Two lipids in the diet, rather than cholesterol, are responsible for heart failure and stroke

Heart and coronary artery cells are among the 60 trillion endothelium cells that make up the human body [1]. Endothelium cells are built from eight essential amino acids that must come from the diet [2]. All eight of these essential amino acids are only available in animal products, such as eggs, meat, milk and fish. Animal products also contain the most saturated fatty acids (SFAs) and the essential linolenic acid and linoleic acid. These eight essential amino acids are also available in grains and legumes, but not in the correct amounts to build healthy cells. The eight essential amino acids are needed to synthesize ten more amino acids in the liver to make eight apoproteins [3]. Three apoproteins are needed to carry the cholesterol and fat in the plasma as lipoproteins because the blood is made of 98% water, and cholesterol and fat are not water-soluble. Endothelial cells are coated with cholesterol to protect them from the salts and water in the plasma. Cholesterol is so important that the liver makes approximately 2 g of it daily [4]. The fat is used as an energy source. Lipoprotein lipase splits an SFA from VLDL for binding of the SFA to albumin for transport of SFA into the cellular mitochondrial oxidative phosphorylative cycle, with the resultant production of high-energy phosphate that is the cellular fuel. Without this fuel and without cholesterol, the life process comes to a halt [5,6].

This perspective will show that heart failure can develop even without a dietary source of cholesterol. Why LDLs and HDLs should not be used as markers of heart health and why the cholesterol in the egg is not a ‘bad’ cholesterol will be explained. The article will also show how the composition of phospholipids in the arteries change and that sphingomyelin is responsible for this change. This causes a buildup of calcium, which blocks the coronary arteries causing heart failure. How oxysterols are synthesized, how they injure the arterial cells and contribute to the calcification of the arterial wall will be discussed. Oxysterols increase thromboxane release, increasing the risk of blood clotting and oxidation products increase with severity of heart failure. Consumers are being told to eat more polyunsaturated fatty acids (PUFAs) and fewer SFAs, by health authorities, to protect against cardiovascular disease (CVD). The US Department of Agriculture data indicates that this switch is occurring. Since PUFAs are easily oxidized and cause the oxidation of cholesterol into oxysterols, too many PUFAs in our diet will enhance thromboxane in the platelets. This perspective will show that both PUFAs and SFAs are very important to nourishing all the cells in our bodies and how the partially hydrogenated fat, which contains trans fatty acids (TFAs), inhibit...
the synthesis of prostacyclin. The US FDA still allows trans fat in products, thus inhibiting the synthesis of prostacyclin. The total ban of trans fat in our food supply is the only way to help prevent heart failure.

Composition of human plasma
Plasma can be separated by centrifugation into four different fractions (Table 1) [7].

Two of these fractions are LDL and HDL, which are currently used as markers for determining the health of a patient’s heart. The major component in LDL is ApoB, which is an incomplete protein because it does not contain one of the essential amino acids, tryptophan (Tables 2 & 3), and a high LDL number is interpreted as ‘bad’ cholesterol. The major components in HDL are ApoAI and ApoAII, making it a complete protein and a high HDL number is interpreted as ‘good’ cholesterol. In 1961, the American Heart Association (AHA) used LDL and HDL to establish heart health standards, and these standards are still used today [16]. LDL and HDL do not reflect the health of the heart. They only reflect the essential amino acid intake. Physicians recommend eating grains instead of animal protein and grains are an incomplete protein. Grains would have to be eaten with legumes (beans), which is also an incomplete protein, to make a complete protein to provide all the essential amino acids.

Egg cholesterol
Another facet of current heart health parameters involves the amount of cholesterol in the blood. The hypothesis that cholesterol is the major risk factor in heart disease was first based on a study in 1906 by a Russian professor of pathology named Anitschkov [17]. He fed rabbits cholesterol or eggs and noticed atherosclerosis in the rabbits’ coronary arteries that resembled the atherosclerosis in human coronary arteries of people who had died of heart disease. As a result, eggs became associated with high cholesterol levels. In 1975, the Federal Trade Commission held a hearing on the role of eggs and cholesterol in heart disease [18]. All the cardiologists who testified agreed that cholesterol was the cause of heart disease and recommended that eggs should not be eaten. Only two spoke for eggs – Michael DeBakey, the preeminent heart surgeon, and I, a biochemist. Both of our testimonies were disregarded because we were not cardiologists. The Federal Trade Commission’s ruling forbid the egg industry to mention the nutritional value of the egg unless it also stated that cardiologists considered eggs a source of heart disease [19]. Several meta-analyses have been inconclusive on the issue of cholesterol in the diet. Even today, eggs are not given their proper role in good nutrition. For example, Gillinov and Nissen recommended in their 2012 book, Heart 411, that no more than four eggs a week should be eaten [20]. I believe that the easiest way to consume all of the essential amino acids that are needed by the liver, is to eat an egg every day.

Oxidized cholesterol is synthesized in the liver and derived from food
A study by Staprans et al. compared rabbits fed commercial cholesterol with those fed oxidized cholesterol diets; one diet contained 0.33% non-oxidized cholesterol (control diet) and the other was the same diet containing 0.33% cholesterol of which 5% was oxidized (oxidized diet) [21]. The serum cholesterol levels increased to a similar extent in both groups, with the majority of cholesterol in the β-VLDL fraction. There were significant increases in the oxidized cholesterol levels in the serum β-VLDL fraction and liver. Feeding a diet of oxidized cholesterol resulted in a 100% increase in fatty streak lesions in the aorta. This study demonstrated that oxidized cholesterol in the serum of rabbits was both synthesized endogenously (in the liver) and derived from food.

