpected features of self-activation (by hydroperoxide product) and self-catalyzed inactivation (suicide reaction) that added constraints beyond the simple limit of availability of HUFA substrates. Recognizing that important non-steroidal anti-inflammatory drugs (NSAIDs), aspirin and indomethacin, act irreversibly (Smith & Lands, 1971) when they block prostaglandin formation (Vane, 1971) started us on 20 years of study about how to cut the excessive formation and action of n-6 prostaglandins that was linked to the pathology of inflammatory and thrombotic processes.

We found that the oxygenase need for hydroperoxide activator gave cellular peroxidases an anti-inflammatory role in suppressing formation of prostaglandins (Cook & Lands, 1976; Hemler, Graff & Lands, 1978; Hemler, Cook & Lands, 1979; Hemler & Lands, 1980). We also found that common pharmacological inhibitors of prostaglandin formation had one of three basic types of mechanism: antagonizing substrate access; antagonizing hydroperoxide activation; time-dependent alteration of the enzyme site (Rome & Lands, 1975; Lands, 1981, 1985; Lands & Hanel, 1982; Hanel & Lands, 1982; Kulmacz & Lands, 1985).

Recognizing Different Outcomes for n-3 and n-6 Acids

In spite of diverse mechanisms that control prostaglandin formation (Lands, 1979, Marshall, Kulmacz & Lands, 1986), simple competitions among fatty acids and NSAIDs at the reaction site of cycloxygenase still provide a major tactic to decrease excessive formation and function of n-6 prostaglandins. We first described in 1972 how competition by n-3 analogs inhibits prostaglandin formation from 20:4n-6 (Lands et al., 1973). Certain competing acetylenic acid analogs also interacted with cyclooxygenase in an irreversible suicide reaction (Vanderhoek & Lands, 1973), as did the naturally occurring 20:5n-3 (Culp et al., 1979). Gradually, the different impacts of n-3 and n-6 HUFA in tissues appeared increasingly important worldwide (Lands, Pitt & Culp, 1980).

In many instances, the formation and function of n-6 eicosanoids is more vigorous than that of n-3 eicosanoids and can be undesirable in chronic diseases that have excessive n-6 eicosanoid actions.

For the past twenty five years, I have urged Americans to eat more n-3 and less n-6 fats to prevent unwanted over-responses. The differences between

n-3 and n-6 HUFA and their derived autacoids has remained the principal focus of my research. In fact, my last laboratory report (Kulmacz, Pendleton & Lands, 1994) gave a detailed comparison of the kinetic differences between 20:4n-6 and 20:5n-3 in forming prostaglandins.

After thromboxane was seen as a key mediator in heart attacks, we designed animal studies to show the preventive action of added dietary n-3 in competing with n-6 actions (cerebral infarction, Black et al., 1979; myocardial infarction, Culp et al., 1980; inflammatory leukotrienes, Murphy et al., 1981). As in earlier membrane lipid studies, we saw again that criteria for esterifying HUFA into membrane lipids were independent of the many biological actions that occur after HUFA are mobilized. A significant preventive action by dietary supplements of n-3 HUFA on thrombocyte activity in humans (Lands et al., 1985) convinced me that we should learn how much dietary change is needed to shift tissue HUFA composition to healthier proportions of n-3 and n-6 HUFA.

Fortunately, our previous success in interpreting anti-inflammatory drug actions led to an unrestricted Pfizer Biomedical Research Award with which we explored quantitative dynamics of diet-tissue relationships. The funding helped us develop rapid automated analyses of fatty acid composition (Ohta et al., 1990) and develop quantitative empirical equations that predict diet-tissue relationships for laboratory animals (Lands, Morris & Libelt, 1990a) and humans (Lands et al., 1992). As a result, a subsequent invited review on prostaglandin biosynthesis discussed how voluntary food choices worldwide likely cause different observed mortality rates among different nations (Lands, 1991). Sadly, changing dietary patterns among Japanese suggest that some n-6-mediated chronic disorders are likely to become worse in Japan in the future (Lands et al., 1990b).

