

Review Article

Interaction between sphingomyelin and oxysterols contributes to atherosclerosis and sudden death

Fred A Kummerow

Burnsides Research Laboratory, Department of Comparative Biosciences, College of Veterinary Medicine, University of Illinois, 1208 W. Pennsylvania Avenue, Urbana, IL 61801, USA

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Abstract: Despite major public health efforts, coronary heart disease continues to be the leading cause of death in the United States. Oxidized lipids contribute to heart disease both by increasing deposition of calcium on the arterial wall, a major hallmark of atherosclerosis, and by interrupting blood flow, a major contributor to heart attack and sudden death. Oxidized cholesterol (oxysterols) enhances the production of sphingomyelin, a phospholipid found in the cellular membranes of the coronary artery. This increases the sphingomyelin content in the cell membrane, which in turn enhances the interaction between the membrane and ionic calcium (Ca^{2+}), thereby increasing the risk of arterial calcification. Patients undergoing bypass surgery had greater concentrations of oxysterols in their plasma than cardiac catheterized controls with no stenosis, and had five times more sphingomyelin in their arteries than in the artery of the placenta of a newborn. The oxysterols found in the plasma of these patients were also found in the plasma of rabbits that had been fed oxidized cholesterol and in frying fats and powdered egg yolk intended for human consumption. Together these findings suggest that oxysterols found in the diet are absorbed and contribute to arterial calcification. Oxidized low-density lipoprotein (OxLDL) further contributes to heart disease by increasing the synthesis of thromboxane in platelets, which increases blood clotting. Cigarette smoke and *trans* fatty acids, found in partially hydrogenated soybean oil, both inhibit the synthesis of prostacyclin, which inhibits blood clotting. By increasing the ratio of thromboxane to prostacyclin, these factors interact to interrupt blood flow, thereby contributing to heart attack and sudden death. Levels of oxysterols and OxLDL increase primarily as a result of three diet or lifestyle factors: the consumption of oxysterols from commercially fried foods such as fried chicken, fish, and french fries; oxidation of cholesterol *in vivo* driven by consumption of excess polyunsaturated fatty acids from vegetable oils; and cigarette smoking. Along with the consumption of *trans* fatty acids from partially hydrogenated vegetable oil, these diet and lifestyle factors likely underlie the persistent national burden of heart disease.

Keywords: Sphingomyelin, oxysterols, thromboxane, prostacyclin, *trans* fatty acids, calcium, stenosis

Introduction

Pohjantahti et al. [1] summarized 50 studies and concluded that the elevation of oxidized low-density lipoprotein (OxLDL) is a key event in the development of atherosclerosis. Diets enriched in oxidized fatty acids increase fatty streak lesions in the aorta of cholesterol-fed rabbits. Staprans et al. [2] fed rabbits a chow diet to which 0.33% cholesterol was added. The control group received cholesterol that had been stored at -70°C under N_2 to prevent oxidation. A second group received the same diet except approximately 5% of the total added cholesterol was oxidized. These rabbits

received 25 mg oxidized cholesterol per day. Five oxysterols were found in the plasma of these rabbits: 7 α -hydroxycholesterol, 7 β -hydroxycholesterol, beta-epoxycholesterol, alpha-epoxycholesterol, and 7-ketocholesterol. The percentage of aortic area covered by fatty streaks was twice as great in the rabbits receiving oxidized cholesterol as in the controls. In my laboratory, these five oxysterols were also found in elevated concentrations in the plasma of human patients who had undergone coronary artery bypass grafting (CABG) surgery, suggesting that they are important in the development of atherosclerosis in both animals and humans. Staprans, et al. demonstrated that oxidized