Imai et al. found that angiotoxicity and atherosclerosis was due to contaminants of United States Pharmacopeia-grade (commercial) cholesterol [22]. Evidently, the Staprans et al. study was performed with contaminated cholesterol.

Table 1. Composition of lipoproteins.

<table>
<thead>
<tr>
<th>Lipoproteins</th>
<th>Total</th>
<th>Lipids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protein (%)</td>
<td>Lipid (%)</td>
</tr>
<tr>
<td>VLDL</td>
<td>90</td>
<td>50</td>
</tr>
<tr>
<td>LDL</td>
<td>75</td>
<td>10</td>
</tr>
<tr>
<td>HDL</td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>VHDL</td>
<td>40</td>
<td>12</td>
</tr>
</tbody>
</table>
However, it did show greater increases of oxidized cholesterol in the plasma as well as in the liver. Enzymatic oxidation of cholesterol in vivo mainly occurs in the liver. The radical species responsible for cholesterol oxidation were derived from activated oxygen, which could occur in a variety of tissues [23], including the artery cell wall [24–27]. It was also demonstrated, in the livers of rabbits, that the absorption of dietary oxidized cholesterol occurred within 24 h after feeding trace amounts of radiolabeled oxidized cholesterol. Similar absorption of oxidized cholesterol has also been described in humans [28]. Western diets contain high concentrations of oxidized cholesterol products.

Oxysterols, not cholesterol, were responsible for injury to human arterial smooth muscle cells

The cytotoxicity of pure cholesterol in cultured smooth muscle cells from human umbilical arteries showed that cholesterol, up to 10 µg/ml, did not affect cell growth over 5 days of culture [29]. By contrast, oxysterols at a concentration of 2.5 µg/ml caused a decrease in the cell number, cell viability and DNA and protein content within 2 days of culturing. Reducing the oxysterol concentration to 0.5 µg/ml still resulted in a significant decrease in cell number in 3 days of culturing. These results suggested that oxysterols, not cholesterol, were responsible for injury to human arterial smooth muscle cells.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Isoleucine</td>
<td>0</td>
<td>2</td>
<td>48</td>
<td>3</td>
<td>1</td>
<td>0</td>
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<td>37.2</td>
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<tr>
<td>Luecine</td>
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<td>16</td>
<td>107</td>
<td>6</td>
<td>10</td>
<td>5</td>
<td>109</td>
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<tr>
<td>Lysine</td>
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<td>18</td>
<td>71</td>
<td>9</td>
<td>5</td>
<td>6</td>
<td>48</td>
<td>58.8</td>
</tr>
<tr>
<td>Methionine</td>
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<td>2</td>
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<td>1</td>
<td>2</td>
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<td>6.3</td>
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<tr>
<td>Phenylalanine</td>
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<td>3</td>
<td>3</td>
<td>4</td>
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<td>3</td>
<td>10</td>
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<tr>
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<td>38</td>
<td>3</td>
<td>2</td>
<td>28</td>
<td>3.2</td>
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<tr>
<td>Valine</td>
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<td>2</td>
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<td>50.2</td>
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<tr>
<td>Total</td>
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<td>70</td>
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<td>28</td>
<td>36</td>
<td>31</td>
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<td>3</td>
<td>9</td>
<td>10</td>
<td>108</td>
<td>50.7</td>
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<tr>
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<td>1</td>
<td>2</td>
<td>2</td>
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<td>6</td>
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<td>5</td>
<td>6</td>
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<tr>
<td>Glutamic acid</td>
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<td>9</td>
<td>18</td>
<td>10</td>
<td>233</td>
<td>99.4</td>
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<tr>
<td>Glycine</td>
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<td>1</td>
<td>3</td>
<td>3</td>
<td>58</td>
<td>33.8</td>
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<tr>
<td>Histidine</td>
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<td>1</td>
<td>0</td>
<td>1</td>
<td>13</td>
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<td></td>
</tr>
<tr>
<td>Half-cystine</td>
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<td>2</td>
<td>7</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>9.7</td>
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</tr>
<tr>
<td>Proline</td>
<td>10</td>
<td>8</td>
<td>42</td>
<td>1</td>
<td>5</td>
<td>2</td>
<td>27</td>
<td>66.9</td>
</tr>
<tr>
<td>Serine</td>
<td>15</td>
<td>12</td>
<td>78</td>
<td>7</td>
<td>11</td>
<td>11</td>
<td>54</td>
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<tr>
<td>Tyrosine</td>
<td>7</td>
<td>8</td>
<td>30</td>
<td>6</td>
<td>2</td>
<td>14</td>
<td>25.9</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>149</td>
<td>84</td>
<td>506</td>
<td>29</td>
<td>59</td>
<td>48</td>
<td>661</td>
<td>433.5</td>
</tr>
<tr>
<td>Grand total</td>
<td>245</td>
<td>154</td>
<td>896</td>
<td>57</td>
<td>95</td>
<td>79</td>
<td>1003</td>
<td>719.3</td>
</tr>
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### Table 3. Major and minor apoproteins.

<table>
<thead>
<tr>
<th>Lipoproteins</th>
<th>Major</th>
<th>Minor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chylomicrons</td>
<td>ApoB, ApoC-I, ApoC-II, ApoC-III</td>
<td>ApoA-I, ApoA-II</td>
</tr>
<tr>
<td>LDL</td>
<td>ApoB</td>
<td></td>
</tr>
<tr>
<td>VHDL</td>
<td>ApoA-I, ApoA-II</td>
<td></td>
</tr>
</tbody>
</table>
Pathology of coronary heart failure

In 1993, Ross stated: “Coronary heart disease of the extremities is most apparent at branching points of the arterial tree where blood flow is irregular with current and back currents. The cellular events that occur during the progression of lesions in hypercholesterolemic animals are almost exactly mirrored by those observed in human atherosclerotic coronary arteries in hearts removed in transplant operations” [30].