How People Die

Describing my concerns at an international conference in Japan on nutrition in cardio-cerebrovascular diseases led me to show a figure linking two preventable dietary imbalances that cause fatal outcomes (Lands, 1993). An animated, updated version of this figure now appears at the distance learning website http://efaeducation.nih.gov/sig/chainofevents.ppt. The figure indicates how two easily prevented imbalances create mediators of morbidity and mortality, how selected medications intervene and how plasma cholesterol is a distal associative biomarker of the imbalanced processes rather than a mediator of the vascular injury and the fatal events.

There is a tendency for some people to neglect known transient mediators or to attribute a mediator role to an associated biomarker. This trend is evident in the lack of mention of the harmful role of non-esterified fatty acids that are released in plasma whenever there is increased circulation of VLDL, which also gives much-discussed plasma LDL biomarkers. The trend also includes a lack of mention of the harmful role of prenylated proteins in signaling processes during an increased formation of isoprenoids, which also gives much-discussed plasma cholesterol biomarkers. The animated figure attempts to remind readers of the mediators involved. Neglect of these mediators has continued for decades, while continued promotion of excess intakes of food and n-6 fats gave greater transient mediators of post-prandial oxidative stress, vascular injury and death.

The long sequence of enzyme-catalyzed events between food ingestion, post-prandial endothelial inflammation and injury, plaque accumulation, thrombosis, ischemia, arrhythmia and death involves transient changes in many mediators that are difficult to monitor in large clinical trials. Each mediator along the way sets the stage for the downstream mediators to act, and all have causal roles. The overwhelming evidence that the origins of heart attacks begin in children and progressively accumulate with time (Strong et al., 1999) has not yet mobilized effective preventive interventions.

Prevention of harm comes from decreasing the true causal mediators (especially the most upstream ones) rather than the distal biomarkers indirectly associated with the mediators. Long experience in interpreting roles for membrane lipids urges caution in attributing causal roles to often-discussed markers when seldom-discussed attributes may be true mediators of the event being evaluated. Scientists need to see the details of how things occur if they plan to prevent the occurrences. Unfortunately, stories about associated markers sometimes gain political attention and divert resources away from preventing the true mediators.

Following my retirement from university research in 1991, I continue to speak and publish about the impact of diet choices on human health with uncertain effect on the scientific community. For example, a difficult-to-access proceeding was published (Lands, 1994) after the American Heart Association asked me to give the keynote talk titled ''State of the art: Where are we and where are we going?'' at a scientific conference on ''Omega-3 Fatty Acids in Nutrition, Vascular Biology, and Medicine''. I doubt that many have read it. The main point is that the proportion of n-6 in tissue HUFA is a biomarker of the essential fatty acids eaten, a predictor of the probable intensity of n-6 eicosanoid formation and function and a risk factor for coronary heart disease.

Recent talks on this topic were published in specialized journals addressing topics like nutraceu-

ticals (validation of the empirical equation; Lands, 2003a), lipids (analysis of risk levels and MRFIT outcomes; Lands, 2003b), atherosclerosis (comparing known mediators of disease; Lands, 2003c). All gave some of the evidence for the link between dietary imbalances in n-3 and n-6 fats and disease processes, but they are not easily accessed by everyone. Poorly informed food choices by Americans are creating tissue compositions that cause unintended responses, but the public remains poorly informed of the causal imbalances and the corrections that could be made.

To facilitate use of the empirical diet-tissue relationship in planning more effective clinical studies, the equation was embedded in a convenient interactive spreadsheet and put on an easily accessed distance learning website (http://efaeducation.nih.gov/sig/dietbalance.html). Then, to help identify specific food choices that maintain tissue proportions of n-3 and n-6 HUFA at a personally desired level, the spreadsheet was combined with the USDA nutrient database to let people make interactive personalized menu plans that meet each individual's preferences in taste and level of risk aversion. It is downloadable free from http://efaeducation.nih.gov/sig/kim.html.

