How is tissue fatty acid composition maintained?

Seminar to the Polyunsaturated Lipid Function Special Interest Group
Wednesday February 12, 2003

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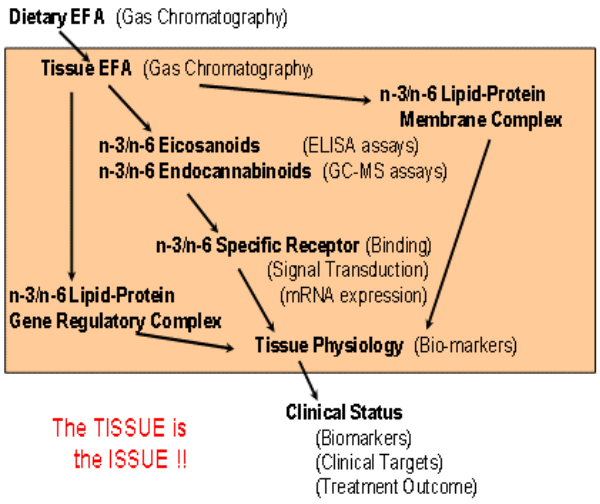
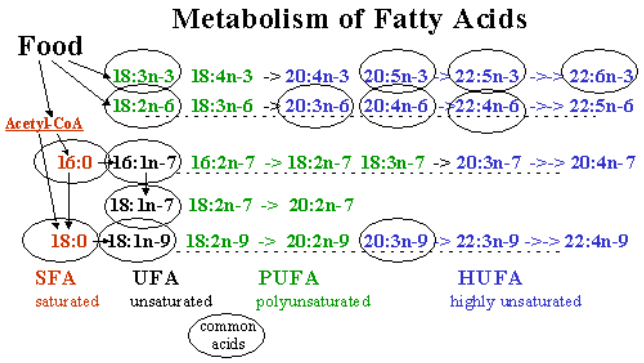
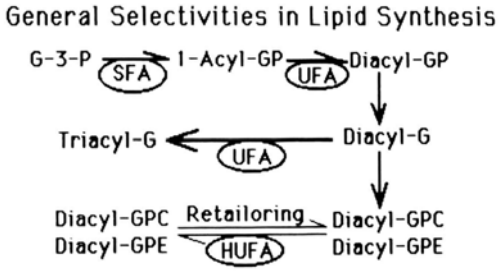
Essential fatty acids (EFA) are vitamin-like nutrients that cannot be formed from other foods and are needed by vertebrates for full, healthy life. The EFA form three different types of lipid-protein complexes that act through signaling pathways and influence tissue physiology and overall clinical status. This talk focuses on the process and consequence of maintaining certain proportions of EFA in tissues with regard to eicosanoid-mediated physiology and microbial growth and death.



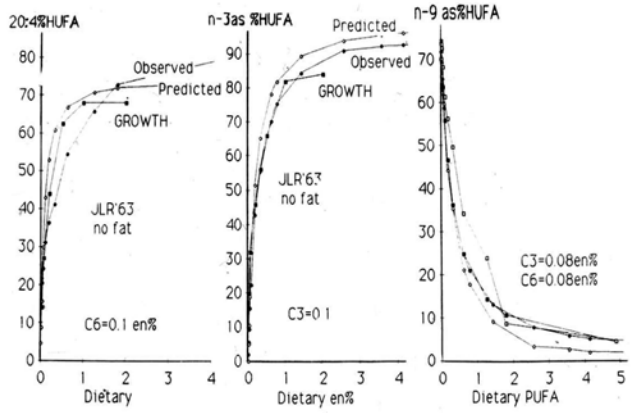


The metabolic selectivities by which enzymes common to all living tissues maintain typical fatty acid compositions in glycerolipids support the naming of four general types of natural acyl chains: saturated fatty acids (SFA); unsaturated fatty acids (UFA); polyunsaturated fatty acids (PUFA); and long-chain highly unsaturated fatty acids (HUFA).

