

# openheart Omega-6 vegetable oils as a driver of coronary heart disease: the oxidized linoleic acid hypothesis

James J DiNicolantonio, James H O'Keefe

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The intake of omega-6 vegetable oils, particularly soybean oil, began to increase in the USA starting in the early 1900s at a time when the consumption of butter and lard was on the decline.<sup>1</sup> This caused a more than two-fold increase in the intake of linoleic acid, the main omega-6 polyunsaturated fat found in vegetable oils, which now makes up around 8% to 10% of total energy intake in the Western world. The omega-6 fat linoleic acid should not be confused with conjugated linoleic acid found in pastured animal foods.

A systematic review of studies measuring the changes in linoleic acid concentration in subcutaneous adipose tissue in the USA revealed an approximate 2.5-fold increase in linoleic acid increasing from 9.1% to 21.5% from 1959 to 2008.<sup>2</sup> Importantly, the concentration of linoleic acid in adipose tissue is a reliable marker of intake as the half-life of linoleic acid is approximately 2 years in adipose tissue. The authors of the study also noted that the increase in adipose tissue linoleic paralleled the increase in the prevalence of diabetes, obesity and asthma.<sup>2</sup>

The amount of linoleic acid in adipose tissue, but also in platelets, is additionally positively associated with coronary artery disease (CAD), whereas long-chain omega-3 (eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) levels in platelets are inversely related to CAD.<sup>3</sup> This provides rather compelling evidence that omega-3s protect whereas omega-6 linoleic acid promotes heart disease. Importantly, the increased consumption of omega-6 polyunsaturated fat linoleic acid can reduce omega-3 in the body as it competes with the alpha-linolenic acid for metabolism to longer chain polyunsaturated fats. It has been known for decades that linoleic acid, as a *percentage* of total fatty acids in lipids, is reduced in patients with CAD, and this has been used as an argument to suggest that low intakes of linoleic acid may cause heart disease.<sup>4</sup> However,

total fatty acid concentrations, as opposed to percentages, are independent of changes in other fatty acids and hence are more reliable markers of linoleic acid intake (although likely less reliable compared with adipose tissue). Importantly, linoleic acid concentrations in both serum cholesteryl esters and phospholipid fatty acids are in fact higher in patients with CAD compared with those without CAD coronary artery disease.<sup>4</sup> Again, since linoleic acid cannot be synthesised in the body, this suggests that patients who have heart disease consume more omega-6 linoleic acid than those without heart disease. Indeed, the authors of the study concluded, "(...) cholesteryl linoleate is widely believed to decrease in patients with CAD. Such decreases, however, represent decreases only in relative terms. We have shown in this study that linoleate actually is present in a higher concentration in individuals with CAD than in those without CAD".<sup>4</sup>

The low-density lipoprotein (LDL) oxidation hypothesis gained traction during the 1980s because it was noted that in general, native unoxidised LDL does not cause foam cell formation. In other words, LDL had to become oxidised first in order for atherosclerosis to develop. Indeed, it was later discovered that oxidised LDL (oxLDL) caused direct toxic effects to the cell, recruitment and entry of monocytes into the subendothelial layer and increased foam cell formation<sup>5</sup> leading to increased atherosclerosis and inflammation.<sup>6</sup> Moreover, oxLDL was found to be higher in patients with CAD compared with normal patients and oxLDL was able to better identify patients at an elevated risk of heart disease.<sup>7-9</sup> Moreover, OxLDL and auto-antibodies to oxLDL are found in atherosclerotic lesions.<sup>10-12</sup> Furthermore, patients with progressive carotid atherosclerosis have more antibodies to oxLDL versus those without progression.<sup>9</sup> Thus, the evidence is resounding that oxLDL is important in



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Department of Preventive Cardiology, Saint Luke's Mid America Heart Institute, Kansas City, Missouri, USA