De Bakey et al. had noted thickening at branching and bifurcations during coronary artery bypass (CABG) surgery [31]. Thickening in the branching arteries in aging pigs on a cholesterol-free diet has also been noted [32]. It did not differ significantly in sphingomyelin composition from that of the nonbranching adjacent tissue of pigs at 6 months of age (Figure 1). By 18 and 48 months of age, however, the sphingomyelin content was significantly higher at the thickened branching areas than at the nontthickened segment of the arteries. The same results were seen when studying the branching and nonbranching segments of arteries of men and women, at autopsy [32]. They contained more sphingomyelin at branching sections than nonbranching sections. The phospholipid composition of the arteries from young men contained four-times more sphingomyelin than the arteries isolated from human umbilical cords, indicating that the sphingomyelin content of arteries increases with age. Plasma from people who had bypass surgeries by 40 years of age were obtained and their plasma analyzed. It contained as much sphingomyelin as plasma from those

Figure 1. Composition of sphingomyelin in a nonbranching segment compared with the branching segment of the same artery at bifurcations of 6-, 18- and 48-month-old swines kept on a cholesterol/saturated fat-free diet.

*Means are statistically different at p < 0.05 compared with the data from nonbranching segment at the same age.
**Means are statistically different at p < 0.05 compared with the data from the same artery at 6 months.
***Means are statistically different at p < 0.05 compared with the data from the same artery at 18 months.

Data taken from [32].
who had CABG surgery at 70 years of age. This data indicated that a fundamental disturbance in lipid metabolism had occurred. I studied this fundamental disturbance for six decades as will be discussed.

From 1976 to 1986, my laboratory focused on using the transmission electron microscope to examine the structure of arteries with heart disease. With the help of pathologists from Japan, the disturbance of lipid metabolism of pigs from fetus to birth and from birth to 3 years of age was followed. At the transmission electron microscope level, the aortic tissue in 3-year-old pigs fed a low-fat cholesterol-free diet was identical to the aortic tissue from humans of all ages who had died of heart disease. A perspective summarizing six papers on the electron microscope showed that a dietary source of cholesterol was not necessary for the development of heart failure [3].

**Composition of arteries from CABG patients**

Arteries from patients who had CABG surgeries had changes in the phospholipid composition of their arteries [33]. Human arterial cells form a structural backbone of biological membrane in the arteries. It is provided by a lipid bilayer of five phospholipids in which the fatty acyl chains form the hydrophobic core of the membrane and their polar heads group facing the aqueous environment at either side of the bilayer [34]. Arteries obtained from human umbilical cords and discarded veins from CABG surgeries were subjected to phospholipid analysis [33]. An analysis of the artery from the placenta is shown in Figure 2. Figure 2 shows that the artery from the cord contained 10% sphingomyelin and 43% phosphatidylcholine, and other phospholipids. It also shows how the composition of phospholipids changed in patients who needed CABG surgery. Sphingomyelin composition increased to 48% and phosphatidylcholine decreased to 27%. Patients who had 48.0% sphingomyelin in their coronary arteries, had no plaques in their arteries, but CABG patients with 60.0% sphingomyelin in their carotid arteries had plaques in them. It is evident that sphingomyelin had changed the composition of the arteries of patients who had CABG surgery.

The lipid composition and calcium concentration in plaque tissue of the carotid and coronary arteries that were made available from CABG surgery were also analyzed. The total phospholipid concentration in the plaque of the carotid arteries of the CABG surgery patients did not differ from that in the nonplaque area from

![Figure 2. Composition of phospholipids in the arterial cell membranes.](image)

**Figure 2. Composition of phospholipids in the arterial cell membranes.**

CABG: Coronary artery bypass. Data taken from [33].
the same patient. However, the percentages of individual phospholipids were changed. The percentage of sphingomyelin in the plaque was more than 20% higher than in the nonplaque tissues from carotid arteries. In coronary arteries, an almost 20% increase of sphingomyelin was also observed in the hard areas that would form a plaque later. In these areas, the calcium concentration also significantly increased to 23.6 ± 12.1 mg/g tissue, compared with 5.0 ± 1.02 mg/g tissue in the surrounding soft areas.

**Oxysterol levels in the plasma**

Lipid analysis of the plasma and arterial tissue obtained prior to and during CABG surgery revealed, when compared with patients who had been cardiac catherized and had no apparent heart disease (controls), a significantly higher concentration of seven oxysterols in the plasma

**Figure 3. Oxysterols in the plasma from coronary artery bypass patients compared with controls.**

Results are expressed as ng oxysterols/ml plasma and given as mean ± standard deviation. Means are statistically different with their controls at levels of *p < 0.05 and **p < 0.01.


Data taken from [38].
Oxysterols contribute to arterial calcification

Calmarz et al. stated in 2013, that LDL oxidation and its association with carotid artery intimal–media thickness was a cardiovascular risk factor [36]. A study demonstrating this was carried out on human arterial cells cultured in five purchased oxysterols (the controls) and compared with the plasma of those awaiting CABG surgery. The control plasma enriched with oxysterols increased influx of $^{40}\text{Ca}^{2+}$ to the same degree as plasma from CABG patients [25]. Therefore, the purchased oxysterols acted the same way as the oxysterols in the plasma of patients who had had bypass surgery. Oxysterol levels are important to calcium binding.

