

## **Omega-6 Fatty Acids and Cardiovascular Disease: Friend or Foe?**

**Running title:** *Harris et al.; Omega-6 and CVD*

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The once-settled roles of all dietary fatty acid classes vis-à-vis coronary heart disease (CHD) seem to be under fire these days. For decades it had been received wisdom that “saturated fats are bad,” and that margarines should replace butter to reduce risk for heart attacks. But a recent *Time* magazine cover that screamed, “EAT BUTTER“ illustrates this changing perspective<sup>1</sup>. Olive oil, the poster child of the “Mediterranean Diet” and a rich source of oleic acid, has long been nearly worshiped as cardioprotective, but recent meta-analyses<sup>2</sup> and animal feeding studies<sup>3</sup> are challenging this view. Similarly, the marine-derived omega-3 fatty acids, which have historically found a place among the “healthiest” of all dietary fats have fallen on hard times based on the null findings in several recent randomized trials<sup>4</sup>, and now linoleic acid (LA), the principal vegetable-oil derived omega-6 fatty acid – once taken as a medicine by the tablespoon to lower cholesterol - is now being accused of causing, not preventing heart disease<sup>5</sup>. The only class that seems to be holding its own is the industrially-produced *trans* fats which, although clearly promoting CHD, are also slowly disappearing from the American diet<sup>6</sup>. Understandably, the American public is becoming jaded when it comes to official proclamations of what constitutes a “healthy fat.”

In this context, careful examination of the dietary patterns of large numbers of individuals followed for many years as they relate to CVD can help bring perspective. Such is the contribution of Farvid et al. in this issue of *Circulation*<sup>7</sup>. These investigators performed the largest systematic review and meta-analysis to date examining the relations between omega-6 fatty acid (essentially LA) intake and CHD morbidity and mortality. Utilizing data from both published and unpublished studies (via direct investigator contact), Farvid et al. included 12 cohort studies involving about 290,000 individuals among whom occurred nearly 11,000 CHD events and about 4500 CHD deaths. Intakes of LA were estimated by a variety of dietary

questionnaires, and follow-up ranged from 5 to 30 years. Comparing the highest to the lowest intake groups, risk for CHD events was lower by 14% and for CHD death by 17%, both statistically significant. The effects on total mortality would have been of interest as well, but such data were not available. The authors note that several studies have sought effects of dietary LA on cancer outcomes but have found none.

The fact that these relations were observed using such blunt instruments as dietary questionnaires suggests the findings are robust. The observation that replacing either saturated fats or carbohydrates with vegetable oils produced essentially the same CHD benefit suggests that it is not the nutrient being replaced by LA that affords the benefit but the LA itself. Finally, since the LA effect was independent of the intake of alpha-linolenic acid (ALA, the plant omega-3 fatty acid found primarily in soybean oil, which provides about 6% of total fatty acids as ALA and 54% as LA), the benefit observed cannot be attributed to consumption of the ALA alone as some have hypothesized<sup>5</sup>.

These findings contrast with of a recent paper by Chowdhury et al.<sup>2</sup> who reported, based on the results of 8 prospective cohort studies, that there was no association [hazard ratio (HR) of 0.98, 95% confidence interval (CI) 0.94 to 1.02] between omega-6 fatty acid intake (not LA, per se) and “coronary disease” (defined as fatal or nonfatal myocardial infarction, coronary heart disease, coronary insufficiency, coronary death, angina, and/or angiographic coronary stenosis). Some of the possible reasons for this discrepancy are discussed in Farvid et al.

#### *Omega-6 are Proinflammatory?*

As noted earlier, some investigators have proposed that LA intakes in America are excessive<sup>8</sup>, and far from reducing risk for inflammatory diseases like CHD, may actually be increasing risk<sup>5</sup>. This perspective builds upon the following logic: since 1) LA is a precursor for arachidonic

acid (AA), 2) AA is the substrate for the production of certain pro-inflammatory eicosanoids, and 3) CHD is a disease with major inflammatory components, then 4) higher intakes of LA may increase risk for CHD. Although not intrinsically illogical, this perspective fails to consider several inconvenient facts. First, as noted by Farvid et al. wide variations in LA intake do not materially affect circulating or cellular AA levels. Rett and Whelan performed a systematic review of 36 studies in which dietary LA was either reduced by up to 90% or increased by as much as 6-fold<sup>9</sup>. In neither case were plasma phospholipid levels of AA altered. The authors concluded, “Our results do not support the concept that modifying current intakes of dietary linoleic acid has an effect on changing levels of arachidonic acid in plasma, serum or erythrocytes in adults consuming Western-type diets.”

