Chapter 15

The Effects of Hydrogenation on Soybean Oil

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Additional information is available at the end of the chapter

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1. Introduction

Soybeans are very versatile, both as a food product and an ingredient in many industrial products. The oil produced by soybeans is contained within many foods we eat every day. Natural soybean oil contains several essential fatty acids that our body needs to work properly, including linoleic and linolenic acids. However, much of the soybean oil consumed in many parts of the world has been partially hydrogenated; that is, it's chemical composition has been changed. This hydrogenation removes the necessary essential fatty acids contained within the original oil. Some of the partially hydrogenated soybean oil has been converted to trans fatty acids.

Trans fatty acids have been shown to increase the risk of atherosclerosis and coronary heart disease due to their in vivo effects in two ways. They effect the levels of prostacyclin and thromboxane, which increases the risk of thrombosis, and they increase sphingomyelin production by the body, which then causes calcium influx into the arterial cells to increase, leading to atherosclerosis. Consumption of partially hydrogenated soybean oil can be harmful to the body.

2. Soybeans

Soybeans have many uses. When processed, a 60-pound bushel will yield around 11 pounds of crude soybean oil and 47 pounds of meal. Soybeans are about 18% oil and 38% protein. Because soybeans are high in protein, they are a major ingredient in livestock feed. Most soybeans are processed for their oil and protein for the animal feed industry. A smaller percentage is processed for human consumption and made into products including soy milk, soy protein, tofu and many retail food products. Soybeans are also used in many non-food (industrial) products [1].

Biodiesel fuel for diesel engines can be produced from soybean oil by a process called transesterification. Soy biodiesel is cleaner burning than petroleum-based diesel oil. Its use reduces particle emissions, and it is non-toxic, renewable and environmentally friendly. Soy crayons are made by replacing the petroleum used in regular crayons with soy oil, making them non-toxic and safer for children. Candles made with soybean oil burn longer but with less smoke and soot. Soy ink is superior to petroleum-based inks because soy ink is not toxic, renewable and environmentally friendly, and it cleans up easily. Soy-based lubricants are as good as petroleum-based lubricants, but can withstand higher heat. More importantly, they are non-toxic, renewable and environmentally friendly [1]. Soy can also be used in paint and plasticizers, and used in bread, candy, doughnut mix, frozen desserts, instant milk drinks, gruel, pancake flour, pan grease extender, pie crust, and sweet goods. Non-food items made with soybeans include anti-corrosives, anti-static agents, caulking compounds, core oils, diesel fuel, disinfectants, electrical insulation, epoxies, fungicides, herbicides, printing inks, insecticides, oiled fabrics, and waterproof cement [2].

Soybean oil is normally produced by extraction with hexane. The production consists of the following steps. The soybeans are first cleaned, dried and de-hulled prior to extraction. The soybean hulls need to be removed because they absorb oil and give a lower yield. This dehulling is done by cracking the soybeans and a mechanical separation of the hulls and cracked soybeans. Magnets are used to separate any iron from the soybeans. The soybeans are also heated to about 75^{o} C to coagulate the soy proteins to make the oil extraction easier. To extract the oil, first the soybeans are cut into flakes, which are put in percolation extractors and emerged in hexane. Counter flow is used as extraction system because it gives the highest yield. After removing the hexane, the extracted flakes only contain about 1% of soybean oil and are used as livestock feed, or to produce food products such as soy protein. The hexane is recovered and returned to the extraction process. The hexane free crude soybean oil is then further purified [3].

World production of soybean oil in 2010-2011 rose 8.0% to a new record high of 41.874 million metric tons. The U.S. accounts for 20.6% of world soybean oil production, while Brazil produces 15.8% and the European Union accounts for 5.8%. The consumption of soybean oil rose 9.2% worldwide in 2010-2011, with the U.S. accounting for 18.6%, Brazil accounting for 12.4%, India accounting for 6.9%, and the European Union accounting for 6.4% of demand [4].

3. Uses for soybean oil

Of the total of 18 million pounds of soybean oil consumed in 2011, approximately 9 million pounds was used for cooking and salad oil. 3.75 million pounds was used for baking, and 3.6 million pounds on industrial products. The remaining 900,000 pounds is used in various other edible products. The high smoke point of soybean oil makes it often used as a frying oil. If overused, however, it causes the formation of free radicals.

Soybean oil contains 52.5% linoleic (18:2 $\Delta^{9,12}$) acid, which is also known as 18:2n^{6} or omega–6. It also contains 7.5% linolenic (18:3 $\Delta^{9,12,15}$) acid also known as 18:3n^{3} or omega-3. The des-