The saturated acid, palmitate, is the first to form an ester in making new lipids. The resulting 1-acyl-glycerol phosphate is then acylated by different enzyme(s) (that use either 18-carbon UFA or PUFA fairly indiscriminantly) and then hydrolyzed to diacyl glycerols (DG) by a phosphatase. The DG units are readily converted to phospholipids, typically with 50% esters as SFA and 50% as UFA. Vertebrates have a retailoring process with choline and ethanolamine lipids that favors entry of HUFA at the 2-position of the phospholipids, and reversal of the DG phosphotransferase reaction gives DG with HUFA at the 2-position. The enzyme selecting an acyl-CoA ester to convert DG to triacyl glycerol seems quite promiscuous in using almost any available UFA, PUFA, or HUFA. The



result is fat typically with 33% SFA and 66%UFA. The overall result creates impressions of a vague selectivity of Nature with relative indifference to any fine details in acyl chain structure, certainly with no evidence for much discrimination between n-3 and n-6 EFA in forming and cleaving ester bonds at the 2-position. Entry of dietary 18:2n-6 and 18:3n-3 into the "flexible" pools of tissue TG tends to be in simple linear relationship to dietary abundance, whereas their entry into phospholipid HUFA seems definitly preferred with a high retention during low supplies of precursors and "saturable" limits above which dietary supply has little influence.

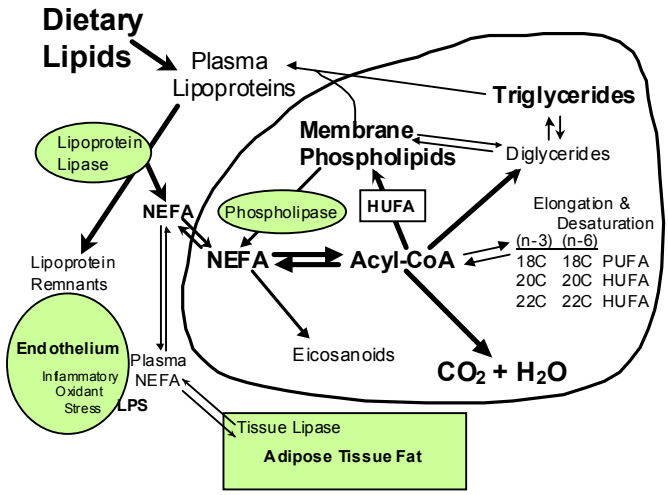


PUFA in Serum Lipids

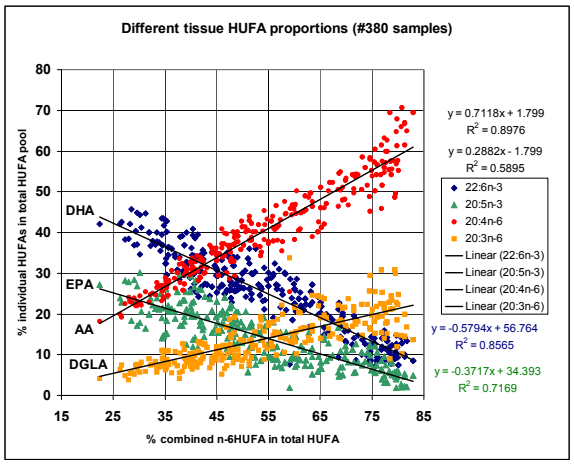
Dietary 18:2n-6 (% cal)	Serum Lipids 18:2 (mol %)	20:4 (mol %)
1.6	6.7	15.6
4.0	11.6	17.5
7.7	23.7	16.3
13.6	29.3	17.4

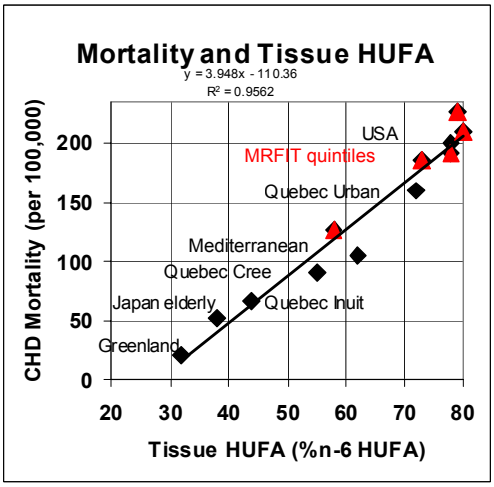
Unresolved is the issue of whether the well-known competitive hyperbolic relationship first shown by Mohrhauer & Holman (1963) is due to constraints in the elongation-desaturation systems or of the retailoring system. I tend to believe both act, with the former perhaps "stronger" than the latter.

This brief overview indicates that general selectivities occur with acyl-CoA esters during lipid formation, but fine details of acyl chain structure (such as n-3 and n-6 arrays) seem unrecognized by the enzyme proteins that maintain the various fatty acids in tissue lipids. As a result tissue esters and resultant physiology are influenced greatly by dietary supplies. In contrast, selective acyl chain interactions seem evident for n-3 and n-6 HUFA reacting with cycloxygenases in forming prostanoids (but not much with lipoxygenases in forming leukotrienes), and for some eicosanoids signaling through certain receptors. The resulting impact of n-3 and n-6 eicosanoids on health is likely a relatively unregulated consequence of uninformed food choices from which there may be no natural remedy other than knowledge of the consequence. Indiscriminate accumulation of whatever fatty acid is supplied in the environment gives flexible tissue compositions, but may not sustain life. Does a living cell have any way of knowing whether the supplied NEFA nutrient will succeed or fail? Can a cell "recognize" and "choose" the "right stuff" for survival, or does it just use any available acyl-CoA esters and live or die as a result? [Lands (1980) Dialog Between Membranes and their Lipid-Metabolizing Enzymes. Trans. Biochem. Soc. 8, 25-27.]