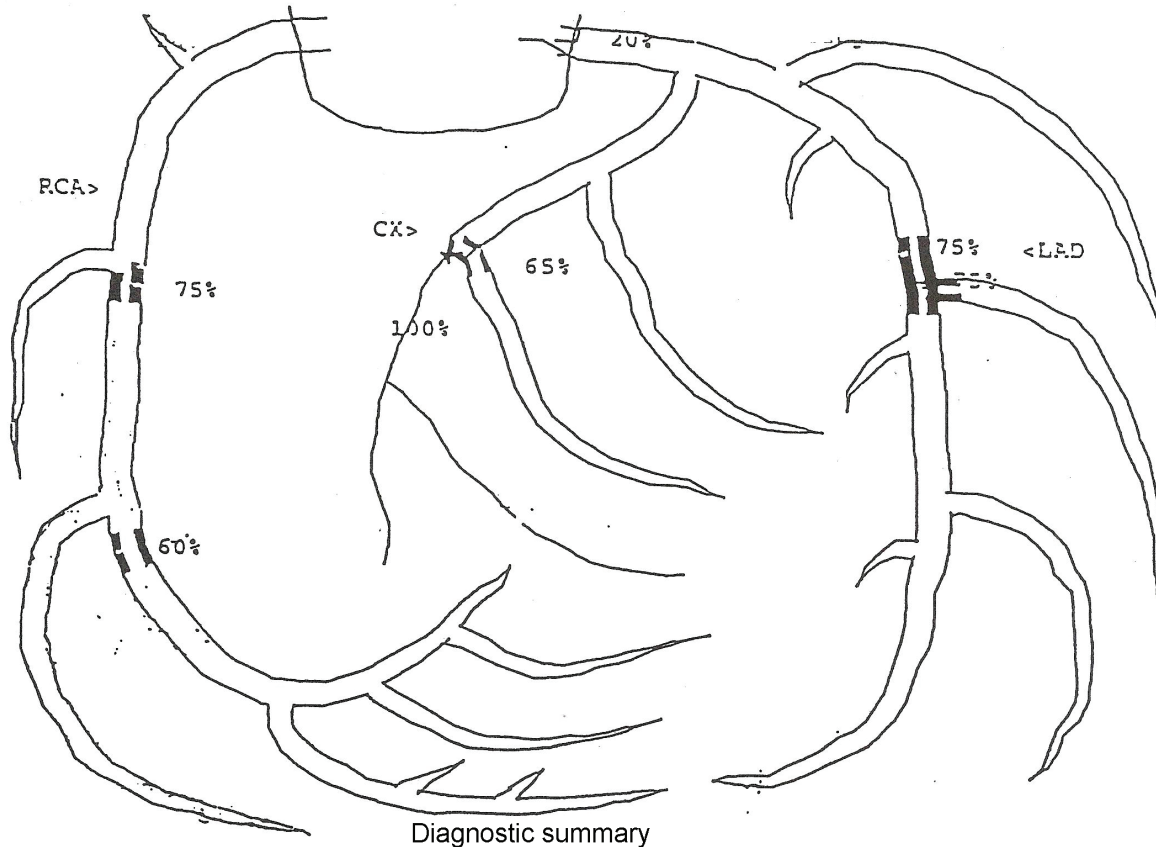


Figure 1. Diagnostic summary of the cardiac catheterized heart of a patient needing a coronary heart bypass grafting (CABG) surgery. The right coronary artery is 75% occluded. Circumflex artery is 100% occluded. The left one is 75% occluded and the right. (This figure is courtesy of Dr. Bruce Handler).

cholesterol in the serum of rabbits is both synthesized endogenously and derived from food. Oxysterols are synthesized endogenously via enzymatic or radical-mediated oxidation. Enzymatic oxidation mainly occurs in the liver and steroidogenic tissues. The radical species responsible for cholesterol oxidation are derived from activated oxygen, which could occur in a variety of tissues [3], including the artery cell wall [4-6]. It was also demonstrated that the absorption of dietary oxidized cholesterol occurred within 24 hours, after feeding trace amounts of radiolabeled oxidized cholesterol, in the livers of rabbits. Similar absorption of oxidized cholesterol has also been described in humans [7]. Thus, oxidized cholesterol in the diet as well as dietary and lifestyle factors that promote the endogenous oxidation of cholesterol likely play important roles in the development of atherosclerosis. This review describes research from my laboratory suggesting that these dietary and lifestyle factors contribute to

atherosclerosis through two mechanisms: sphingomyelin-mediated arterial calcification and thromboxane-mediated interruption of blood flow.

The role of sphingomyelin and calcium in the pathogenesis of atherosclerosis

In a review article entitled "The pathogenesis of coronary heart disease: Perspectives for the 1990s" [8], 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". These lesions are shown in (Figure 1) of a patient needing CABG surgery. The right coronary artery is 75% and 60% occluded, cir-