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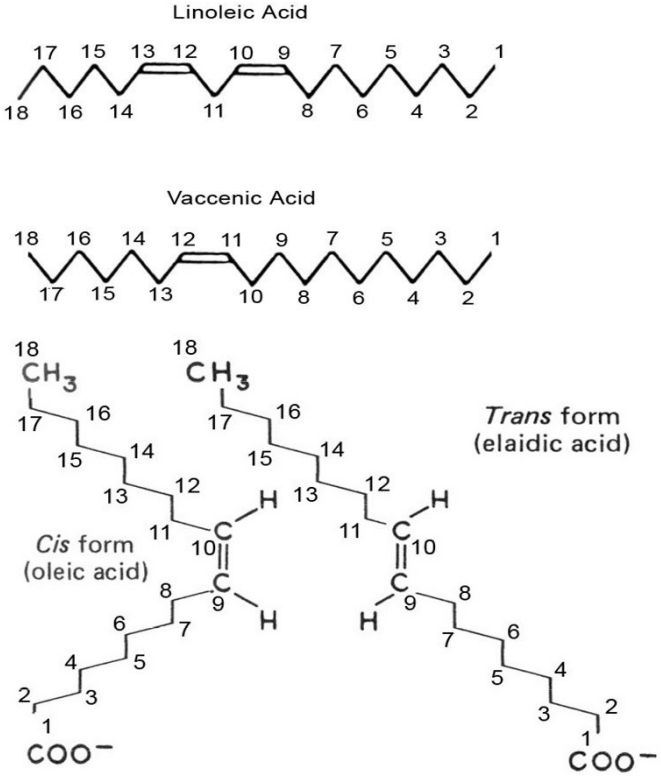
ignation 18:2 $\Delta^{9,12}$, and 18:3 $\Delta^{9,12,15}$ means that these two fatty acids have double bonds (points of unsaturation) at position 9 and 12 or 9,12 and 15 at which hydrogen can be added. In the late 1800s, a French chemist discovered that an unsaturated fatty acid can be converted to a saturated fatty acid by bubbling hydrogen through a heated vegetable oil in a closed vessel. If completely hydrogenated, they become stearic acid. The commercial use of partially hydrogenation of soybean oil began in the early 1900s. The exact fatty acid composition of the partially hydrogenated soybean oil was essentially unknown until the development of gas chromatography (GC) by James and Martin in 1952. The Food and Drug Administration, using the American Oil Chemists Society method, labeled the isomers in partially hydrogenated fat as only one peak (elaidic acid). It is only with a GC equipped with a 200 meter column that it is possible to further separate the fatty acid isomers of partially hydrogenated fat into at least 14 separate isomeric fatty acids [5].



During hydrogenation, the double bond at any of these 9,12 or 9, 12, 15 positions can be shifted to form new cis and trans unsaturated fatty acid isomers not present in soybean oil.

The double bond of the cis-natural linoleic and linolenic fatty acids can also change the configuration from cis to trans, creating a geometric isomer like trans Δ^{11}-18:1 vaccenic acid in butter fat. Oleic acid, the largest percentage of the natural fatty acid in the human body, is cis Δ^{9}-18:1 (the number after delta indicates the position of the double bond at the 18 carbon atom chain counting from the carboxyl group).

Oleic acid goes through geometrical isomerisation during hydrogenation to trans Δ^{9}-18:1 acid known as elaidic acid; thus the "natural" oleic acid is turned into elaidic acid during the hydrogenation process, and becomes an "unnatural" fatty acid. It twists into a new form and can be both a cis and/or a trans fatty acid. In addition to geometrical isomerisation, the double bond of either cis or trans fatty acids can theoretically migrate along the 18 carbon chain of either oleic, linolenic, and linoleic acid, changing their position from Δ^{9}, $\Delta^{9,12,15}$, or $\Delta^{9,12}$, creating five monoene cis positional isomers, 6 trans monoene isomers and 3 trans diene positional isomers. Thus hydrogenated soybean oil contains 24.1% trans monoenes, 6.2% trans dienes and 9.4% cis monoene isomers or a total of 39.7% isomeric fatty acids. They were identified as cis and trans octadecenoic and octadecadienoic isomers on a GC equipped with a 200 meter column and by their mixed melting points with authentic octadecenoic and octadecadienoic acids. None of these fatty acids are present in natural soybean oil. The 14 isomers in hydrogenated fat can be used as a source of energy but they cannot substitute for EFA because they do not have the required double bond structure [5].

4. Nutrition

It was unknown until 1930 that linoleic (18:2 n^{6}) and linolenic (18:3 n^{3}) acids were essential fatty acids (EFA), and like the nine essential amino acids and the vitamins, cannot be synthesized in the human body; they must come from a diet that includes natural fats and oils. In one study, pregnant rats were fed linoleic, linolenic, and arachidonic acids by dropper. This was a sufficient amount for the mother rats to wean their young, but those pups from mothers fed only linolenic acid died before weaning. Although linolenic acid is considered an essential fatty acid, these data indicate that it may not be an essential fatty acid [6].

An increase in the sales of soy food is largely credited to the Food and Drug Administration's approval of soy as a cholesterol-lowering food [7]. A 2001 literature review argued that these health benefits were poorly supported by available evidence, and noted that data on soy's effect on cognitive function of the elderly existed [8].

The FDA issued the following claim for soy: "25 grams of soy protein a day, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease." [9]. Solae also submitted a petition on the grounds that soy can help prevent cancer. On February 18, 2008, Weston A. Price Foundation submitted a petition for the removal of this health claim. 25 g/day soy protein was established as the threshold intake because most trials used at least this much protein and not because less than this amount is inefficacious [10]. An American Heart Association review of a study of the benefits of soy protein casts doubt on the FDA claim for soy protein. However, AHA concludes "many soy products should be beneficial to

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cardiovascular and overall health because of their high content of polyunsaturated fats, fiber, vitamins, and minerals and low content of saturated fat" [11].

EFA are required to synthesize the eicosanoids that are needed to regulate blood flow in the arteries and veins. Linoleic acid (n-6) is synthesized into arachidonic acid, and linolenic acid (n-3) is synthesized into eicosapentaenoic acid. Both in turn are made into prostacyclin or thromboxane. Prostacyclins are synthesized in the endothelial cells that line the blood vessel wall. Thromboxanes are synthesized in the platelets in the blood. The balance between prostacyclin for flow and thromboxane for clotting is a very delicate one and can be changed by different diets and different drug prescriptions. Fish have already converted the linolenic acid they get from seaweed into eicosapentaenoic acid. Hence, fish oil is often recommended as a dietary supplement. Prostacyclin and thromboxane can be made from linoleic acid as well. The least expensive source of omega-3 and omega-6 is soybean oil, which is sold as vegetable oil in a supermarket [12].

However, this vegetable oil is stripped of Vitamin E, which is then sold in capsules. The removal of Vitamin E leaves the oil more susceptible to oxidation, which harms the natural fatty acids that are needed for good health.