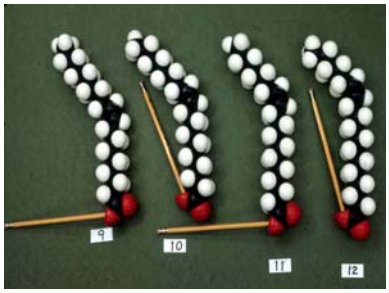


Results from various populations show that tissue phospholipid HUFA may range from 15% to 90% n-6 HUFA, depending on the voluntary food choices of normal humans. The relative non-selectivity of the metabolic enzymes allows n-3 and n-6 competition with abundance of supply influencing the levels maintained in tissues as shown at the left, below. The consequence of having different proportions of n-6 HUFA in he HUFA pool is shown at the right, below. There seems to be no "corrective" metabolic response to prevent fatal tissue combinations from being developed. As much as humans might wish for some protective re-adjustment of the metabolic promiscuity, the enzymes seem to continue assembling harmful and harmless combinations in response to supplies ingested - - - without much regard to or feedback from the consequences.





Believing that the acyltransferase activity esterifying the 1-position of phospholipids preferred SFA, we studied various CoA esters and found that many "fluid" UFA 8-, 10-, and 12- cis-18:1 react well - - but NOT the naturally occurring 9- and 11-cis-18:1. The sharp discrimination between adjacent isomers was unexpected and unexplained. Clearly, the protein was "recognizing" favorably some structural aspect that is not simply described by the term "SFA".



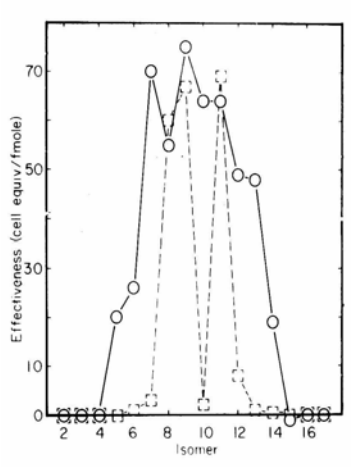
Although much flexibility occurs around the cis-, trans-, yne-, and cyclopropyl group inserted in the acyl chain, rat liver acyltransferases interacted with the acyl-CoA esters with high specifity not recognized earlier using common natural acyl-CoA esters. The cis- and cyclopropyl- chains were treated nearly identically, whereas a striking one-carbon shift in selectivity occurred with the acetylenic (yne-) acids.

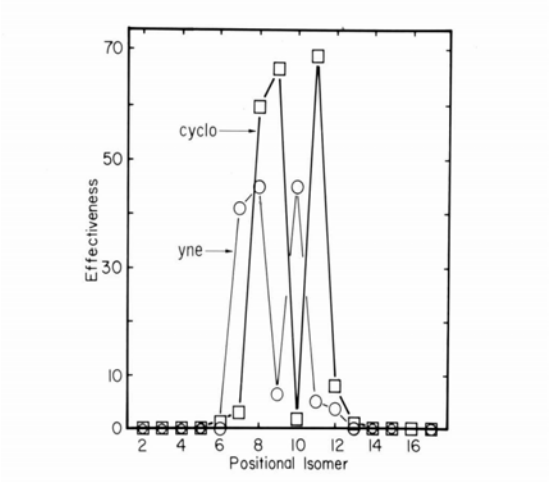
Rat liver acyltransferase selectivities with acyl-CoA esters:

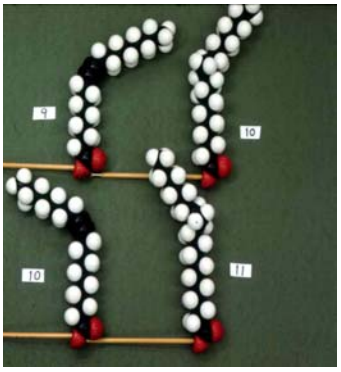
Reitz et al. (1969) cis-Octadecenoyl-CoA. Biochim. Biophys. Acta 176, 480-490.