As previously stated, the arterial wall includes five phospholipids, two of these, sphingomyelin and phosphatidylcholine, were affected by oxysterols, which lead to calcium binding. Deposition of calcium in the coronary arteries was found to be directly proportional to an elevated sphingomyelin concentration in the coronary arteries [37,38]. This was demonstrated by culturing arterial cells from plasma of a healthy person [39] and comparing it with the cultures from bypassed patients (those with heart disease). The plasma of the healthy subject did not cause any change in the phospholipid composition in the arterial cells. By contrast, the sphingomyelin level increased in the plasma of bypassed patients. The increase of sphingomyelin leads to more calcification on the arterial wall [40]. The calcification can continue until the blood flow is completely blocked off.

Oxysterols enhance thromboxane synthesis

Oxidized LDL (OxLDL) and oxysterols increase the amount of thromboxane released from platelets and are responsible for blood clotting. Platelets were isolated from blood from a local blood bank and a portion of it treated with either normal LDL (nLDL) or oxLDL. Both nLDL and oxLDL were isolated. Thromboxane release by platelets was triggered by a low concentration of thrombin, and which component of oxLDL is responsible for that activation was determined. Platelets treated with oxLDL also contained significantly higher levels of oxysterols than platelets treated with nLDL. Our results indicated that oxysterols are among the LDL oxidation products responsible for enhanced thromboxane release from platelets [41,42]. As shown in Table 4, oxLDL contained high levels of oxysterols that are also present in the platelets obtained from the plasma of patients who had CABG surgery (Figure 3) [33]. This study, therefore, indicated that the same reaction in vitro and in vivo had occurred. Steinberg noted in 2007 that there were over 3000 papers published relating to oxLDL and its possible role in atherogenesis [43].

Oxidation products of cholesterol increased with the severity of heart disease

The concentration of cholesterol, lipid oxidation products of cholesterol and total antioxidant capacity in the plasma of 2000 cardiac catheterized patients with 0, 10–69 and 70–100% stenosis of their arteries were analyzed [44]. The results showed that lipid oxidation products increased with the severity of stenosis and the total antioxidant capacity decreased with the severity of stenosis. The plasma cholesterol concentration, however, was not significantly different between these groups of patients. Therefore, the concentration of oxidation products rather than the concentration of cholesterol in the plasma increased with the severity of coronary heart disease.

Health authorities’ recommendations on diet & heart disease

To protect against CVD, the AHA, NIH and international health authorities recommend

<table>
<thead>
<tr>
<th>Cholesterol (ng/mg protein)</th>
<th>nLDL</th>
<th>oxLDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>7α-hydroxycholesterol</td>
<td>135</td>
<td>5220</td>
</tr>
<tr>
<td>7β-hydroxycholesterol</td>
<td>210</td>
<td>4380</td>
</tr>
<tr>
<td>β-epoxycholesterol</td>
<td>78</td>
<td>480</td>
</tr>
<tr>
<td>α-epoxycholesterol</td>
<td>80</td>
<td>1100</td>
</tr>
<tr>
<td>Cholestanetriol</td>
<td>100</td>
<td>1300</td>
</tr>
<tr>
<td>7-ketocholesterol</td>
<td>250</td>
<td>13,500</td>
</tr>
<tr>
<td>Total oxidized cholesterol</td>
<td>853</td>
<td>25,980</td>
</tr>
</tbody>
</table>

nLDL: Normal LDL; oxLDL: Oxidized LDL. Data taken from [41].
replacing dietary SFAs with PUFAs. As the food market is dominated by products rich in omega-6-PUFA, this advice may result in many adverse health effects [45]. Both SFAs and PUFAs are needed; SFAs to provide the cellular fuel and small amounts of PUFA to provide the linolenic acid and linoleic acid, which converts to arachidonic acid and then to prostacyclin, which keeps the blood flowing [46]. Another concern is that these groups fail to specify the type of PUFA; all PUFAs are not equal. High intake of PUFA has been associated with an increased risk of cancer and other diseases [45].

Consumption of polyunsaturated fat has increased in the USA

Data from the US Department of Agriculture (Table 5) indicated that from 1912 through to 2011, the consumption of polyunsaturated fat had increased from 11.3 to 64.5 pounds per capita and saturated fat had decreased from 28 to 13.4 pounds per capita in the same period [49]. Therefore, a sixfold increase in polyunsaturated fat and a decrease of twofold in saturated fat has unbalanced the diet. Evidently, Mozaffarian et al. had not realized that we need both SFAs and unsaturated fatty acids. They stated: “a shift toward greater population PUFA consumption in place of SFA would significantly reduce rates of coronary heart disease (CHD).” [46]. No clinical trial has succeeded in lowering the risk of CVD using an increased intake of omega-6-PUFA. Meta-analyses on the roles of both PUFA and SFA in CVD have been inconclusive [50].

According to Astrup et al., “more research is needed to clarify the role of SFAs compared with specific forms of carbohydrates in CHD risk and to compare specific foods with appropriate alternatives” [51–53]. The linoleic and linolenic acid in PUFAs, in small amounts, are essential fatty acids needed in the diet to synthesize prostacyclin [54]. However we also need SFAs to feed all the 60 trillion cells in our body [1].

Composition of partially hydrogenated vegetable oil

With the partial hydrogenation of oil in 1910, TFAs came into the picture [55]. It took nearly five decades before the biochemical structure of TFAs was understood. In 1952, the gas chromatography was made available [56] and could identify the components of soybean oil. The hydrogenation of soybean oil added atoms of hydrogen to 50% of the bonds 9,12 in linoleic and the bonds 9,12,15 in linolenic acid, converting them to 50% stearic acid. A total of 40–50% of the double bonds of fatty acids in linoleic and linolenic acid were shifted to different positions.