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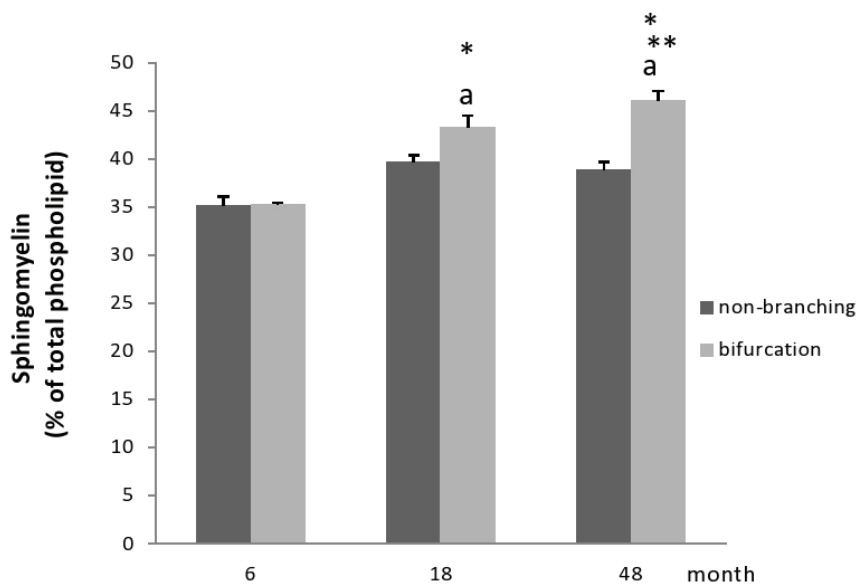


Figure 2. Composition of sphingomyelin in a non-branching segment compared to the branching segment of the same artery at bifurcations of 6, 18 and 48 month old swine kept on a cholesterol/saturated fat free diet. Means with a letter “a” are statistically different at $P < 0.05$, compare to the data from non-branching at the same age, Means with a symbol * or ** are statistically different at $P < 0.05$, compare to the data from the same artery at 6 or 18 months, respectively.

cumflex is 100% and 65% occluded and the left one is 75% occluded.

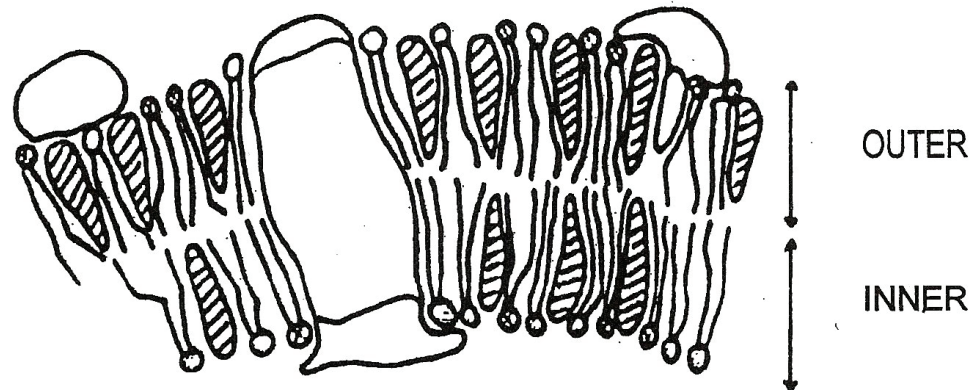
DeBakey et. al. [9] had noted thickening at branching and bifurcations during CABG surgery. Kummerow et. al. [10] also noted thickening in the branching arteries in aging pigs on a cholesterol-free diet. It did not differ significantly in sphingomyelin composition (**Figure 2**) from that of the non-branching adjacent tissue of pigs 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 branching points. Lipids extracted from both pig and human arteries indicated that aging is a factor that increases sphingomyelin. The non-branching segment of the aorta, obtained on autopsy from six men 21-27 years of age, contained four times more sphingomyelin than arteries isolated from human placenta, 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, the disease itself caused a premature increase in sphingomyelin in non-atheromatous arterial

cells in CABG patients, pointing to a fundamental disturbance in phospholipid metabolism in their arterial cells.





Kummerow et. al. [11] found patients who had CABG surgery sometimes needed a second CABG surgery because the vein used in the first surgery had become occluded. During this second surgery, an unoccluded vein from the same patient was used to replace the occluded vein. Approval for collection of discarded veins was provided by the University of Illinois and the Carle Foundation Institutional Review Boards. The occluded veins contained significantly more sphingomyelin and Ca^{2+} than the unoccluded veins. 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 need of the initial CABG surgery.