Second, studies testing the effect of LA on inflammatory status in humans have routinely found nothing. Johnson and Fritsche<sup>10</sup> reviewed 15 trials meeting their inclusion criteria and concluded that “virtually no evidence is available from randomized, controlled intervention studies among healthy, non-infant human beings to show that addition of LA to the diet increases the concentration of inflammatory markers.” Indeed, when Asp, et al. gave 35 postmenopausal women with type 2 diabetes mellitus additional safflower oil for 16 weeks, they observed significant *decreases* in CRP and HbA1c and increases in HDL-cholesterol<sup>11</sup>. The 8 g of oil per day increased their LA intake from 6.8% to 9.8% energy and raised plasma phospholipid levels by 2-4 percentage points

Another concern with increasing LA intake is that it will lower blood levels of the long chain omega-3 fatty acids EPA and DHA<sup>12</sup>, presumably by slowing conversion of ALA to EPA, and/or by competing with these two fatty acids for esterification sites in membrane phospholipids. To the extent that EPA/DHA are cardioprotective, such an effect would be

counterproductive. However again, there is a gap between theory and fact. For example, Liou et al.<sup>13</sup> increased the LA intake from 3.8% to 10.5% of total energy in the diets of 22 men for 4 weeks. The high LA diet did lower plasma phospholipid EPA levels slightly (from 1% to 0.6%), but it raised DHA levels by the same amount (from 3% to 3.4%), leaving the sum of EPA+DHA (4%) unaffected. They also observed no effects on inflammatory markers or platelet aggregation with the high LA diet.

When Chowdhury, et al.<sup>2</sup> examined the relations between *circulating* fatty acids (i.e., biomarkers) and CHD, the omega-6 story became more complicated. There was no association between LA levels and disease, but contra the “omega-6-are-inflammatory” hypothesis, in 10 studies including some 23,000 individuals with over 3700 CHD events, higher levels of circulating AA – the presumed toxic mediator - were associated with *lower* risk for CHD events (HR, 0.83, 95% CI, 0.74 to 0.92).

#### *Linoleic Acid Metabolites*

As noted, the “LA is harmful” hypothesis depends heavily on the view that an LA metabolite, AA, is converted to potent pro-inflammatory signaling molecules. But AA is not the only LA metabolite with potential effects on CHD – LA itself can be converted to a wide variety of bioactive molecules. For example, nitrated LA (LNO<sub>2</sub>) has been shown to have cardioprotective effects<sup>14</sup>. It can reduce cardiac ischemic injury by facilitating mitochondrial uncoupling, and it has been reported to inhibit platelet and neutrophil function, inhibit LPS-induced cytokine release from monocytes, improve insulin sensitivity, and relax pre-constricted aortic rings<sup>14</sup>. In addition, LNO<sub>2</sub> is a powerful ligand for PPAR- $\gamma$ <sup>15</sup>, a nuclear transcription factor that controls cell differentiation as well as production of metabolic and anti-inflammatory signaling molecules. At physiologically-relevant levels LNO<sub>2</sub> rivals the effects of the thiazolidinediones on PPAR- $\gamma$ <sup>15</sup>.

LNO<sub>2</sub> (as well as nitrated AA) can be esterified in cell membrane (and lipoprotein) phospholipids and cholesteryl esters. Hence the possibility of targeted delivery of these signaling molecules via lipoprotein receptors exists. Beyond that, LA can also be converted to a growing number of oxygenated metabolites (i.e., oxylipins) by cyclo-oxygenase, lipoxygenases, and/or cytochrome P-450 epoxygenases (**Figure 1**). As just one example of the potential physiological effects of LA oxylipins, the anti-hypertensive effects of LA may be mediated, at least in part, by LA diols and triols which may inhibit tubular sodium reabsorption and thereby facilitate sodium excretion in salt-sensitive individuals<sup>16</sup>. LA can also be metabolized to DGLA (from which other bioactive lipids can be produced), and further to AA, which is the well-known precursor prostaglandin E<sub>2</sub>, thromboxane A<sub>2</sub>, and leukotriene B<sub>4</sub>, all “pro-inflammatory” mediators. But if one takes off the blinders and examines the entire AA metabolome, one finds a constellation of metabolites including a variety of prostaglandins, leukotrienes, ligands for endocannabinoid receptors, lipoxins, isoprostanes, nitrated AA, and epoxides, among others (**Figure 1**). Some are “proinflammatory” but some are “anti-inflammatory” or promote the resolution of inflammatory insults. Often these effects have only been observed in certain cell/tissue types and under potentially non-physiological conditions, and their effects in normal physiology or those of other metabolites remain to be discovered. The net impact on human metabolism (and CHD risk) of this multitude of products will ultimately be determined by their interaction among themselves (and with their omega-3 fatty acid analogs), and is virtually impossible to predict. Hence, to label the entire class of omega-6 fatty acid metabolites as “proinflammatory” is painfully naïve.