Table 5. Fats and vegetable oils consumption in the USA since 1912 per capita (in pounds).

<table>
<thead>
<tr>
<th>Items</th>
<th>1912 Total</th>
<th>Per cap</th>
<th>1950 Total</th>
<th>Per cap</th>
<th>1999 Total</th>
<th>Per cap</th>
<th>2011 Total</th>
<th>Per cap</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn oil</td>
<td>53.0</td>
<td>0.6</td>
<td>223.0</td>
<td>1.47</td>
<td>1416.9</td>
<td>5.2</td>
<td>1620.0</td>
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</tr>
<tr>
<td>Cottonseed oil</td>
<td>950.0</td>
<td>10.0</td>
<td>1445.0</td>
<td>9.51</td>
<td>832.8</td>
<td>3.1</td>
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<td>1.2</td>
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<td>0.17</td>
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<td>Palm kernel oil</td>
<td>0.0</td>
<td>0.0</td>
<td>26.0</td>
<td>0.17</td>
<td>233.2</td>
<td>0.9</td>
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<td>0.68</td>
<td>1524.7</td>
<td>5.6</td>
<td>202.7</td>
<td>0.6</td>
</tr>
<tr>
<td>Canola oil</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.00</td>
<td>111.2</td>
<td>0.4</td>
<td>4249.0</td>
<td>13.6</td>
</tr>
<tr>
<td>Safflower oil</td>
<td>0.0</td>
<td>0.0</td>
<td>5.1</td>
<td>0.03</td>
<td>15.8</td>
<td>0.1</td>
<td>60.9</td>
<td>0.2</td>
</tr>
<tr>
<td>Sesame oil</td>
<td>0.0</td>
<td>0.0</td>
<td>5.0</td>
<td>0.03</td>
<td>15.8</td>
<td>0.1</td>
<td>27.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>16.0</td>
<td>0.2</td>
<td>1446.0</td>
<td>9.51</td>
<td>8029.6</td>
<td>29.4</td>
<td>9000.0</td>
<td>28.8</td>
</tr>
<tr>
<td>Sunflower oil</td>
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<td>0.0</td>
<td>0.5</td>
<td>0.00</td>
<td>393.7</td>
<td>1.4</td>
<td>395</td>
<td>1.3</td>
</tr>
<tr>
<td>Total unsaturated oils</td>
<td>1070.0</td>
<td>11.3</td>
<td>3355.6</td>
<td>22.1</td>
<td>13319.5</td>
<td>48.8</td>
<td>20128.1</td>
<td>64.5</td>
</tr>
<tr>
<td>Lard</td>
<td>1069.0</td>
<td>11.2</td>
<td>1891.0</td>
<td>12.60</td>
<td>202.0</td>
<td>0.7</td>
<td>480.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Butter</td>
<td>1579.0</td>
<td>16.6</td>
<td>1648.0</td>
<td>10.70</td>
<td>1307.0</td>
<td>4.8</td>
<td>1510.0</td>
<td>4.8</td>
</tr>
<tr>
<td>Tallow</td>
<td>22.0</td>
<td>0.2</td>
<td>69.0</td>
<td>0.45</td>
<td>996.0</td>
<td>3.6</td>
<td>1050.0</td>
<td>3.4</td>
</tr>
<tr>
<td>Coconut</td>
<td>0.0</td>
<td>0.0</td>
<td>69.0</td>
<td>0.45</td>
<td>927.0</td>
<td>3.4</td>
<td>1155.1</td>
<td>3.7</td>
</tr>
<tr>
<td>Total saturated fats</td>
<td>2670.0</td>
<td>28.0</td>
<td>3677.0</td>
<td>24.2</td>
<td>3432.0</td>
<td>12.6</td>
<td>4195.1</td>
<td>13.4</td>
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<td>US population in millions</td>
<td>95</td>
<td>152</td>
<td>273</td>
<td></td>
<td>312</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

†Totals in millions of pounds.
Cap: Capita.
Data from [47–49].
on the carbon chain, making nine different synthetic TFAs and five different cis fatty acids. These were cis and trans isomers of octadecenoic and octadecadienoic acids that are not present in animal fats or plant oils. They interfere with the action of two isoforms, a constitutive COX-1 and an inducible COX-2 enzyme. COX-2 is the enzyme that recognizes the isomers produced during hydrogenation as a foreign substrate and reacts to them by causing inflammation and inhibition of linoleic to arachidonic acid. COX-2 is the inducible form of isoform of COX. COX-1 is present constitutively, while COX-2 is expressed primarily after an inflammatory insult. A total of 10% of the linolenic and linoleic acid was left in its original state at position 9,12 in linoleic and 9,12,15 in linolenic acid. The 14 synthetic fatty acids were a source of energy, but interfered with the conversion of linoleic acid to arachidonic acid and prevented the synthesis of prostacyclin. Without prostacyclin to keep the blood flowing, the end result would be sudden death [57].

Since 1957, I have been trying to call attention to the TFAs present in margarines and shortenings with some success [58,59]. Samples from tissue obtained from human autopsies were shown to contain up to 14% TFAs. Samples of fat from human placental, maternal, fetal and baby tissue were also examined for the presence of TFAs. While the maternal tissue contained considerable amounts of TFAs, these lipids were not found to any measurable extent in placental, fetal or baby fat [60]. This was also shown in rats that were fed trans fat. When the trans fat was removed from the diet, their tissue metabolized the trans fat and their tissue no longer contained trans fat [61]. The results of these studies indicated that the TFAs present in human tissue apparently arise solely from dietary fat, and they do not normally appear in the tissues unless a source of TFAs is included in the diet.