Hopefully, easy internet access to such tools will help researchers and clinicians gather more effective evidence of the impact of tissue HUFA on health and design better interventions than they have from the published articles sitting on dusty library shelves. Each year brings new evidence for the merit of Americans altering their current diet by eating more n-3 and less n-6 fats to prevent excessive n-6 eicosanoid formation and function. A recent collaboration links the relative dietary intake of n-6 linoleate (18:2n-6) to the psychiatric status of humans (Hibbeln, Nieminen & Lands, 2004). The future seems certain to hold many new insights into the way that membrane fatty acid diversity affects human health and cardiovascular integrity.

Fifty years of learning how fatty acids come to be placed in membrane lipids and the consequences of those placements taught me to be alert to the environmental supply of essential fatty acids available to our relatively promiscuous enzymes. We have no assurance that our genes code for proteins that will always select the acids that give the best possible outcome overall. As a result, we have good reason to exert well-informed choices of foods to reach the health goals we likely intend for ourselves, our families and our friends.

References

Barber, E.D., Lands, W.E.M. 1973. Quantitative measurement of the effectiveness of unsaturated fatty acids required for growth of *S. cerevisiae*. *J. Bacterial.* **115:**543–551

Beerthuis, R.K., Nugteren, D.H., Pabon, H.I.J., van Dorp, D.A. 1968. Biologically active prostaglandins from some new odd-numbered essential fatty acids. *Red. Trav. Chim. Pays. Bas* **87:**461–475

Bergstroem, S., Danielsson, H., Klenberg, D., Samuelsson, B. 1964. The enzymatic conversion of essential fatty acids into prostaglandins. *J. Biol. Chem.* **239:**PC4006–4008

Black, K.L., Gulp, B.R., Madison, D., Randall, O.S., Lands, W.E.M. 1979. The protective of effects of dietary fish oil on focal cerebral Infarction. *Prostaglandins and Medicine* **3:**257–268

Brandt, A.E., Lands, W.E.M. 1967. The effect of acyl group composition on the rate of acyltransferase catalyzed synthesis of lecithin. *Biochim. Biophys. Acta* **144:**605–612

Cook, H.W., Lands, W.E.M. 1976. A Mechanism for the suppression of cellular biosynthesis of prostaglandins. *Nature* **260:**630–632

Culp, B.R, Lands, W.E.M., Lucchesi, B.R., Pitt, B., Romson, J. 1980. The effect dietary supplementation of fish oil on experimental mlyocardial infarction,. *Prostaglandins* **20:**1021–1031

Culp, B.R, Titus, B.R., Lands, W.E.M. 1979. Inhibition of prostaglandin biosynthesis by eicosapentaenoic acid. *Prostaglandins and Medicine* **3:**269–278

Ellingson, J.S., Hill, E.E., Lands, W.E.M. 1970. The control of fatty acid composition glycerolipids of the endoplasmic reticulum. *Biochim. Biophys. Acta* **196:**176–192

Ellingson, J.S., Lands, W.E.M. 1968. Phospholipid reactivation of plasmalogen metabolism. *Lipids* **3:**111–120

Graff, G., Lands, W.E.M. 1976. A shift from phospholipid to triglyceride synthesis when cell division is inhibited by *trans*-fatty acids. *Chem. Phys. Lipids* **17:**301–314

Graff, G., Sauter, J., Lands, W.E.M. 1983. Selective mutational loss of mitochondrial function can be caused by certain unsaturated fatty acids. *Arch. Biochem. Biophys.* **224:**342–350

Hamberg, M., Svensson, J., Samuelsson, B. 1975. Thromboxanes: a new group of biologically active compounds derived from prostaglandin endoperoxides. *Proc. Natl. Acad. Sci. USA* **72:**2994–2998

Hanel, A.M., Lands, W.E.M. 1982. Modification of antiinflammatory drug effectiveness by ambient lipid peroxides. *Biochem Pharmacol.* **31:**3307–3311

Hemler, M.E, Cook, H.W., Lands, W.E.M. 1979. Prostaglandin Biosynthesis Can Be Triggered by Lipid Peroxides. *Arch. Biochem. Biophys.* **193:**340–345