In humans, excess oxysterols stimulated the synthesis of sphingomyelin and inhibited sphingomyelin metabolism [12]. By using a radiolabeled methyl- ^3H choline, the time- and dose-dependent effects of 27-hydroxycholesterol on sphingomyelin synthesis could be observed. The increased radioactivity in sphingomyelin was accompanied by a decreased radioactivity in phosphatidylcholine. This result indicated that 27-hydroxycholesterol increased the trans-

LIPID COMPONENTS OF CELL MEMBRANE



MAJOR COMPONENTS

-  - CHOLESTEROL
-  - SPHINGOMYELIN (SP)
-  - PHOSPHATIDYL CHOLINE (PC)
-  - PHOSPHATIDYL ETHANOLAMINE (PE)

MINOR COMPONENTS






-  - PHOSPHATIDIC ACID (PA)
-  - CHOLESTEROL ESTER (CE)
-  - LYSOPHOSPHATIDYLETHANOLAMINE (LPE)
-  - PHOSPHATIDYLSERINE (PS)
-  - LYSOPHOSPHATIDYLCHOLINE (LPC)

Figure 3. Lipid Components of Cell Membrane. Cell membrane consists of 43% PC, 19% PE, 14% PS, 10% SP, and 3% LPC in the artery of an umbilical cord.

fer of choline from phosphatidylcholine to sphingomyelin. It is known that oxysterols could enhance $^{45}\text{Ca}^{2+}$ uptake and cytosolic free Ca^{2+} . The enhanced Ca^{2+} uptake has been thought to come from greater membrane permeability by oxysterols [13, 14]. By measuring parallel cytosolic $^{45}\text{Ca}^{2+}$ uptake, cytosolic free Ca^{2+} , choline label in sphingomyelin and cytotoxicity in the presence of 27-hydroxycholesterol we observed that enhanced $^{45}\text{Ca}^{2+}$ uptake and cytosolic free Ca^{2+} level occurred earlier than cytotoxicity, but later than increased radioactivity in sphingomyelin. This finding suggests that enhanced $^{45}\text{Ca}^{2+}$ uptake may result from an increase in sphingomyelin synthesis and not from the cytotoxicity of 27-hydroxycholesterol. This is further supported by evidence from previous studies [15-17]. Deposition of calcium in the coronary artery has been found to be directly proportional to an elevated sphingomyelin concentration [16]. Just as with 27-hydroxycholesterol, 25-hydroxycholesterol increased both $^{45}\text{Ca}^{2+}$ uptake and sphingomyelin composition

[15]. Since sphingomyelin is located on the exterior of the plasma membrane [17] and has an exposed polar head group accessible to the aqueous environment, the negative charge on the exterior side on sphingomyelin would be accessible for ionic bonding with Ca^{2+} .

27-Hydroxycholesterol at a level of $0.1\mu\text{g}/\text{mL}$, which is within the range of its plasma concentration in healthy adults [18] had no obviously stimulating effect on the incorporation of choline label into sphingomyelin from phosphatidylcholine during 15d of treatment. When the level was increased to $0.5\mu\text{g}/\text{mL}$, however, it took only 3d for 27-hydroxycholesterol to increase radioactivity in sphingomyelin. These results indicate that 27-hydroxycholesterol only increases transfer of choline from phosphatidylcholine into sphingomyelin when it is present in concentrations higher than those found in healthy adults. This was consistent with the reports that both 27-hydroxycholesterol and sphingomyelin increase in atherosclerosis [19].

Spingomyelin, oxysterols, atherosclerosis and sudden death

Table 1. Oxysterols in the plasma from CABG patients compared to controls legend

	WOMEN UNDER 60 YEARS		MEN UNDER 60 YEARS		WOMEN OVER 60 YEARS		MEN OVER 60 YEARS	
	Controls	Patients	Controls	Patients	Controls	Patients	Controls	Patients
Number	7	7	8	8	8	8	9	9
7 α -OHC	22.5 \pm 5.7	27.2 \pm 8.8	21.3 \pm 5.4	29.1 \pm 4.6 ^b	26.6 \pm 4.2	26.8 \pm 8.1	22.1 \pm 3.6	26.3 \pm 6.9
7 β -OHC	17.7 \pm 4.4	18.6 \pm 2.7	14.4 \pm 2.8	21.5 \pm 4.4 ^b	18.6 \pm 6.5	23.4 \pm 3.9	18.1 \pm 5.2	21.5 \pm 6.6
β epoxy-C	25.2 \pm 6.9	42.4 \pm 9.5 ^b	24.0 \pm 4.6	34.6 \pm 7.4 ^b	31.4 \pm 9.3	36.4 \pm 8.6	29.1 \pm 8.5	37.8 \pm 8.6 ^a
α epoxy-C	16.5 \pm 4.6	20.7 \pm 3.1	17.7 \pm 2.5	19.1 \pm 1.7	20.8 \pm 5.9	21.5 \pm 6.2	18.8 \pm 3.7	21.8 \pm 5.5
Triol-C	26.1 \pm 4.8	36.9 \pm 17.2	20.6 \pm 7.5	34.6 \pm 6.3 ^b	29.2 \pm 8.3	38.1 \pm 3.9 ^a	29.3 \pm 9.3	35.7 \pm 9.4
7keto-C	27.4 \pm 6.5	35.9 \pm 12.2	23.0 \pm 3.6	31.9 \pm 7.1 ^b	30.8 \pm 5.5	39.1 \pm 6.2 ^a	29.3 \pm 8.4	34.5 \pm 8.6
27-OHC	20.6 \pm 3.9	23.5 \pm 6.9	19.4 \pm 4.8	20.9 \pm 5.0	20.3 \pm 4.9	25.8 \pm 2.4 ^a	20.4 \pm 2.1	24.2 \pm 2.1 ^b
Total	156.3 \pm 24.2	200.8 \pm 31.1 ^a	141.0 \pm 18.7	191.7 \pm 20.2 ^b	177.9 \pm 24.2	211.3 \pm 13.4 ^b	167.2 \pm 23.5	201.8 \pm 25.3 ^b