**Partially hydrogenated fat has different properties than animal fat or vegetable oil**

An in vitro study showed that the fatty acids in partially hydrogenated fat had different properties than the fatty acids in animal fat or vegetable oil [62]. This was shown in a study where endothelial cells were cultured for 3 days in media with high (adequate) or low (inadequate) amounts of magnesium plus various concentrations of TFAs, in partially hydrogenated vegetable fat or cis fatty acids, in animal fats or soybean oil.

The cells were then harvested and the amount of $^{45}Ca^{2+}$ incorporated into the cell was determined. The percentage of TFAs incorporated into the endothelial cells was proportional to the amount added to the culture medium. Adequate magnesium was crucial in preventing calcium influx into endothelial cells. Without an adequate amount of magnesium in the culture medium, trans acids, even at low concentrations, increased the incorporation of $^{45}Ca^{2+}$ into the cells, whereas cis acids did not incorporate $^{45}Ca^{2+}$ into the cells.

**Partially hydrogenated fat inhibited the synthesis of PUFAs**

An in vivo study also showed that the TFAs inhibited the synthesis of PUFAs in the phospholipid membrane of arterial cells [63]. In this study, five groups, each with six pregnant pigs, were fed from day 35 of gestation and during lactation. The basal diet contained 2% corn oil (control). The other two diets included 10% butter or 10% hydrogenated fat added. Plasma, milk fatty acids, aortic phospholipid fatty acids, phospholipid composition and calcium content of the aorta from the piglets were determined.

At 48 ± 2 days of age, the aorta phospholipid of piglets from pigs fed hydrogenated fat contained a significantly higher concentration of linoleic acid, less arachidonic acid, less long chain PUFAs and 3% trans fat. The aortic calcium content data showed a significant interaction of calcium concentration with age. Piglets from pigs fed either butter fat or the control (corn oil) diet had no change in the linoleic acid and arachidonic acid and no change in the composition of the aorta phospholipids. The changes in composition in piglets from pigs fed hydrogenated fat indicated that trans fat inhibits the metabolic conversion of linoleic acid to arachidonic acid and to other n-6 PUFAs. At 48 days of age the piglets had accumulated 11.3% TFA in their plasma. The bulk of the TFA was transferred to the piglets from their mother’s milk. The composition of the sows’ milk was 15.8% TFA at the birth of the piglets and decreased to 0.5% TFA after the birth. Human milk has been shown to contain up to 18% TFAs and breast fed babies at 2 months of age had up to 15.6% TFAs in their plasma [64]. Stary found calcium deposits in young people as granules of microscopic size in atherosclerosis lesions defined histologically as type IV [65]. I concluded: that dietary trans fat perturbed essential fatty acid (EFA) metabolism, which led to changes in the phospholipid fatty acid composition in the aorta, the target tissue.
of atherogenesis; and this inhibition of EFA to PUFA by the isomeric fatty acids in hydrogenated fat is a risk factor in the development of coronary heart disease because arachidonic acid is needed to synthesize prostacyclin.

**Why natural trans fats are different from manufactured ones**

Enzymes in the stomachs of cows eating grass change the *cis* form in vaccinic acid to a *trans* form. Grass naturally contains linoleic and linolenic fatty acids. When cows eat the grass, the enzymes in their stomachs change part of the *cis* form in vaccinic acid to a *trans* form [66], therefore, meat fat, milk fat and butter contains from 2 to 4% *trans* fat. It was believed by the FDA for years that partially hydrogenated soybean oil *trans* fat had the same chemical structure and worked in the same way in our bodies as natural vaccinic acid. As previously explained, these two *trans* fat sources have entirely different properties in vivo as well as *in vitro*.

**Concerns regarding the composition of margarines & shortenings**

In 1965, I was a member of an advisory group of the AHA, which was created to update the 1961 recommendation on what to eat. Because of this position, in 1967, I obtained the composition of eight different margarines and three different shortenings from WH Meyer, Manager of the Professional and Regulatory Relations for The Proctor and Gamble Company. These were all that were available in the market at the time. In summary, all the margarines contained between 39 and 50% TFAs and shortenings contained between 19 and 30%.

The concerns regarding *trans* fats were highlighted to Dr Campbell Moses, Medical Director of the AHA, with the suggestion that he meet with the president of the Institute of Shortening and Edible Oils to reduce the amount of *trans* fat in the partially hydrogenated fat to zero. In 1968, Dr Moses, and the president of the Institute of Shortening and Edible Oils met to change the industry standards [Moses C, Pers. Comm. (1968); 67].

The first version of their industry standards stated: “Partial hydrogenation of polyunsaturated fats results in the formation of *trans* forms, which are less effective than *cis, cis* forms in lowering cholesterol concentrations. It should be noted that many currently available shortenings and margarines are partially hydrogenated and many contain little polyunsaturated fat of the natural *cis, cis* form.” The Institute of Shortening and Edible Oils objected to this version. The second version, which was distributed to the public, omitted reference to *trans* and *cis* fatty acids. It stated: “Margarines that are high in polyunsaturated usually can be identified by listings of a ‘liquid oil’ first among the ingredients. Margarines and shortenings that are heavily hydrogenated or contain coconut oil, which is quite saturated, are ineffective in lowering the serum cholesterol.” The industry agreed to lower the TFAs and increase the level of EFA in shortening and margarines [68]. After this ruling, the percentage of TFAs in margarine decreased on average from 44 to 27%, while the percentage of linoleic acid increased from 11 to 25% (Table 6).