**Correspondence to**  
James J DiNicolantonio;  
jjdinicol@gmail.com

the formation of atherosclerosis. A caveat to the oxLDL hypothesis of heart disease is that oxLDL can increase on atherosclerotic plaque regression and hence an increase in oxLDL does not always suggest an increased cardiovascular risk.<sup>6</sup>

However, the oxLDL hypothesis of coronary heart disease does not get at the root cause, that is, what causes LDL to become oxidised in the first place? It was later discovered that the oxidation of LDL was initiated by the oxidation of linoleic acid contained within the LDL particles.<sup>13</sup> Indeed, linoleic acid is the most common oxidised fatty acid in LDL.<sup>14</sup> Once linoleic acid becomes oxidised in LDL, aldehydes and ketones covalently bind apoB, creating LDL that is no longer recognised by the LDL receptors in the liver but is now recognised by scavenger receptors on macrophages leading to the classic foam cell formation and atherosclerosis.<sup>13 15 16</sup> Hence, the amount of linoleic acid contained in LDL can be seen as the true 'culprit' that initiates the process of oxLDL formation as it is the linoleic acid that is highly susceptible to oxidation. Additionally, an increase in the intake of linoleic acid increases the linoleic acid content of very-low-density lipoprotein (VLDL) and high-density lipoprotein (HDL) increasing their susceptibility to oxidise, which further increases the risk of cardiovascular disease.<sup>17-19</sup> Thus, expanding on the oxLDL theory of heart disease, a more comprehensive theory, the 'oxidised linoleic acid theory of coronary heart disease', is as follows: dietary linoleic acid, especially when consumed from refined omega-6 vegetable oils, gets incorporated into all blood lipoproteins (such as LDL, VLDL and HDL) increasing the susceptibility of all lipoproteins to oxidise and hence increases cardiovascular risk.<sup>20</sup>

However, oxidised cholesterol was also considered a culprit as it was contained in atherosclerotic plaque, which led to the demonisation of dietary cholesterol as a cause of coronary heart disease (CHD). However, cholesterol bound to saturated fat does not readily oxidise; this is not the case with linoleic acid.<sup>21</sup> Moreover, lipids from human atherosclerotic plaques have been found to contain oxidised cholesteryl linoleate (cholesterol esters containing linoleic acid).<sup>21-24</sup> Moreover, the severity of atherosclerosis is noted to increase with increasing oxidised cholesteryl linoleate.<sup>21 25</sup> In other words, cholesterol was protected from oxidation if bound to saturated fat but susceptible to oxidation when bound to linoleic acid. Again, this suggests is that eating more linoleic acid increases the oxidation of cholesterol within LDL particles further increasing atherosclerosis formation and the risk of coronary heart disease. Indeed, healthier regions of aortas have been found to have less oxidised cholesteryl linoleate (5.8%–9.5%) compared with atherosclerotic regions (12.4%–21%).<sup>21</sup>

The most prevalent fatty acid contained in LDL is linoleic acid.<sup>14</sup> On LDL oxidation, linoleic acid is converted to hydroperoxides, which can then be converted to hydroxy acids, such as 9-HODE (9-hydroxy-10,12-octadecadienoic acid). 9-HODE is extremely prevalent in oxidised LDL

and is a good indicator of lipid peroxidation. In fact, 9-HODE is 20 times higher in young patients with atherosclerosis compared with healthy volunteers and 30-fold to 100-fold greater in patients with atherosclerosis aged 69 to 94 compared with young healthy individuals.<sup>14</sup> 9-HODE levels may be a novel way to determine someone's cardiovascular risk and further studies should be performed to test if 9-HODE could be a good risk factor for coronary heart disease, particularly in those over the age of 50.