How soybean oil is used in modern humans was developed in prehistoric humans to assure their survival. There must have been long periods of time between meals, that is fasting periods, and there were times in which they had food available, the "fed" period. During this fed period, carbohydrates were used within two hours as a quick source of energy. Extra carbohydrates were stored first as glycogen in the muscles and liver and then any excess converted to fat and stored in the adipose tissues (the fat around your middle and elsewhere). This stored fat was then available for energy during the long fasting periods. Modern humans have inherited this way of handling these fed and fasting periods. This process assured the survival of prehistoric humans but has now become one way that obesity is developing in humans today. Too much food is available all hours of the day and night, and eating it is a pleasure.

To avoid adding fat to your body, any carbohydrates you eat should be used up as a calorie source before the next meal. Any carbohydrates that have already turned into fat and any fat in your diet itself should be used for energy within the cell during the fasting period. Eating a snack between meals means adding additional carbohydrates into the system before any of the fat from the previous meal has been used for energy. It ends up adding to your adipose tissue. If you weighed yourself before a hearty meal and again the next day, you may find you have gained a pound or two, the amount depending on how much food you ate and the fat you stored. As such a meal may also contain excess salt, some of the weight gain can be due to excess water you stored. Millions of dollars are spent to try to get rid of this stored fat, and the government is planning to spend millions more dollars to solve the obesity problem. Prehistoric humans had no choice in controlling the time between fasting and fed periods because they had no refrigerators, fast food outlets, or supermarkets to run to. Modern humans do have this choice. More time between the fed periods, that is between meals, may help with the obesity problem [12].

The fat in the intestinal tract is first converted into tiny droplets of fat (chylomicrons) by the intestinal cells. The intestinal tract is not just a through highway, but is actively involved in the process of metabolizing fat so that the body can use it. The chylomicrons diffuse from the intestinal tract into the lymph system and into the veins through the thoracic duct and end up in the blood. The blood, during the fed period, carries these chylomicrons for deposit where they are resynthesized into adipose tissue and stored fat around the stomach, hips, and other locations. The fat (triglycerides) in adipose tissue is "mobilized" when the glycogen in the muscle and liver has been reduced.

The glycerin portion goes to the liver. The free fatty acids take a different route and are combined with a protein named albumin. Therefore, there must be enough albumin in the blood to carry the free fatty acids in the blood. This fatty acid albumin complex is water-soluble enough in the blood to be carried to cells of all kinds that use the fatty acid portion as an energy source. Any excess fatty acid goes to the liver and is remade into triglycerides. The cellular organelle (the endoplasmic reticulum) in the liver cells participates in coating the very small triglyceride droplets with protein and adds phospholipid and cholesterol to produce very low density lipoprotein (VLDL), which furnishes the fatty acid for the approximately 50 thousand trillion cells in the body [12].

Correction of the inhibition of lipoprotein lipase by protein binding of free fatty acids permits normal protein transport of FFA into the cellular mitochondrial oxidative phosphorylative cycle with the resultant production of high-energy phosphate which is the cellular fuel. Without this fuel, in addition to oxygen, the life process comes to a halt. Bacteria have used this method of providing energy for at least two billion years (Ratz).

5. Fried foods

Another issue with fats is the preparation of foods by frying them in fat. There are problems with deep fat fried food that affect our nutrition. These problems occur because of chemical alterations in the fat that happen as a consequence of deep fat frying food. This frying process is as follows:

1. Food picks up oxygen from the air during frying that negatively alters the fat composition.
2. The foods fried in these fats pick up those altered fats.
3. These altered foods have a direct, negative influence on the nutritional value of the fat.

The changes in the fat are dependent on at least four factors:

1. The length of time it was exposed to heat—in commercial operations, the length of time a food is fried leads to how much fat is absorbed on the cooked food item;
2. The temperature of the fat;
3. The exact composition of the fat used, such as corn oil, cottonseed oil, soybean oil, beef tallow, or hydrogenated fat, and

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4. What is being fried, e.g., chicken or fish.

Feeding the fats fried at varying lengths of time led to very different outcomes in the nutrition of animals. Those fed the fats fried the shortest period of time were healthier than those fed the fats fried for the longest times. Those fed fats heated at higher temperatures were not as healthy as those fed on fat heated to lower temperatures. It was interesting also that animals fed on heated margarine did not grow as well as those on fresh margarine and that their plasma cholesterol level increased. Those fed on heated butter oil grew as well as those on fresh butter oil.

Oil from commercial fat fryers was used in a set of experiments that clearly showed that poor nutrition resulted. This is important because used fat from commercial operations is typically collected and fed to animals, such as pigs, to provide energy for rapid growth. When we conducted experiments feeding the commercially used fat for frying to rats, they did not do well. When we added protein to their diets, the effect of the "bad" heated fat was countered because the added protein provided more adequate nutrition. We tried to fortify the diets with adequate vitamins, but that could not counter the growth-depressing effect of the heated oil. A few vitamins, such as riboflavin, helped a bit.

Fish contain high amounts of polyunsaturated fat that are not present in the fat of chicken or beef. Thus, when fish are fried, the polyunsaturated fat in them can leak into the frying fat, causing the fat to be changed more radically into a less healthy version. Chicken and hamburger have less of this polyunsaturated fat and thus are healthier choices to fry.

Eating excessive amounts of fried food also slows down digestion. People may get stomachaches as a result. As early as 1946, a link that heated fats may lead to cancer was shown. What we don't know yet is whether heated fats by themselves lead to cancer or whether the heated fat combined with specific foods cause cancer. Animals fed heated fat combined with a known carcinogen developed cancer, whereas those fed fresh fat combined with a known carcinogen did not. Thus the heated fat was a co-carcinogen.