Hemler, M.E., Graff, G., Lands, W.E.M. 1978. Accellerative autoactivation of prostaglandin biosynthesis by PGG_2. *Biochem. Biophys. Res. Comm.* **85:**1325–1337

Hemler, M.E., Lands, W.E.M. 1980. Evidence for peroxide initiated free radical mechanism of prostaglandin biosynthesis. *J. Biol. Chem.* **255:**6253–6261

Hibbeln, J.R., Nieminen, L.R., Lands, W.E.M. 2004. Increasing homicide rates and linoleic acid consumption among five Western countries, 1961-2000. *Lipids* **39:**1207–1213

Hill, E.E., Lands, W.E.M. 1968. Incorporation of long-chain polyunsaturated acids into phosphatidate and phosphatidylcholine. *Biochim. Biophys. Acta.* **152:**645–648

Hill, E.E., Husbands, D.H., Lands, W.E.M. 1968a. The selective incorporation of ^{14}C-glycerol into different species of phosphatidic acid phosphatidylethanolamine, and phosphatidylcholine. *J. Biol. Chem.* **243:**4440–4451

Hill, E.E., Lands, W.E.M., Slakey, P.M. 1968b. The incorporation of ^{14}C-glycerol into different species of diglycerides and triglycerides in rat liver slices. *Lipids* **3:**411–416

Holub, B.J., Lands, W.E.M. 1975. Quantitative effects of unsaturated fatty acids in microbial mutants. IV. Lipid composition of *S. cervisiae* when growth is limited by unsaturated fatty acid supply. *Can. J. Biochem.* **53:**1262–1277

Husbands, D.H., Lands, W.E.M. 1970. Phosphatidate synthesis by sn-glycerol-3-phosphate acyltransferase in pigeon liver particles. *Biochim. Biophys. Acta* **202:**129–140

Jezyk, P., Lands, W.E.M. 1968. The effect of solvent and temperature upon acyl-CoA: phospholipid acyltransferase specificity. *J. Lipid Res.* **9:**525–531

Kulmacz, R.J., Lands, W.E.M. 1985. Stoichiometry and kinetics of the interaction of prostaglandin H synthase with antiinflammatory agents. *J. Biol. Chem.* **260:**12572–12578

Kulmacz, R.J., Pendleton, R.B., Lands, W.E.M. 1994. Interaction between peroxidase and cyclooxygenase activities in prostaglandin-endoperoxide synthase. *J. Biol. Chem.* **269:**5527–5539

Lands, W.E.M. 1958. Metabolism of glycerolipids: A comparison of lecithin and triglyceride synthesis. *J. Biol. Chem.* **231:**883–888

Lands, W.E.M. 1960. Metabolism of glycerolipids II. The enzymatic acylation of lysolecithin. *J. Biol. Chem.* **235:**2233–2237

Lands, W.E.M. 1965a. Effect of double bond configuration in lecithin synthesis. *J. Am. Oil Chem. Soc.* **42:**465–467

Lands, W.E.M. 1965b. Lipid metabolism. *Ann. Rev. Biochem.* **34:**313–346

Lands, W.E.M. 1979. The biosynthesis and metabolism of prostaglandins. *Ann. Rev. Physiol.* **41:**633–652

Lands, W.E.M. 1980a. Dialog between membranes and their lipid-metabolizing enzymes. *Trans. Biochem. Soc.* **8:**25–27

Lands W.E.M. 1980b. Fluidity of Membrane lipids. *In:* Control of Membrane Fluidity. Kates M., Kuksis A. (eds). Humana Press, Inc., Cliffton, NJ pp. 69–73

Lands, W.E.M. 1981. Actions of antiinflammatory drugs. *Trends Pharmacol. Sci.* **2:** 78–80

Lands, W.E.M. 1985. Mechanisms of action of antiinflammatory drugs. *In*:Adv. Drug Res. 14 (B. Testa, ed.). Academic Press London. pp. 147–164.