In 1952, Glavind and colleagues published a paper showing that aortic lipid peroxides positively correlated with atherosclerosis.<sup>25</sup> These findings were confirmed in 1970 by Brooks *et al* who found large amounts of 9-HODE and 13-hydroxy-9,11-octadecadienoic acid (13-HODE) derived from linoleic acid hydroperoxides in aortic plaques.<sup>22</sup> In 1991, Wang and Powell found increased amounts of 9-HODE and 13-HODE in the aortas and LDL of atherosclerotic rabbits.<sup>26</sup> That same year, Belkner and colleagues found oxygenated cholesterol esters (cholesteryl linoleate) in atherosclerotic plaques of human aortas, the degree of which correlates with the stage of atherosclerosis.<sup>21</sup> To sum up, the increase in linoleic acid hydroperoxides in atherosclerotic plaques coincide with a greater severity of atherosclerosis versus normal regions. In other words, the more oxidised linoleic acid you have in atherosclerotic plaque, the worse the severity of CAD.

In 1987, Halliwell and Grootveld found that many diseases were hallmarked by an increase in lipid peroxidation products.<sup>27</sup> Malondialdehyde, an oxidation product of both linoleic acid and arachidonic acid, is generally used as an indicator of lipid peroxidation as it is easier to determine compared with lipid hydroperoxides. Malondialdehyde reacts with thiobarbituric acid and forms a coloured substance of which the fluorescent intensity of the addition product can be measured. And numerous studies have found increased lipid peroxidation products, measured via thiobarbituric acid reactive substances, in patients with atherosclerosis.<sup>14</sup>

In 1984, both Steinbrecher *et al* and Morel *et al* discovered that endothelial cells can oxidise LDL and that this process involves lipid peroxidation.<sup>28 29</sup> Oxidised LDL was found to be atherogenic and toxic to endothelial cells. In 1990, Miyazawa *et al* confirmed elevated levels of hydroperoxides from linoleic acid in human LDL,<sup>30</sup> which was also elevated in human plasma.<sup>31 32</sup> Later in 1992, it was discovered by Weisser *et al* that patients with atherosclerosis have more oxidised LDL versus healthy patients. Thus, numerous lines of evidence implicate the oxidation of linoleic acid as a major cause for increased oxidised LDL and hence an increased risk for coronary heart disease.

Chylomicrons and VLDL can both be acted on by lipoprotein lipase at the endothelium causing the release of harmful products such as linoleic acid free fatty acids and oxidised lipids from linoleic acid (such as 13-HODE). These oxidised linoleic acid metabolites can then induce

direct toxic effects to the endothelium such as inflammation, reactive oxygen species and adhesion molecules causing endothelial activation and permeability and a greater number of lipoproteins entering into the subendothelium leading to atherosclerosis.<sup>33 34</sup> Indeed, exposure of the endothelium to linoleic acid has been found to increase LDL transfer across the endothelium, which is considered an essential step in the atherosclerosis process.<sup>35</sup> This provides a novel mechanism for why marine omega-3s (EPA/DHA) may be cardioprotective considering their ability to dramatically lower triglycerides and triglyceride-rich lipoproteins such as VLDL and intermediate-density lipoprotein. Thus, marine omega-3s likely reduce the number of these lipoproteins that are acted on by endothelial lipoprotein lipase, reducing the release of harmful oxidised linoleic acid metabolites and at the same time reduce the number of these lipoproteins penetrating into the subendothelium. **Box 1** provides a summary of the evidence implicating omega-6-rich vegetable oils as a causative factor in atherosclerosis and coronary heart disease.

