

# This Week's Citation Classic

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**Holman R T.** The ratio of trienoic:tetraenoic acids in tissue lipids as a measure of essential fatty acid requirement. *J. Nutrition* 70:405-10, 1960. [Dept. Physiological Chemistry, Univ. Minnesota, Minneapolis, and Hormel Inst., Austin, MN]

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**Increasing dietary linoleate causes progressive changes in polyunsaturated acids of tissue lipids in rats. A trienoic to tetraenoic acid ratio of 0.4 or less indicates that the minimum nutrient requirement for linoleate, 1 percent of dietary calories, has been met. [The SC<sup>®</sup> indicates that this paper has been cited over 230 times since 1961.]**

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"At the 1958 meeting of the American Medical Association, Zoe Anderson and I had a lengthy discussion about the essential fatty acid (EFA) content of butterfat. I asserted the opinion that its content of EFA would not meet the EFA requirement, and she objected that I could not make such statements without data! Immediately upon my return to the Hormel Institute, I made a nutritional comparison of butterfat and a linoleate-rich oil to get that data. The question was answered, but the experiment also began a new approach to study EFA requirement. The experiment included seven groups of rats fed three levels of dietary fat and three kinds of dietary fat. Interpretation of the data from such diverse treatments was extremely difficult until I arranged the groups according to dietary linoleate, irrespective of kind or amount of dietary fat. The tetraenoic acid content of tissue lipids (arachidonic acid) increased, and the trienoic acid decreased as the dietary linoleate was

increased. To express both effects in one term, the 'trienoic/tetraenoic' acid ratio was calculated. When the ratios for these tissues were plotted, all points fell on the same hyperbolic curve in which an abrupt change in slope occurred at 1 percent of linoleate calories, indicating the minimum requirement for linoleate. Butterfat provided this amount!

"This experiment was the first biochemical *response to dose level* of EFA. The requirement was set from objective quantifiable biochemical changes rather than subjective and complex effects on skin and growth. Dose-response studies with single pure EFA, combinations of these, or complex mixtures of fats have verified the validity of the initial results. Gas chromatography with higher resolution and greater sensitivity described the phenomena in greater detail than was possible with alkaline isomerization used in the first experiment. Through dose-response studies, we have set the quantitative EFA requirement for EFA in animals and humans, provided an estimate of EFA status, and developed a diagnosis for EFA deficiency. Recently, the upper limit of normal ratio for human serum phospholipids was revised to 0.2.<sup>1</sup> The 'triene/tetraene' ratio has proven to be quite applicable to nutritional problems in both animals and humans. Abnormalities of EFA metabolism detectable by this ratio are being found frequently in diseases in man.

"Many colleagues have contributed to our program on EFA, but two have made special efforts related to the dose-response approach. W.O. Caster's mathematical approaches have been especially helpful, and Hans Mohrhauer has had major responsibility for studies with purified EFA. I am also especially grateful to Zoe Anderson for provoking me sufficiently to do the critical experiments."

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1. Holman R T, Smythe L & Johnson S. Effect of sex and age on fatty acid composition of human serum lipids. *Amer. J. Clin. Nutr.* 32:2390-9, 1979.