Commercial frying of food has increased worldwide since our studies on heated fats. In Germany, fat fryers are required by law to test their frying fat for its freshness by a method approved by the German government. In the U.S. a test is also available, but its use is not mandatory [12].

6. Free radicals

Free radicals are produced from oxidized linoleic (n-6) and linolenic acid (n-3); they are fragments of unsaturated fatty acids. This is especially likely to happen when the essential fatty acids are heated, especially the n-3 variety. All oils change structures when they are heated, but hose high in n-3 fatty acids have more problems than those high in n-6. Free radicals provide another reason to avoid fried food. The first sign of fats becoming free radicals is that they are rancid, and they begin to smell "off" and their taste becomes bitter. Roasted peanuts, for example, can become rancid and then shouldn't be eaten.

Free radicals are "bad" since they destroy vitamins A, D, C, and E, thus preventing these vitamins from doing positive things in the body. Free radicals also destroy both the essential fatty acids and the essential amino acids. They oxidize the LDL into something called oxidized low density lipoproteins (oxLDL). These oxLDL are very powerful components in the blood that have been considered since about 1990 as involved in the development of heart disease [12].

Essential fatty acids do more than regulate the blood; they are also a key to reproduction. Since the 1930's, we've known that reproduction always fails on fat-free diets. In studies on rats, reproduction continues under low fat conditions because the rats have enough linoleic acid stored in their bodies. They manufacture arachidonic acid from the linoleic acid in their own fat, so they can reproduce healthy young even after a fat-free diet. If the rats did not have enough linoleic acid stored in their bodies (such as rats born to mothers on fat-free diets), we found they could not make enough of the arachidonic acid needed for healthy reproduction, and their young die. Women need the essential fatty acids for reproduction. The easiest way to supply them is from plant oils [5].

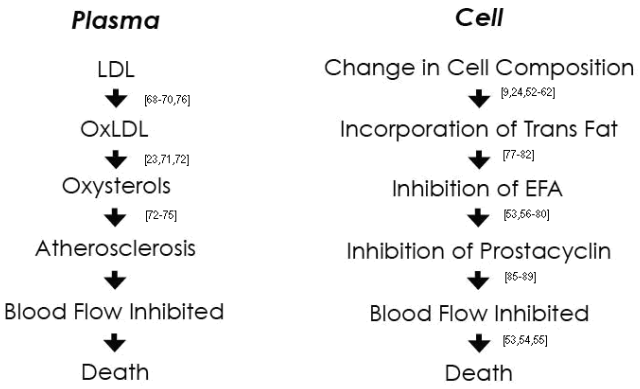
Data from ADM shows the composition of three different hydrogenated fats, based on a serving size of 14 grams. The first two were made of enzymatically interesterified soybean oil, and contained 0 grams of trans fat per serving. The third was made of partially hydrogenated soybean and/or cottonseed oil, and contained 4.5 grams of trans fat per serving. The take away message is that due to effective food industry lobbying, food labeling rules allow foods with up to half a gram of trans fat per serving to be labeled "0 trans fat". So look for "partially hydrogenated vegetable oil" on the label.

Several researchers have documented the effects of foods without trans fat and their positive effects on lowering CHD. Mozaffarian et al. showed that n-3 PUFAs from both seafood and plant sources may reduce CHD risk, with little apparent influence from background n-6 PUFA intake. They found lower death rates among those with high seafood and plant-based diets. Plant-based n-3 PUFAs may particularly reduce CHD risk when seafood-based n-3 PUFA intake was low, which has implications for populations with low consumption or availability of fatty fish. Kris-Etherton et al. found that nuts and peanuts routinely incorporated in a healthy diet with a composite of numerous cardioprotective nutrients reduced the risk of CHD. They also suggested that higher intake of trans fat could adversely affect endothelial function, which might partially explain why the positive relationship between trans fat and cardiovascular risk is greater than one would predict based solely on its adverse effects of plasma lipids [12].

7. Two mechanisms involved in coronary heart disease

Two mechanisms may be involved in CHD: One, the oxidation of the fatty acids and cholesterol in LDL leading to a change in sphingomyelin concentration in the arteries, which is a process that occurs over a life time; two, the deposition of trans fat in the cardiovascular system. Trans fat calcifies both the arteries and veins and causes blood clots. Trans fat leads to

the reduction of prostacyclin that is needed to prevent blood clots in the coronary arteries. A blood clot in any of the coronary arteries can result in sudden death.



8. Mechanism one

When sufficient biological antioxidants are not present in the plasma, the LDL is oxidized to oxLDL and cholesterol is oxidized to oxysterol. Oxysterols incorporated into the endothelial layer of the arteries and veins can change the phospholipid cell membrane composition so that more sphingomyelin incorporates into the membrane which becomes “leaky” to calcium infiltration. Oxysterols were present at higher concentrations in the plasma of patients who had coronary artery bypass grafting (CABG) surgery. These patients had 40 times more calcium in their bypassed veins than normal veins in the same patient. When purchased oxysterols were added to plasma from patients who did not need CABG surgery, endothelial cells cultured in their blood and tested with radioactive calcium the incorporation of radioactive calcium did not differ from that of plasma from CABG patients. This indicates that oxysterols stimulated calcification. When endothelial cells were cultured with oxysterols in a standard culture media, the cells became calcified in a similar way to those of the CABG patient. The oxidation of cholesterol and deposition of calcium is the primary cause for the development of atherosclerosis in the arteries and veins.

In a review article entitled "The pathogenesis of atherosclerosis: Perspectives for the 1990s" Ross stated "Atherosclerosis 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 al-

most exactly mirrored by those observed in human atherosclerotic coronary arteries in hearts removed in transplant operations" [13]. De Bakey et al. have noted similar atherosclerosis (thickening) at branching and bifurcation during coronary artery bypass grafting (CABG) surgery [14].