**Effects of the early formulations of trans fat products**

Versions of various margarines cited above were formulated by the author and their effects on prostacyclin production were tested [68]. Soybean oil and hydrogenated soybean oil (coating fat) were mixed in different proportions to yield seven fat mixtures with proportions of linoleic acid ranging from 54.1 to 5.7% and *trans*-18:1 acid ranging from 0.4 to 43.9%. Human endothelial cells were cultured in each of the mixtures, and were then separated by gas chromatography and analyzed for prostacyclin levels (Table 7).

Repeating soybean oil with coating fat (43% *trans* fat) dose-dependently decreased (p < 0.0001) the proportion of linoleic (18:2Δ6) and arachidonic (20:4Δ6) acids, but increased the proportion of elaidic acid (*trans*-18:1), in the phospholipid membranes of vascular endothelial cells. Consistent with these changes, coating fat also dose-dependently suppressed prostacyclin release (p < 0.0001). The lowest proportion of coating fat used (23% coating fat providing 13.4% *trans* fat) suppressed prostacyclin by 35% (p < 0.05), while pure coating fat (providing 43.9% *trans* fat) nearly abolished prostacyclin release, suppressing it by 98% (p < 0.05). Shortening (Crisco) became available in 1920, and it contained the same kind of fat as margarine fat. The results demonstrated that the pre-1968 formulas inhibited prostacyclin production. The post-1968 formulas had fewer *trans* fats and while they still inhibited prostacyclin production, they did so to a lesser degree.

Four margarines (Table 8) were purchased from the supermarket in 2013 for comparison to the current industry standards. The proportions
of linoleic acid and TFAs were similar to those reported in 1968, suggesting that the industry standards set in 1968 are still being met. The linoleic acid in these margarines labeled as containing 1.5–2.5 g of trans fat per serving ranged from 25.6–40.7%, while the trans acid ranged from 15.2 to 28.7%. These proportions are similar to those in the mixtures containing 23–50% coating fat used in the present study, which lowered prostacyclin release by 35–54% (p < 0.05). These are the margarines currently available in the world market.

Many commercial foods, misleadingly, labeled as trans-free contain hidden sources of trans fats that could contribute to the suppression of prostacyclin production, especially if multiple servings are consumed, or if these foods are eaten in addition to foods containing margarine and shortening with greater amounts of trans fat. Even processed foods labeled as 0% trans fat may provide hidden sources of trans fat that contribute to prostacyclin suppression.

### Presence of trans fat suppresses the production of prostacyclin

Clearly, the presence of trans fat suppresses the production of prostacyclin. The trans fat present in the mixtures used increased its own concentration in membrane phospholipids at the expense of linoleic and arachidonic acids. Linoleic acid is a precursor to arachidonic acid, from which prostacyclin is derived, and would thus be expected to support the production of this critical prostanoid [69]. The linoleic acid present in partially hydrogenated soybean oil, however, is clearly insufficient to overcome the suppressive effect of the TFAs present in these partially hydrogenated oils.

### CDC on deaths from heart failure data

Data from the CDC [72] indicated that in 1900, there were 27,424 deaths due to heart failure & stroke.
failure in the USA, with an age-adjusted rate of 265.34/100,000. By 1962, the total number of deaths had increased to over 350,000 or 556.9/100,000 (age-adjusted rate) (Figure 4). In my opinion, the gradual increase in the death rate was due to the presence of 44% TFA in the margarine, which prevented the synthesis of prostacyclin. The death rate gradually decreased after 1968. This appears to be due to the use of a margarine containing lower levels of TFAs, which was introduced in 1968. This new margarine did not suppress prostacyclin production as heavily, which reduced cardiac events.

Cardiac events have declined dramatically since 1968 [76], yet remain the leading cause of death in the USA, causing almost 600,000 deaths in 2011 [77]. In total, 325,000 of these deaths are from sudden cardiac death, according to Jain [78]. I do not believe that heart disease is a disease, it is a somatic response to a simple error involving not knowing the effect of 44% trans fat in partially hydrogenated fats on prostacyclin synthesis and the presents of oxysterols in the plasma.

**FDA required the labeling of foods that contain TFAs**

It took until 11 July 2003 for a national mandate from the FDA to require the labeling of foods that contain TFAs [79]. The FDA based this directive on 160 scientific articles that showed the bad effects of trans fat. Unfortunately the FDA did not go far enough at the time. First, it allowed a food item that contains less than 0.5 g/serving to be labeled 0% trans [80]. This is a problem because many people eat more than one serving at a time, and because the effect of even small amounts of trans fat consumption can add up. Fifteen samples of food items were collected that claimed 0 g of trans fat. One contained 0 g trans fat, one contained more than 0.5 g, and 13 contained just a shade under 0.5 g of trans fat. The FDA also allows any amount of trans fat as long as it is stated on the label. Second, it treats hydrogenated trans fat the same as natural trans fat when in fact they work differently in the body. Trans fats that come from partially hydrogenated oil change the composition of the arteries, while natural trans fats do not [81]. The WHO concluded: “Policies aimed at restricting the TFA content of food were associated with significant reductions in TFA levels, without increasing total fat content. Such policies are feasible, achievable and likely to have an effect on public health” [82].