### *Randomized Trials*

Of course, the most direct way to test the hypothesis that higher LA intakes reduce risk for CHD is to perform a randomized controlled trial (RCT). This has been attempted many times, and nearly as

many meta-analyses have been employed to summarize their findings. Depending on which trials one includes, there is either a significant reduction in risk<sup>17</sup>, no effect<sup>2</sup>, or a trend towards increased risk<sup>5</sup> associated with higher omega-6 intakes. Space does not allow a consideration of the pros and cons of each approach. Suffice it to say that large-scale, multi-year intervention trials in which one major dietary component is (must be) substituted for another are both difficult to conduct and to interpret owing to the multiple variables involved. Hence, it has been argued that prospective cohort data should be given the same evidentiary weight as RCTs in nutrition because each has relevant strengths and weaknesses<sup>18</sup>. The report from Farvid et al.<sup>7</sup> makes an important contribution in this regard. Importantly, their data (shown in Figure 5) continue to support the recommendation of many health authorities for 5-10% of energy as LA<sup>19,20</sup>.

**Conflict of Interest Disclosures:** WSH is the President of OmegaQuant Analytics, LLC and a Senior Research Scientist at Health Diagnostic Laboratory, Inc. Both of these laboratories offer fatty testing, the former for researchers and consumers, and the latter for clinicians. He is also a consultant for Omthera Pharmaceuticals, Aker Biomarine Antarctica, and Tersus Pharmaceuticals. GCS has received support from the California Walnut Commission for investigator-initiated research.

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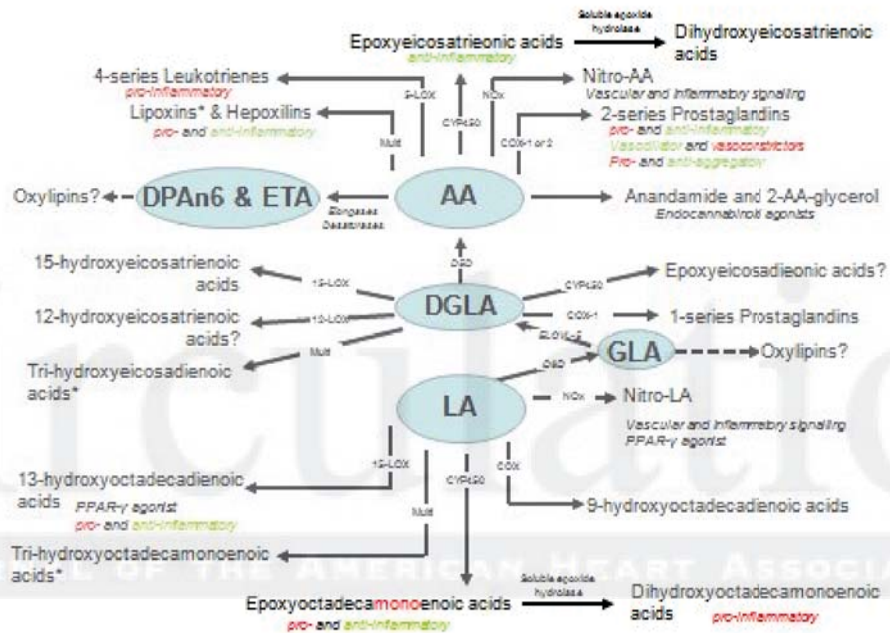
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### Figure Legend:

**Figure 1.** An overview of metabolites known (or likely) to be derived from linoleic acid.

Abbreviations: AA, arachidonic acid; COX, cyclo-oxygenase; CYP, cytochrome; D5(6)D, delta-5(6) desaturase; DGLA, dihomo-gamma-linolenic acid; DPAn-6, docosapentaenoic acid (22:5 n-6); DTA, docosatetraenoic acid (22:4 n-6); ELOVL, elongase; ETA, eicosatrienoic acid (20:3 n-6); LA, linoleic acid; LOX, lipoxygenase; Multi, multiple enzymes involved; PPAR, peroxisome proliferation-activated receptors. \*As triols, these are analogous to the resolvins and protectins derived from the long-chain omega-3 fatty acids.



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