Keaney stated that the gene expression pattern in the arterial wall is subject to influence by modified forms of LDL [15], which altered both scavenger reception (CD36) expression and the expression of pro-inflammatory genes [16]. The disturbed laminar flow pattern of fluids occurs near branch points [17], bifurcations, at major curves and at arterial geometries [18] that are typically associated with the earliest appearance (and subsequent progression) of atherosclerotic lesions [19]. An endothelial receptor for oxLDL, a designated lectin-like oxLDL receptor (LOX-1) [20], was identified [21]. The transient application of shear stress showed that the initial stimulation of shear stress was sufficient for induced expression of LOX-1 and that sustained application of shear stress was not required [22]. The over-expression of LOX-1 receptors at the bifurcation and the higher level of modified LDL and oxysterols in the plasma of persons needing CABG surgery could lead to a higher uptake of modified LDL, resulting in a greater delivery of oxysterols to the endothelial cells at the bifurcations. The levels of sphingomyelin in plasma have been shown to be higher in patients with coronary heart disease and those with left ventricular dysfunction [23]. Furthermore, it was found that sphingomyelin levels in the blood correlate with and can be used to accurately predict coronary artery disease [24]. Sphingomyelin has long been known to accumulate in atheromas of both humans and animals, and contributes to the formation of atherosclerosis [25].

Thickening [26] was noted in the branching arteries in aging porcine on a non-cholesterol diet. It did not differ significantly in sphingomyelin composition from that of the non branching adjacent tissue of porcine at 6 months of age. By 18 and 48 months of age, however, the sphingomyelin content was significantly higher at the thickened branching areas than at the non thickened segment of the arteries. This indicated that during aging of the arteries, there was a striking increase in the amount of sphingomyelin in the membrane of the cells at the branching points of arteries [26]. Lipid extracted from both porcine and human arteries indicated that aging is a factor that increased sphingomyelin. There was more sphingomyelin in the aging arteries of both porcine and human arteries.

The non branching segment of the aorta obtained, on autopsy, from six men 21-27 years of age contained four times more sphingomyelin than in arteries isolated from human umbilical cords, indicating that the sphingomyelin content of arteries increases with age. Aging is not the only factor that increased the sphingomyelin composition of arterial cells. Women and men under 40 years of age who had been subjected to CABG surgery contained the same high percentage of sphingomyelin in their non atheromatous arterial cells as those over 40 years of age. Therefore, heart disease itself seemed to have caused an increase in non atheromatous arterial cells in sphingomyelin composition prematurely in CABG patients, pointing to a fundamental disturbance in phospholipid metabolism in their arterial cells.

The phospholipid composition of a normal arterial cell has less sphingomyelin, and this amount increases until half the artery is sphingomyelin. That is, the more sphingomyelin

was in the arterial cells, the more Ca^{2+} was identified. This is because the hydroxyl group and amide group of sphingomyelin act as both donors and acceptors of hydrogen bonds [27]. Furthermore, Lehninger found that sphingomyelin's long, 18-to-26 carbon atoms chain fatty acids altered the positioning of other phospholipids. Dipalmitoylphosphatidylcholine has no amide bond [28]. As both sphingomyelin and dipalmitoylphosphatidylcholine are largely on the extracellular side of the membrane [29,30], such bilayer asymmetry would enhance binding. These in vitro results showed that sphingomyelin-Ca^{2+} binding goes beyond an isolated individual membrane binding Ca^{2+}, to lattice type matrix binding among adjacent membranes [31]. These results in vitro were simulated in vivo Ca^{2+} deposition (calcification) in arteries and veins.

9. The in vivo effect of sphingomyelin on the composition of the vascular membrane

Patients who had CABG surgery sometimes needed a second CABG surgery because the vein used in the first surgery had been occluded. During this second surgery, an unoccluded vein from the same patient was used to replace the occluded vein. The occluded veins contained, on average, significantly more sphingomyelin and Ca^{2+} than the unoccluded veins [32]. The unoccluded veins contained 24% sphingomyelin and 182 ppm of Ca^{2+} as compared to 48% of sphingomyelin and 6,345 ppm of Ca^{2+} in the occluded veins that had been used in the first CABG surgery. The increased sphingomyelin and Ca^{2+} concentrations in the occluded veins were responsible for the initial formation of atherosclerosis in these patients.

10. Oxysterols increased sphingomyelin and Ca^{2+} deposition in patients with CABG surgery

Ridgway found that 25-hydroxycholesterol stimulated sphingomyelin synthesis in Chinese hamster ovary cells [33]. Similarly in humans, an oxysterol increased sphingomyelin synthesis during the development of atherosclerosis. A significant increase in the concentrations of oxysterols, phospholipids, and Ca^{2+} were noted in patients who had CABG surgery [26, 32]. Patients who had cardiovascular disease had increased oxysterol levels in their plasma compared with the controls; that is, by comparison to cardiac catheterized patients with no stenosis [32]. The plasma from CABG patients had a higher concentration of oxysterols than was present in the controls. Human endothelial cells were cultured for 72 hours in a medium containing plasma obtained from CABG patients, or from controls patients with addition of 5 types of oxysterols (7-keto-cholesterol, cholestane-3β, 5α, 6β-triol, 7β-hydroxycholesterol, β-epoxy cholesterol, and 7α-hydroxycholesterol). These added oxysterols increased the total oxysterol level in the controls equivalent to that in the CABG plasma.