Lands, W.E.M. 1991. Biosynthesis of prostaglandins. *Annu. Rev. Nutr.* **11:**41–60

Lands W.E.M. 1993. Eicosanoids and health. *In:* The Third International Conference on Nutrition in Cardio-Cerebrovascular Diseases. K.T. Lee, Y. Oike, T. Kanazawa (eds). The New York Academy of Sciences, New York, NY. pp 46–59

Lands, W.E.M. 1994. State of the art: Where are we and where are we going? *In*: Proceedings from the Scientific Conference on Omega-3 Fatty Acids in Nutrition, Vascular Biology, and Medicine. *Am. Heart Assoc.* pp 1-8

Lands W.E.M. 2003a. Functional foods in primary prevention or nutraceuticals in secondary prevention? *Curr. Top. Nutraceutical Res.* **1**(2):113–120

Lands, W.E.M. 2003b. Diets could prevent many diseases. *Lipids* **18:**317–321

Lands, W.E.M. 2003c. Primary prevention in cardiovascular disease: moving out of the shadows of the truth about death. *Nutr. Metab. Cardiovas. Dis.* **13:**154–164

Lands W.E.M. Davis F.S. 1983. Fluidity of membrane lipids. *In*: M. Kates (eds). Biomembranes NY, Plenum Publ. Corp., NY, pp 475–516.

Lands, W.E.M. Graff, G. 1981. Effects of fatty acid structure on yeast cell physiology. *In*: Proceedings V International Symposium on Yeasts. pp. 331–343 Pergamon Press, Toronto

Lands, W.E.M., Hanel, A.M. 1982. Phenolic anti-cyclooxygenase agents and hypotheses of antiinflammatory therapy. *Prostaglandins* **24:**271–278

Lands, W.E.M., Hart, P. 1964. Metabolism of glycerolipids V. metabolism of phosphatidic acid. *J. Lipid Res.* **5:**81–87

Lands, W.E.M., Hart, P. 1965. Metabolism of glycerolipids VI. Specificities of acyl-coA: phospholipid acyltransferases. *J. Biol. Chem.* **240:**1905–1911

Lands, W.E.M., Hart, P. 1966. The control of fatty acid composition in glycerolipids. *J. Am. Oil Chem. Soc.* **43:**290–295

Lands, W.E.M., Merkl, I. 1963. Metabolism of glycerolipids III. Reactivity of various acyl esters of coenzyme A with α-acylglycerol phosphorylcholine and positional specificities in lecithin synthesis. *J. Biol. Chem.* **238:**898–904

Lands, W.E.M., Samuelsson, B. 1968. Phospholipid precursors of prostaglandin. *Biochim. Biophys. Acta* **164:**426–429

Lands, W.E.M., Blank, M.L., Nutter, L.J., Privett, O.S. 1966a. A comparison of acyltransferase activities *in vitro* with the distribution of fatty acids in lecithins and triglycerides in vivo. *Lipids* **1:**224–229

Lands, W.E.M., Gulp, B.R., Hirai, A., Gorman, R. 1985. Relationship of thromboxane generation to the aggregation of platelets from humans: Effects of eicosapentaenoic acid. *Prostaglandins* **30:**819–825

Lands, W.E.M., Hamazaki, T., Yamazaki, K., Okuyama, H., Sakai, K., Goto, Y., Hubbard, V.S. 1990. Changing dietary patterns. *Am. J. Clinical Nutrition* **51:**991–993

Lands, W.E.M., Inoue, M., Sugiura, Y., Okuyama, H. 1982. Selective incorporation of polyunsaturated fatty acids into phosphatidylcholine by rat liver microsomes. *J. Biol. Chem.* **257:**14968–14972

Lands, W.E.M., Lee, R.E., Smith, W.L. 1971. Factors regulating the biosynthesis of various prostaglandins. *Ann. N.Y. Acad. Sci.* **180:**107–122

Lands, W.E.M., LeTellier, P.R., Rome, L.H., Vanderhoek, J.Y. 1973. Inhibition of prostaglandin biosynthesis. *Adv. in Biosci.* **9:**15–27