## CLINICAL STUDIES

### Linoleic acid increases cardiovascular events versus alpha-linolenic acid in a 2-year clinical study

The MARGARIN study (Mediterranean Alpha linolenic enRiched Groningen dietARy Intervention study) was a randomised double-blind placebo-controlled trial tested an alpha-linolenic acid (ALA)-enriched margarine (fatty acid composition 46% LA, 15% ALA) versus a linoleic acid (LA)-enriched margarine (58% LA, 0.3% ALA) for 2 years in 103 moderately hypercholesterolaemic men and women (55 years old). Average ALA intakes were 5.9 g/day (2.3% energy) and 1.0 g/day (0.4% energy) in the ALA and LA groups, respectively. Compared with 0.3% ALA margarine (ie, the LA-enriched margarine), the 15% ALA margarine significantly lowered inflammation (C reactive protein (CRP), net difference after 1 year=-0.53 mg/L and after 2 years=-0.56 mg/L ( $p<0.05$ )) despite being given on top of a LA-rich diet. CRP was reduced after 1 year (-0.10 mg/L) but no change after 2 years in the ALA group, whereas CRP rose in the LA group (+0.2 and +0.3 mg/L, respectively). Since CRP has been independently associated with increased CV risk, this study suggests that ALA may reduce whereas LA may increase the risk of CVD.<sup>36</sup> Moreover, the ALA group also had a lower plasma fibrinogen level after 1 year when using the complete MARGARIN study population (-0.18 g/L; -0.31, -0.04). Fibrinogen increased in the LA group during the 104 weeks of follow-up (0.53 g/L vs 0.32 ( $p=0.01$ )). This further suggests that LA increases whereas ALA decreases the risk of thrombosis and cardiovascular events as just a 0.18 g/L lower plasma fibrinogen level is associated with an 11% reduced risk of ischaemic heart disease.<sup>37</sup> The group provided the LA-enriched margarine had a significantly improved total cholesterol (TC):HDL ratio versus the ALA-enriched margarine, despite the fact

### Box 1 Evidence implicating omega-6-rich vegetable oils as a causative factor in atherosclerosis and coronary heart disease

1. Greater amounts of linoleic acid oxidation products are found in LDL and plasma of patients with atherosclerosis.<sup>14</sup>
2. Greater amounts of linoleic acid oxidation products are found within atherosclerotic plaques and the degree of oxidation determines the severity of atherosclerosis.<sup>22</sup>
3. A diet higher in oleic acid or lower in linoleic acid decreases LDL susceptibility to oxidation.<sup>14</sup>
4. Endothelial cells oxidise LDL forming linoleic acid hydroperoxides.<sup>14</sup>
5. Linoleic acid is the most abundant fatty acid in LDL and is extremely vulnerable to oxidation being one of the very first fatty acids to oxidise.<sup>14</sup>
6. A meta-analysis of randomised controlled trials in humans found that when saturated fat plus trans-fat is replaced with omega-6 fat (high in linoleic acid), there is an increase in all-cause mortality, ischaemic heart disease mortality and cardiovascular mortality.<sup>41</sup>
7. The oxidation of linoleic acid in LDL leads to conjugated dienes (malondialdehyde and 4-hydroxynonenal), which covalently bind to apoB altering its structure creating oxidised LDL. oxLDL is no longer recognised by the LDL receptors on the liver but by scavenger receptors on macrophages causing monocyte infiltration into the subendothelium, foam cell formation and eventual atherosclerosis.<sup>14</sup>
8. Oxidation products of linoleic acid (including 9-HODE and 13-HODE) are found in infarcted tissue.<sup>44</sup>
9. Ultrasound of the carotid arteries in healthy patients who have high 9-HODE in LDL have signs of atherosclerosis.<sup>14</sup>
10. The increase in 9-HODE begins between 40 and 50 years old prior to the clinical manifestation of atherosclerosis.<sup>14</sup>
11. 9-HODE is a good indicator of oxLDL, especially if other causes of inflammation are excluded. An increased oxidised LDL, and hence levels of 9-HODE and 13-HODE in LDL, found in patients with rheumatoid arthritis may explain why they have an increased risk of heart disease.<sup>45</sup>
12. 9-HODE and 13-HODE stimulate the release of interleukin 1B from macrophages.<sup>45</sup>
13. The linoleic acid metabolite 9-HODE is a strong promoter of inflammation<sup>45</sup> and hence may be both a marker and inducer of atherosclerosis.
14. Susceptibility of LDL to oxidation correlates independently with the extent of atherosclerosis.<sup>46</sup>
15. 15) Linoleic acid free fatty acids and hydroxy acids (such as 13-HODE) can induce direct toxic effects to the endothelium causing an increase inflammation, reactive oxygen species and adhesion molecules.<sup>33 34</sup>
16. Exposure of the endothelium to linoleic acid has been found to increase LDL transfer across the endothelium, an essential step in the atherosclerosis process.<sup>35</sup>
17. Oxidised linoleic acid metabolites (OXLAMs) are recognised by immune cells and can recruit monocytes/neutrophils to atherosclerotic lesions.<sup>47</sup> OXLAMs are considered a danger signal activating innate immune cells, which are involved in atherosclerosis formation.<sup>48 49</sup>
18. Linoleic acid is the most abundant fat found in atherosclerotic plaques, and this has been known since at least the 1960s.<sup>50</sup>
19. Oxidised linoleic acid but not oxidised oleic acid is found in atherosclerotic plaques.<sup>51</sup>
20. Consuming more linoleic acid increases the amount of linoleic acid in complicated aortic plaques.<sup>52</sup>