Phospholipid	Human		Porcine	
(%)	younger	older	3 weeks	2 years
Phosphatidylcholine	34.1	19.2	44.74	33.91
Phosphatidylethanolamine	8.8	2.4	25.18	24.76
Sphingomyelin	44.8	68.8	16.06	23.72
Phosphatidylinositol	+			
Phosphatidylserine	5.0	1.6	11.35	14.55
Phosphatidic acid	1.0	0.6		
Lysolecithin	3.9	8.0	trace	1.28

Table 1. Data from Kummerow F.A.. 1987. Factors which may alter the assembly of biomembranes so as to influence their structure or function *In* Membrane Biogenesis. Op den Kamp J. A. F., editor. Springer-Verlag. 95.Phospholipid composition of human and porcine arterial tissues

Oxysterols stimulated sphingomyelin synthesis and inhibited sphingomyelin metabolism [34, 23, 24]. When radioactive Ca^{2+} ($^{45}Ca^{2+}$) influx was measured, significantly higher influx of $^{45}Ca^{2+}$ was noted in the endothelial cells cultured with added oxysterols indicating that oxysterols increased Ca^{2+} influx into endothelial cells [34]. By using a radiolabeled choline, the time- and dose-dependent effects of 27-hydroxycholesterol on sphingomyelin synthesis could be observed. The increased radioactivity in sphingomyelin, which was accompanied by decreased radioactivity in phosphatidylcholine in 27-hydroxycholesterol-treated cells, was higher than that in control cells. This result indicated that 27-hydroxycholesterolincreasedthetransferofcholinefromphosphatidylcholinetosphingomyelin. An interesting finding was that the increased radioactivity in sphingomyelin by 27-hydroxycholesterol was detected first, followed by enhanced Ca^{2+} uptake and the accumulation of cytosolic free Ca^{2+}. Moreover, decreased activities of neutral and acid sphingomyelinase, which hydrolyze sphingomyelin, were also detected in 27-hydroxycholesterol treated cells [35]. Therefore, the cause for calcification was related to the structure and location of sphingomyelin in the cell membrane.

11. The concentration of cholesterol and lipid oxidation products in the plasma of cardiac catheterized patients was also determined [36]

The concentration of cholesterol, lipid oxidation products 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. The results showed that lipid oxidation products increased with the severity of stenosis, they were 2.92 mmol/L at 0% stenosis, 3.19 mmol/L at 10–69% stenosis and 3.48 mmol/l at 70–100% stenosis. The total antioxidant capacity decreased with the severity of stenosis. The plasma cholesterol concentration, however, was not significantly different between these groups of patients. It was 201.9 mg/dL at 0% stenosis, 203.2 mg/dL at

10–69% stenosis and 207.5 mg/dL at 70–100% stenosis. Therefore, the concentration of oxidation products, rather than the concentration of cholesterol in the plasma, increased with the severity of atherosclerosis [36]. In all age groups, all of the women and men with cardiovascular atherosclerosis also had increased individual and total oxysterol levels in their plasma as compared with the controls.

The *in vivo* oxidation was enhanced by sphingomyelin. The oxidation could come from the consumption of too many polyunsaturated fatty acids in soybean oil [32, 36]. Polyunsaturated fats in vegetable oil could provide more oxidized LDL and more oxidized sterols into the plasma, which would increase the possibility of atherosclerosis. Sphingomyelin accumulates in the arterial system of humans and animals, and these increased levels mean an increased likelihood of atherosclerosis formation.

12. Mechanism two

Trans fatty acids are available on every continent. There are at least six hydrogenation plants in the United States alone; there is one in Texas, four in Illinois, and one in New Jersey. The FDA has estimated that daily intake of trans fatty acids in northern Europe to be at around 4.5g-17g/capita, and 1.34-4.9 in southern Europe. In India, 2.7-4.8g/capita/day was estimated, and only 2.7-4.8g/day in Australia and New Zealand. The least amount of trans fatty acids is consumed in Hong Kong, Japan, Korea, and China at 1.5-3g/capita/day. A large hydrogenation plant is located in a suburb of Tokyo that uses both fish and vegetable oils, as well as one in Beijing. These trans fatty acid-filled oils are liquid at room temperature, and similar to olive oil that has been used for centuries in southern Europe as an important source of fat in the diet. Butter, lard and beef tallow are saturated fats that have been used for centuries as a fat source in the diet in northern Europe [37].

The second mechanism that may be involved in CHD is trans fat. Trans fat calcifies both the arteries and veins and causes blood clots. Trans fat inhibits COX-2, an enzyme that converts arachidonic acid to prostacyclin that is needed to prevent blood clots in the coronary arteries. A blood clot in any of the coronary arteries can result in sudden death. The American Heart Association has stated that 42% of victims of a sudden heart attack do not reach a hospital still alive.

A study in 2004, with piglets from mothers fed hydrogenated soybean oil showed that their arteries contained less linoleic acid converted to arachidonic acid than the arteries of piglets from mothers fed butterfat or corn oil. This indicated that the trans fat in hydrogenated soybean oil inhibited the metabolic conversion of linoleic to arachidonic acid. Furthermore, an analysis of the fat embedded in the arteries of the piglets from mothers fed partially hydrogenated soybean oil showed that they contained 3% trans fat incorporated into their phospholipids by 48 days of age [38].

If a mother is breast-feeding her child and also eating foods containing trans fat, she would have a substantial amount of trans fat in her milk supply and pass those to her infant. Preg-

nant porcine fed hydrogenated fat contained 11.3% trans fat in their milk at the birth of their piglets, which decreased during lactation to 4% in 21 days. The plasma of the piglets increased from 5% trans fat three days after birth to 15.3% at six weeks of age. Transferring this result to humans, a human mother would also transfer the trans fat in her milk supply to her infant. The infant would incorporate the trans fat into his/her arterial cells inhibiting arachidonic acid synthesis and prostacyclin secretion [4].