Lands, W.E.M., Libelt, B., Morris, A., Kramer, N.C., Prewitt, T.E, Bowen, P., Schmeisser, D., Davidson, M.H., Burns, J.H. 1992. Maintenance of lower proportions of n-6 eicosanoid precursors in phospholipids of human plasma in response to added dietary n-3 fatty acids. *Biochem. Biophys. Acta.* **1180:**147–162

Lands, W.E.M., Morris, A.J., Libelt, B. 1990a. Quantitative effects of dietary polyunsaturated fats on the composition of fatty acids in rat tissues. *Lipids.* **25:**505–516

Lands, W.E.M., Ohlrogge, J.B., Robinson, J.R., Sacks, R.W., Barve, J.A., Gunstone, F.D. 1977. Quantitative effects of unsaturated fatty acids in microbial mutants. VII. Influence of the acetylenic bond location on the effectiveness of acyl chains. *Biochim. Biophys. Acta* **486:**451–461

Lands, W.E.M., Pieringer, R.A., Slakey, P.M., Zschocke, A. 1966b. A micromethod for the stereospecific determination of triglyceride structure. *Lipids* **1:**444–448

Lands, W.E.M., Pitt, B., Gulp, B.R. 1980. Recent concepts on platelet function and dietary lipids in coronary thrombosis vasospasm and angina. *Herz* **5:**34–41

Lands, W.E.M., Sacks, R.W., Sauter, J., Gunstone, F. 1978. Selective effects of fatty acids upon cell growth and metabolic regulation. *Lipids* **13:**878–886

Lin, J., Yang, R., Tarr, P.T., Wu, P.M., Handschin, C., Li, S., Yang, W., Pei, L., Uldry, M., Tontonoz, P., Newgard, C.B., Spiegelman, B.M. 2005. Hyperlipidemic effects of dietary saturated fats mediated through PGC-1beta coactivation of SREBP. *Cell* **120:**261–273

Marshall, P.J., Kulmacz, R.J., Lands, W.E.M. 1987. Constraints on prostaglandin synthesis in tissues. *J. Biol. Chem.* **262:**3510–3517

Merkl, I., Lands, W.E.M. 1963. Metabolism of glycerolipids IV. Synthesis of phosphatidyl ethanolamine. *J. Biol. Chem.* **238:**905–906

Moncada, S., Gryglewski, R., Bunting, S., Vane., J.R. 1976. An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. *Nature* **263:**663–665

Murphy, R.C., Hammarstrom, S., Samuelsson, B. 1979. Leukotriene C: a slow-reacting substance from murine mastocytoma cells. *Proc. Natl. Acad. Sci. USA* **76:**4275–4279

Murphy, R.C., Pickett, W.C., Culp, B.R., Lands, W.E.M. 1981. Tetraene and pentaene leukotrienes: Selective production from murine mastocytoma cells after dietary manipulation. *Prostaglandins* **22:**613–622

Ohlrogge, J.B, Barber, E.D., Lands, W.E.M., Gunstone F. D. Ismail, I.A. 1976. Quantitative effects of unsaturated fatty acids in microbial mutants. VI. Selective growth responses of yeast and bacteria to *cis*-octadecenoate isomers. *Can. J. Biochem.* **4:**736–745

Ohta, A., Mayo, M.C., Kramer, N., Lands, W.E.M. 1990. Rapid analysis of fatty acids in plasma lipids. *Lipids* **25:**742–747

Okuyama, H., Lands, W.E.M. 1972. Variable selectivities of acyl-CoA: monoacylglycerophosphate acyltransferases in rat liver. *J. Biol. Chem.* **247:**1414–1423

Okuyama, H., Eibl, H., Lands, W.E.M. 1971. Acyl-CoA: 2-Acyl-sn-glycerol-3-phosphate acyltransferase activity in rat liver microsomes. *Biochim. Biophys. Acta* **248:**263–273