Continued

## Box 1 Continued

21. Linoleic acid in adipose tissue and platelets positively associates with CAD, whereas EPA and DHA in platelets are inversely correlated with CAD.<sup>3</sup>
22. Linoleic acid serum concentrations (as opposed to per cent of fatty acids) are higher in patients with CAD.<sup>4</sup>
23. Using the fat-1 transgenic mouse model, which converts omega-6 to omega-3 creating an omega-6:omega-3 ratio of around 1:1 in tissues and organs, reduces atherosclerotic lesions by inhibiting systemic and vascular inflammation.<sup>53</sup>
24. Mice fed fish oil (high in omega-3) as compared with corn oil (high in omega-6) have a significant reduction in atherosclerotic plaque formation possibly due to an increase in antioxidant enzyme activity.<sup>54</sup>
25. There is more thin fibrous cap atheroma, less thick fibrous cap atheroma, less stable plaque and a greater percentage of plaque rupture in patients given sunflower oil (high in omega-6) versus control.<sup>55</sup>
26. An excess dietary intake of linoleic acid causes greater endothelial activation compared with an excess of saturated fat.<sup>56</sup> Linoleic acid can activate vascular endothelial cells, a critical step for inducing atherosclerosis.<sup>57 58</sup>
27. Linoleic acid is inflammatory to the vascular endothelium.<sup>59</sup>
28. Linoleic acid metabolites promote cardiac arrhythmias, cell death, organ failure and cardiac arrest.<sup>60</sup>
29. Patients who have died from sudden cardiac death have more linoleic acid and less omega-3 polyunsaturated fats in their coronary arteries versus control patients who died mostly from traffic accidents.<sup>61</sup> Box 2 summarises the opposing views for (1) why linoleic acid may reduce CHD and (2) why linoleic acid may increase the risk of CHD.

there was an increase in cardiovascular events/deaths in those assigned to the LA-enriched margarine. Indeed, the number of strokes, myocardial infarctions and cardiovascular deaths was seven in patients given the LA-enriched margarine group versus only one in those given the ALA-enriched margarine. Thus, the intake of 6 g of ALA per day may be cardioprotective, whereas the intake of linoleic acid may increase the risk of cardiovascular disease.

A study by Mozaffarian and colleagues found that postmenopausal women with a higher saturated fat intake had less coronary atherosclerosis progression (when measured as per cent stenosis as well as minimal coronary artery diameter),<sup>38</sup> whereas polyunsaturated fatty acid (PUFA) intake was associated with worsening (a decline) in the diameter of the coronary artery. For each 5% increase in energy intake from PUFA, there was a 0.17 mm greater decline in minimal coronary artery diameter and a 5.8% greater progression in mean percentage stenosis. The intake of PUFA was also associated with greater atherosclerosis progression when replacing saturated fat ( $p=0.02$ ). Moreover, a greater intake of saturated fat was not associated with adverse cardiovascular outcomes (myocardial infarction (MI) or CHD death or unstable angina). In summary, the consumption of omega-6 PUFA is associated with coronary atherosclerosis progression,

whereas the intake of saturated fat is associated with less plaque progression.<sup>38</sup> This is in stark contrast to marine omega-3, where modest fish intake is associated with less coronary atherosclerosis progression in postmenopausal women.<sup>39</sup>