**In 2006 the FDA justified its trans labeling mandate**

On 1 January 2006, the FDA justified its trans labeling mandate by stating: “This regulation will provide information on food labels about the amount of trans fat in foods so that consumers can select foods with lower levels of trans fat and thereby lower their intake of trans fat as part of a heart-healthy diet.” [80,83]. The FDA estimates that, “3 years after the effective date, trans fat labeling would prevent from 600 to 1200 cases

![Figure 4. Leading causes of death in the USA since 1900–2010. Data taken from [73–75].](image)
of heart disease and 250–500 deaths each year.” Furthermore, economists at the FDA stated, “based on this estimate, this rule will realize a cost saving of US$900 million to $1.8 billion per year in medical costs, lost productivity, and pain and suffering.” A review by a research group at Harvard Medical School, entitled ‘TFAs and CVD’ concluded with the statement, “10–19% of CHD events in the USA could be averted by reducing the intake of trans fat. Thus given the 1.2 million annual myocardial infarctions and death from CHD in the USA, near-elimination of industrially produced trans fat might avert between 72,000 (6%) and 228,000 (19%) CHD events each year.” [84].

“Blood levels of TFAs in white adults in the US population decreased by 58% from 2000 to 2009,” according to one CDC study published in the 8 February 2012 edition of the Journal of the American Medical Association [85]. This is the first time that CDC researchers have been able to measure trans fats in human blood. Christopher Portier, director of CDC’s National Center for Environmental Health [86] stated, “Findings from the CDC study demonstrate the effectiveness of these efforts in reducing blood TFAs and highlight that further reductions in the levels of trans fats must remain an important public health goal.”

**Manufactured trans fats banned**

On 7 November 2013, the FDA announced proposed measures to ban all manufactured trans fats. On 8 November 2013, the Federal Register printed the FDA’s tentative determination regarding partially hydrogenated oils; request for comments and for scientific data and information [87].

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**Executive summary**

**Background**

- Two lipids present in the diet, oxysterols and trans fats, and not cholesterol in the plasma are responsible for heart failure.

**Discussion**

- Cholesterol is necessary for life; the liver makes approximately 2 g a day to provide enough for the body to use. If the body does not have enough cholesterol to make cells, there are negative health consequences.
- Oxidized cholesterol, called oxysterols, are synthesized both in the liver and derived from food.
- Heart failure can develop without cholesterol from the diet present.
- The cellular events that occur during the progression of heart failure are as follows:
  - Changes first occur at branching points;
  - There is a significant increase of sphingomyelin at branching points of the arteries;
  - Those with heart disease have an increase in sphingomyelin in their arterial walls.
- Oxysterols increase sphingomyelin synthesis, which leads to calcium binding in the arterial wall. Eventually this causes the blockage of blood flow in the coronary arteries.
- Oxysterols are produced during excessive frying of food; cholesterol gets converted into oxysterols during that process.
- Oxysterols are responsible for an increase of thromboxane released from the platelets. Thromboxane is necessary for blood clotting, but too much can lead to sudden heart failure.
- The consumption of too much polyunsaturated fat can overtax the liver. The excess polyunsaturated fat trips the mechanism that turns cholesterol into oxysterols.
- Polyunsaturated fats contain two essential fatty acids, linoleic and linolenic acids, which are needed to synthesize prostacyclin. Prostacyclin keeps the blood flowing, also an important factor in heart failure.
- Trans fatty acids inhibited the conversion of linoleic acid to arachidonic acid, thus preventing the synthesis of prostacyclin.
- Trans fats come from the diet; they are not made in the body, but rather created by partially hydrogenating vegetable oil.
- The exact composition of partially hydrogenated vegetable oil was not known until 1952.
- Partially hydrogenated oil had different properties than the fatty acids in animal fat or vegetable oil.
- Margarines and shortenings over the decades have contained trans fats in varying amounts up to 50%.
- In 1968, these sources of fat were reformulated to have less trans and more linoleic acid. However, they did not go far enough to make the fats ‘safe’ for consumption.
- The US FDA proposed a ban on trans fat on 7 November 2013. It should be instituted fully.

**Conclusion & future perspective**

- The most effective way to prevent coronary heart failure is to eat moderate amounts of food, particularly avoiding commercially fried foods and too many polyunsaturated fats.
- The banning of partially hydrogenated fat would eliminate trans fatty acids from human consumption.
- The FDA should further mandate that soybean oil be fully hydrogenated and diluted with soybean oil that has been extracted by pressing that will include vitamin E, an antioxidant.
Conclusion
This article has shown that cholesterol is not responsible for heart failure. Excessive oxidation of cholesterol is responsible for the increased synthesis of sphingomyelin in the arterial wall, which increases calcification of the coronary arteries, which inhibits the blood flowing through them. In addition, the presence of oxLDL and oxysterols in excessive amounts causes an increase in thromboxane synthesis in the platelets. A deficiency of sphingomyelin and enhancement of thromboxane can lead to heart failure and sudden death.

No one knew what effect the introduction of hydrogenation would have on the synthesis of prostacyclin. The most effective way to prevent cardiac events and sudden death according to these conclusions is to eat fewer commercially fried foods and fewer polyunsaturated fats. We should eat foods that contain all of the essential amino acids, even though they contain cholesterol. More vegetables and fruit should also be eaten because they are a source of antioxidants that prevent the oxidation of LDL in the liver.

Future perspective
Commercial frying fats should not be used if they are composed of vegetable oils that contain polyunsaturated fats because they cause the oil to oxidize. Frying fat should be tested every day for freshness. The test is available now but is not mandatory in the USA, although it is mandatory in Germany. Soybean oil, because it is the only oil presently being hydrogenated, should be completely hydrogenated to stearic acid and diluted with soybean oil that has been extracted by pressing, not solvent extracted, thus keeping all of the vitamin E, an antioxidant, in the oil.

Financial & competing interests disclosure
The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

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Papers of special note have been highlighted as:
* of interest
** of considerable interest
* Heart failure can occur even without a dietary source of cholesterol.
Two lipids in the diet, rather than cholesterol, are responsible for heart failure & stroke | PERSPECTIVE


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