Furthermore, calcium deposition into the endothelial cells could be enhanced. To date, the FDA has not considered the daily intake of trans fat relevant to the health of small children since they do not exhibit overt heart disease. In cases where children have died of unknown causes and had been autopsied, 99% of them showed the beginning stages of hardening (calcifications) of the arteries, which ultimately can lead to heart disease [39].

13. The effects of trans fatty acids on calcium influx into human arterial epithelial cells

The influence of trans fatty acids and magnesium on cell membrane composition and on calcium influx into arterial cells. The percentage of fatty acids 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, linoelaidic and elaidic acids, even at low concentrations, increased the incorporation of $^{45}Ca^{2+}$ into the cells, whereas stearic acid and oleic acid did not. A diet inadequate in magnesium combines with trans fat may increase the risk of calcification of endothelial cells [40].

Vaccenic acid in butter did not inhibit the metabolic conversion of linoleic to arachidonic acid. Epidemiological studies of intake of ruminant trans fat and risk of coronary heart disease (CHD) indicated that the intake of ruminant trans fatty acid was innocuous or even protective against CHD. Thus a study with an animal model has shown that trans-fat decreased synthesis of arachidonic acid from linoleic acid. This study was carried a step further with endothelial cells in the first layer of the artery. They were cultured in a medium that contained the fatty acids of soybean oil or in a medium that contained the fatty acids of hydrogenated soybean oil. The latter cells contained trans-fat in their membrane phospholipid and significantly less arachidonic acid and secreted less prostacyclin than endothelial cells that had been cultured with the fatty acids from unhydrogenated soybean oil [5].

We found that in the cells cultured with trans fat, the free arachidonic acid released by phospholipase action was shunted to metabolism by another pathway leaving less free arachidonic acid available as substrate for prostacyclin synthesis. Cyclooxygenase (COX) is the enzyme that is necessary to make prostacyclin to keep the blood flowing, thus lowering the potential for a heart attack. Vane et al. have shown that COX is the enzyme that converts arachidonic acid to prostaglandin H_2, is further metabolized to prostanoids. Vane et. al. stated two isoforms of COX existed, a constitutive (COX-1) and an inducible (COX-2) enzyme. COX-2 may be the enzyme that recognizes the isomers produced during hydrogenation as a

foreign substrate and reacts to them by causing inflammation and reduction of prostacyclin. COX-2 is the inducible isoform of COX. COX-1 is present constitutively while COX-2 is expressed primarily after the inflammatory insult [41].

The ability to form prostacyclin from arachidonic acid was assayed using a radioimmunoassay kit. Trans-fat depressed the synthesis of prostacyclin. The addition of an excess amount of linoleic acid to this hydrogenated soybean oil fatty acids did not increase the secretion of prostacyclin in endothelial cells. The concentration of trans fatty acid rather than the concentration of linoleic acid was therefore responsible for regulating the synthesis and secretion of prostacyclin in endothelial cells. The trans fat in hydrogenated fat not only depressed the synthesis of prostacyclin that regulated the clotting of blood but also, could not serve as precursors for prostacyclin synthesis. The trans fat "incorporated" into the membrane lipids of blood vessels and muscle tissues and displaced the essential linoleic, linolenic and arachidonic acids.

In another study, rats were fed either corn oil, butter, hydrogenated vegetable oil, or coating fat for 10 weeks at 10g/100g diet. In the group fed coating fat, arachidonic acid was found to be significantly lower in the phospholipid fatty acid content of the platelets, aorta, and heart. The ratio of 20:3(n-9)/20:4(n-6) was greater than in the groups fed corn oil, butter, or hydrogenated vegetable oil, indicating that the group fed coating fat was essential fatty acid deficient. The composition of coating fat was 33% trans fat and only 0.3% linoleic acid, whereas hydrogenated oil was made up of 18% trans fat and 32.8% linoleic acid. It was then concluded that the consumption of hydrogenated fats high in trans 18:1 acids with adequate amount of linoleic acid had no effect on the amount of thromboxane or prostacyclin by platelet or aorta in vitro. The coating fat is dangerous because of its lack of linoleic acid [42].

To demonstrate the process of calcification, endothelial cells cultured with/without trans fat showed that trans fatty acid calcify arterial cells. One with a trans fatty acid added as the "unnatural" elaidic acid (t18:1 n^9) and the other with a cis fatty acid added as the "natural" oleic acid (cis 18:1 n^9) and testing with radioactive calcium. More radioactive calcium infiltration occurred into the endothelial cells cultured with elaidic acid than with oleic acid. An autopsy of 24 human specimens showed that human subjects that had died of heart disease contained up to 12.2% trans fat in their adipose tissue, 14.4% in liver, 9.3% in heart tissue, and 8.8% in aortic tissue and in atheroma.

14. The trans fatty acids in partially hydrogenated fat can cause blood clots

Partially hydrogenated soybean oil contained 14 cis and trans isomers that were formed during hydrogenation [4, 5]. They inhibited cyclooxygenase, an enzyme required for the conversion of arachidonic acid to prostacyclin, a molecule which prevents blood clots [43]. Moreover, oxidized fat enhanced thromboxane synthesis [44, 45], which caused the formation of a blood clot. Trans fatty acids in partially hydrogenated vegetable oil decreased pros-

tacyclin synthesis by inhibiting cyclooxygenase. Oxysterols enhanced thromboxane synthesis [44, 45]. Both prostacyclin and thromboxane are involved in sudden cardiac death.