The Anti-Coronary Club trial found that more people died overall and due to heart disease when saturated fat was replaced with polyunsaturated fat.<sup>40</sup> Recovered data from the Sydney Diet Heart Study also found that replacement of dietary saturated fats with omega-6 linoleic acid (from safflower oil and margarine) increased all-cause mortality, cardiovascular mortality and CHD mortality.<sup>41</sup> Finally, recovered data from the Minnesota Coronary Experiment indicated that replacing saturated fat with omega-6 linoleic acid (from corn oil and margarine) significantly lowered serum cholesterol but did not reduce mortality and may have increased the risk of death in older adults.<sup>42</sup> In fact, for each 30 mg/dL reduction in serum cholesterol, there was a 22% higher risk of death. More troubling was a significantly greater incidence of at least one MI confirmed by autopsy in the omega-6 intervention. The overall clinical trial evidence suggests no benefit of replacing saturated fat with omega-6 polyunsaturated fat and even possible harm.

Summarising the clinical studies, Ramsden and colleagues performed a meta-analysis of randomised controlled trials comparing mixed omega-3/omega-6 PUFA to omega-6-specific PUFA compared with a combination of saturated fat and trans-fat. Both omega-3 and omega-6 were specifically increased in four data sets. When saturated fat plus trans-fat was replaced by omega-3 and omega-6, there was a significant 22% reduction in non-fatal MI plus CHD death, whereas the trials that specifically increased omega-6 caused a 13% non-significant increase. The risk of non-fatal MI plus CHD death was significantly increased in trials of increased omega-6 intake compared with trials of mixed omega-3/omega-6 PUFA ( $p=0.02$ ). All-cause mortality also tended to be higher in trials of omega-6 versus saturated fat plus trans-fat (+16%). The authors concluded, "Advice to specifically increase n-6 PUFA intake, based on mixed n-3/n-6 RCT data, is unlikely to provide the intended benefits, and may actually increase the risks of CHD and death"<sup>43</sup> In an updated meta-analysis published in 2013, replacing saturated fat plus trans-fat with omega-6 PUFA was found to increase all-cause mortality as well as deaths from CHD and deaths from cardiovascular disease.<sup>41</sup>

## SUMMARY

The consumption of the omega-6 polyunsaturated fat linoleic acid has dramatically increased in the western world primarily in the form of vegetable oils. OxLDL is thought to play an important role in atherosclerosis formation; however, it is the oxidised linoleic acid contained in LDL that leads to harmful OXLAMs, which induces atherosclerosis and CHD. Thus, reducing the amount of dietary linoleic acid, mainly from industrial

## Box 2 Oposing views around linoleic acid and the risk of coronary heart disease (CHD)

Arguments for why linoleic acid *reduces* the risk of CHD

- ▶ Reduces TC and LDL.<sup>62</sup>

Arguments for why linoleic acid *increases* the risk of CHD

- ▶ Increases susceptibility of LDL and other lipoproteins to oxidation.<sup>63</sup>
- ▶ Increases small-dense LDL.<sup>64</sup>
- ▶ Reduces in HDL.<sup>62 65</sup>
- ▶ Increases TGs.<sup>66</sup>

vegetable/seed oils, will reduce the amount of linoleic acid in LDL and likely reduce oxLDL as well as the risk for CHD coronary heart disease.

In summary, numerous lines of evidence show that the omega-6 polyunsaturated fat linoleic acid promotes oxidative stress, oxidised LDL, chronic low-grade inflammation and atherosclerosis, and is likely a major dietary culprit for causing CHD, especially when consumed in the form of industrial seed oils commonly referred to as 'vegetable oils'.

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**Correction notice** This article has been corrected since it published Online First. The second sentence in the introductory page should read as 'This caused a more than two-fold increase in the intake of linoleic acid...'

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