Okuyama et al (1969) Cyclopropane Acyl-CoA. J. Biol. Chem. 244, 6514-6519.
Okuyama et al. (1972) trans-Ethylenic Acyl-CoA. Biochemistry 11, 4392-4398.
Tamai et al. (1973) Acetylenic Acyl-CoA. Biochim. Biophys. Acta 296, 563-571.

To see if such selective lipid-protein interactions might affect cell life, we fed the various isomeric acids to auxotrophic bacteria for whom UFA was essential for growth and survival. Surprisingly, some protein in the E.coli mutant responded to isomeric acyl chains in a highly specific way, making cell growth or death become a consequence of details in acyl chain structure when cells grew with glucose as energy source. Again, the one-carbon shift seen in molecular models informed us that very detailed features of the acyl chain can be "recognized" by some proteins in E.coli. However, when cells were grown in glycerol (or with supplemental cyclic AMP), the fine details disappeared, and cell yields were in proportion to the estimated biophysical contribution of the acyl chain to membrane "fluidity", with substituents in the center of the chain giving greater cells/femtomole of needed nutrient acyl chain.







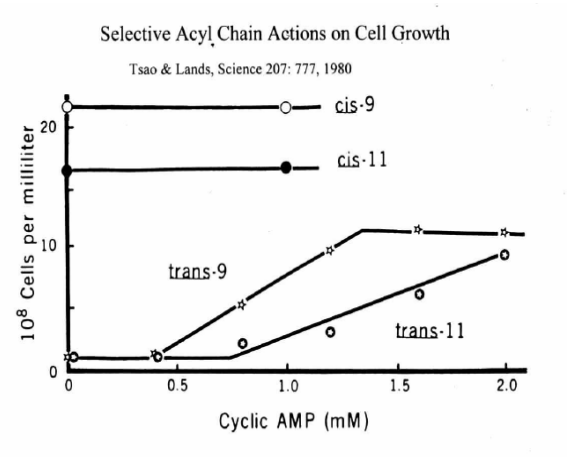
Microbial membrane "fluidity" and DNA replication kinetics:

Barber, E.D., Lands, W.E.M. (1973) Quantitative Growth of S. cerevisiae. J. Bacteriol. 115, 543-551.
Holub, B.J., and Lands, W.E.M. (1975) Quantitative Effects. Can. J. Biochem. 53, 1262-1277.
Ohlrogge et al. (1976) cis-Octadecenoate Isomers. Can. J. Biochem. 54, 736-745.
Lands et al. (1977) Acetylenic Bond Location. Biochim. Biophys. Acta 486, 451-461.
Lands et al. (1978) Cyclopropyl Isomers. Lipids 13, 878-886.
Tsao, Y.K. and Lands, W.E.M. (1979) *trans*-fatty acids, Cyclic AMP and Fluidity. Science, 207, 777-779.
Graff et al. (1983) Loss of Mitochondria, Arch. Biochem. Biophys. 224, 342-350.

Separate estimates of acyl chain "fluidity" in membranes and "functionality" for growth suggested possible kinetic features might keep certain acyl chains from realizing their full capability for supporting cell growth and life. The discriminating protein has never been recognized with new molecular biology methods. It awaits discovery! With regard to teleological stories about what cells "try to do" when they form membrane lipids, the results show many instances in which cells fail to reach optimal growth as the culture dies with unused essential nutrient in the medium. I think it unlikely that preferences for acyl chains coded into protein structure can be readily changed as cells move into crisis conditions. The cells live or die on the basis of the specific essential nutrient acid in their immediate environment - - some acids more helpful than others. We know few strategies for E.coli to "choose" different acids as membranes fail and death nears, and we seem to have few strategies for humans to intelligently "choose" different nutrient acids as trouble nears. Information processing by enzymes and their genes is more limited than information

processed by alert humans. There may be hope in helping humans understand and alter unhealthy proportions of fatty acids in their food environment in ways not available to E.coli.

The last study that my lab reported using the functionality algorithm developed with Bruce Holub was a study of inadequate membrane function with *trans*-isomers. The known biophysical contribution to "fluidity" was significant (about one-half of the *cis*-isomer), but it was not evident in typical growth environments. *Cis*-isomers were fully competent in both supplemented and unsupplemented media, but trans-isomers clearly had impairments beyond simple "fluidity" considerations. Much remains to be done to learn what proteins can discriminate fine details of acyl chain structure when so many proteins seem quite unable to discriminate those details upon which life and death depend.



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Lands, W.E.M. (1965) 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. (1991) Biosynthesis of Prostaglandins. Annual Review of Nutrition 11, 41-60.