According to WebMD, "sudden cardiac death (SCD) is a sudden, unexpected death caused by loss of heart function (sudden cardiac death). It is the largest cause of natural death in the U.S., causing about 325,000 adult deaths in the United States each year. SCD is responsible for half of all heart disease deaths. SCD occurs most frequently in adults in their mid-30s to mid-40s, and affects men twice as often as it does women." [46]

Under the current Food and Drug Administration mandate [47], food items with any amount of trans fatty acids are allowed, as long as they are labeled. Products containing less than 0.5g/serving can be labeled as "trans free" or 0%. This is misleading, because it is easy to circumvent this rule by making the serving size listed on a label small enough to meet the 0.5g threshold. The food industry has taken advantage of this rule by making the serving sizes small enough to contain less than 0.5g/serving of trans fat. Fifteen foods labeled "trans fat free" were analyzed for fat content. Two contained 0% trans fatty acid, two contained higher than 0.5g/serving and the rest contained between 0.014 to 0.25g/serving. If the serving size is increased, foods would contain more than 0.5g of trans fatty acids. In 2003, the daily intake of trans fatty acids for men was estimated by the Food and Drug Administration to be nearly 7 grams per day, and almost 5 grams per day for women [47]. It is possible for people to eat the same amount of trans fatty acids today as in earlier periods, even though they have supposedly been removed from the food supply. A recent article in JAMA, "Levels of Plasma trans-fatty acids in Non-Hispanic White Adults in the United States in 2000 and 2009" listed levels in the year 2000 at 38.0, and in 2009 as 14.0μ/ml, which was considered significant [48].

15. Environmental impact of soybean use

Epidemiological data collected by the Center for Disease Control (CDC) further illustrate the potential harmful effects of trans fat. These data showed that, death from CHD in the USA increased from 265.4/100,000 in 1900 to 581/100,000 population by 1950. During this time period, both margarine and shortening had a high percentage of trans fat (ranging from 39-50%) and a low percentage of linoleic acid (ranging from 6-11%) according to the technical director of the Institute of Shortening and Edible Oils. In 1968 Dr. Campbell Moses, medical director of the AHA, appointed a five member subcommittee on fats of the AHA nutrition committee to revise the 1961 version of “Diet and Heart Disease.” At the time it was known that an increase in EFA composition of a dietary fat would lower plasma cholesterol levels and there was strong evidence that trans fatty acids increased plasma cholesterol levels. The first revised version by the AHA committee 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 members of the

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Institute of Shortening and Edible Oils Inc objected to this version. The second revised and distributed version, omitted references to hydrogenated fat and cis fatty acids stated: "Margarines that are high in polyunsaturates usually can be identified by the 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 trans fatty acids and increase the level of EFA in shortenings and margarine. Dr. R.I. Levy, director of the National Heart, Lung, and Blood Institute at the time, believed 1968 a watershed, as the incidence of CHD has steadily declined in the US since 1968. Why it decreased remained unknown in 1968.

On October 24th, 1978, ten years after the reformulation of hydrogenated fat, the National Institute of Health (NIH) held a conference in Bethesda, Maryland, on the Decline in CHD Mortality. A recent editorial in Circulation cited this symposium. Three major conclusions reached were;

1. The decrease in CHD mortality was real and not a result of artifacts or changes in death certificate coding,
2. Both primary prevention through changes in risk factor fundamentals and clinical research leading to better medical care probably have contributed to but did not fully explain the decline, and
3. A precise quantification of the causes requires further studies.

" In hindsight, the reformulation of hydrogenated fat with its lowering of the trans fatty acids and raising of linoleic acid could have also been responsible for the decline. The per capita consumption of hydrogenated fat continued to increase after 1950. However, the increase in the linoleic acid content in the reformatted 1968 fat and the increasing use of soybean oil in salad dressing and other food items could have helped to keep a decreasing death rate from CHD. The death rate from heart disease dropped substantially during the next decades even though the consumption of hydrogenated fat kept increasing and animal fat was decreasing. Lower trans fat and increased linoleic acid are possible explanations for this change.

The death rate from CHD declined after 1968 from 588.8/100,000 to 217/100,000 in 2004 in the USA. According to AHA data, 451,300 Americans died of CHD in 2004. Heart disease is still the number one cause of death. However, in a population of approximately 300 million, today the deaths would have been 1,480,000 at the 1950 rate according to the National Institute of Health (NIH). A recent study based on the autopsy of young men showed the CHD rate has been increasing since 2004. The recent reformulation of hydrogenated fat raises the trans fatty acid levels from 20% to almost 40%.

In 2003, the metabolism of the trans fat in hydrogenated oil was assumed to follow the same pathway as the natural ruminant trans fat in butterfat. The Food and Drug Administration has stated that the main reason for the trans fat in partially hydrogenated oil to remain in the diet in the USA rested on the generally held belief that trans fat is metabolized the same way as the natural trans (vaccenic acid) in butterfat. The FDA allowed the isomeric fatty

acids in hydrogenated vegetable oils to remain in food products because they assumed that some of that trans fat may be from the natural vaccenic acid that has no harmful effects. Approximately 2.6% of the total daily fat intake is from trans fat and that 50% of the trans may be from vaccenic acid ($18:1n^{11}$).

16. Conclusion

The oil produced by soybeans is widely used by manufacturers of both food products and industrial manufactured goods. Crude soybean oil contains essential fatty acids that our body needs to work properly. However, much of the soybean oil consumed today has been partially hydrogenated. This hydrogenation removes the necessary essential fatty acids contained within the original oil. Additionally, some of the partially hydrogenated soybean oil has been converted to trans fatty acids.

There are two mechanisms that have been shown to lead to heart disease involving the consumption of trans fatty acids. They effect the levels of prostacyclin and thromboxane, which increases the risk of thrombosis, and they increase sphingomyelin production by the body, which then causes calcium influx into the arterial cells to increase, leading to atherosclerosis. Soybeans can be an excellent source of protein, but partially hydrogenated soybean oil can be detrimental to health.

NC Soybean Producers Assn. How soybeans are used. Retrieved from http://www.ncsoy.org/ABOUT-SOYBEANS/Uses-of-Soybeans.aspx

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