Okuyama, H., Lands, W.E.M., Christie, W.W., Gunstone, F.D. 1969. Selective transfer of cyclopropane acids by acyl-coenzyme A: Phospholipid acyltransferases. *J. Biol. Chem.* **244:** 6514–6519

Okuyama, H., Lands, W.E.M., Gunstone, F.D., Barve, J.A. 1972. Selective transfers of trans-ethylenic acids by acyl-Coenzyme A: Phospholipid acyltransferases. *Biochemistry* **11:** 4392–4398

Reitz, R.C., El-Shiekh, M., Lands, W.E.M., Ismail, I.A., Gunstone, F.D. 1969. Effects of ethylenic bond position upon acyltransferase activity with isomeric cis-octadecenoyl-Coenzyme A thiol esters. *Biochim. Biophys. Acta* **176:**480–490

Robertson, A.F., Lands, W.E.M. 1962. Positional specificities in phospholipid hydrolyses. *Biochemistry* **1:**804–808

Rome, L.H., Lands, W.E.M. 1975. Structural requirements for time-dependent inhibition of prostaglandin biosynthesis by anti-inflammatory drugs. *Proc. Natl. Acad. Sci. USA* **72:**4863–4865

Slakey, P.M., Lands, W.E.M. 1968. The structure of rat liver triglycerides. *Lipids* **3:**30–36

Smith W.L., Lands W.E.M. 1971. Stimulation and blockade of prostaglandin biosynthesis. *J. Biol. Chem.* **246:**6700–6702

Smith, W.L., Lands, W.E.M. 1972. Oxygenation of polyunsaturated fatty acids during prostaglandin biosynthesis by sheep vesicular gland. *Biochemistry* **11:**3276–3285

Strong, J.P., Malcom, G., McMahan, C.A., Tracy, R.E., Newman, W.P. 3rd, Herderick, E..E., Cornhill, J.F. 1999. Prevalence and extent of atherosclerosis in adolescents and young adults: implications for prevention from the Pathobiological Determinants of Atherosclerosis in Youth Study. *JAMA* **281:**727–735

Tamai, Y., Lands, W.E.M., Barve, J.A., Gunstone, F.D. 1973. Selective transfers of acetylenic acids to form lecithins. *Biochim. Biophys. Acta* **296:**563–571

Tsao, Y.K., Lands, W.E.M. 1979. Cell growth with trans-fatty acids is affected by cyclic AMP levels as well as by membrane fluidity. *Science*. **207:**777–779

Vandenhoff, G., Gunstone, F.D., Barve, J., Lands, W.E.M. 1975. Inhibition of growth of microbial mutants by trans-octadecenoates. *J. Biol. Chem.* **250:**8720–8727

Vanderhoek, J., Lands, W.E.M. 1973. Acetylenic inhibitors of sheep vesicular gland oxygenase. *Biochim. Biophys. Acta* **296:**374–381

Vane, J.R. 1971. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nat. New Biol.* **231:**232–235

Waku, K., Lands, W.E.M. 1968a. Control of lecithin biosynthesis in erythrocyte membranes. *J. Lipid Res.* **9:**12–18

Waku, K., Lands, W.E.M. 1968b. Acyl-Coenzyme A: 1-Alkenylglycero-3-phosphorylcholine acyltransferase action in plasmalogen biosynthesis. *J. Biol. Chem.* **243:**2654–2659

Walenga, R.W., Lands, W.E.M. 1975a. Effectiveness of various unsaturated fatty acids in supporting growth and respiration in Saccharomyces cerevisiae. *J. Biol. Chem.* **250:**9121–9129

Walenga, R.W., Lands, W.E.M. 1975b. Requirements for unsaturated fatty acids for the induction of respiration in Saccharomyces cerevisiae. *J. Biol. Chem.* **250:**9130–9136

Whittaker, N., Bunting, S., Salmon, J., Moncada, S., Vane, J.R., Johnson, R.A., Morton, D.R., Kinner, J.H., Gorman, R.R., McGuire, J.C., Sun, F.F. 1976. The chemical structure of prostaglandin X (prostacyclin). *Prostaglandins*. **12:**915–928