7 α -OHC: 7 α -hydroxycholesterol, 7 β -OHC: 7 β -hydroxycholesterol, β epoxy-C: β -epoxycholesterol, α epoxy-C: α -epoxycholesterol, triol-C: cholestane-3 β , 5 α , 6 β -triol, 7keto-C: 7-ketocholesterol, 27-OHC: 27-hydroxycholesterol. Results are expressed as ng oxysterols / ml plasma and given as mean \pm SD. ^ameans within the same line with a superscript letter are statistically different at levels of $P < 0.05$. ^b $P < 0.01$, compared with controls.

Table 2. ⁴⁵Ca²⁺ influx into the endothelial cells cultured for 72 hours with the plasma from CABG patients or from age- and sex-matched controls with or without added oxysterols

treatment	⁴⁵ Ca ²⁺ influx (CPM / μ g protein)
control plasma	26 \pm 51 ^{a,b}
patient plasma	68 \pm 7.1 ^a
control plasma \pm oxysterols	60 \pm 6.3 ^b

Results are expressed as mean \pm S.D. from twelve separate experiments. Mean values with a common letter are significantly different at a level of $p < 0.05$. "a" is significant at 1% level, "b" is significant at a 5% level.

Lipid analysis of plasma and arterial tissue obtained from CABG patients

Lipid analysis of the plasma and arterial tissue obtained prior to and during CABG surgery revealed, when compared to controls, a higher concentration of oxysterols in the plasma, and a significantly higher concentration of sphingomyelin in arterial tissue [11] (**Table 1**). Seven oxysterols were found in the plasma; 7 α -hydroxycholesterol, 7 β -hydroxycholesterol, β -epoxycholesterol, α -epoxycholesterol, and 7-ketocholesterol, 27-hydroxycholesterol and cholestane-3 β 5 α , 6 β -triol. Five of these have been identified in the liver of rabbits fed oxidized cholesterol, and the last two were found in fried fat and egg yolk powder [19]. Smith [19] listed 27-hydroxycholesterol, which occurs in fried fats, as "very toxic." These results suggest these oxysterols are absorbed from the diet and may originate from commercially fried and processed foods. Women under 60 years of age who underwent CABG surgery had 45 ng/mL more free oxysterols in their plasma than controls (200.8 ng/mL vs. 156.3 ng/mL; $p < 0.05$). Likewise, men under 60 years of age who underwent CABG surgery had 50.7 ng more free oxysterols in their plasma than controls (191.7 vs. 141.6 ng/mL; $p < 0.01$). Similar

results were found when CABG patients were compared to controls among women (211.3 vs. 177.9 ng/mL; $p < 0.01$) or men (210.8 vs. 167.2 ng/mL; $p < 0.01$) who were over the age of 60. Approximately five times more sphingomyelin was found in the coronary artery tissue from CABG patients (48.2%) than arterial tissue from umbilical cords (10.0%) (**Figure 3**).

Human endothelial cells were cultured for 72 hours in a medium containing plasma obtained from CABG patients or from controls and then pulsed with calcium (⁴⁵Ca²⁺) for one hour. A significantly higher influx of ⁴⁵Ca²⁺ was noted in the endothelial cells cultured in the plasma obtained from CABG patients. Five purchased oxysterols (7-keto-cholesterol, cholestane-3 β , 5 α , 6 β -triol, 7 β -hydroxycholesterol, β -epoxycholesterol, and 7 α -hydroxycholesterol) were then added to plasma from controls to make the total oxysterol level equivalent to that in the plasma from CABG patients. Control plasma enriched with oxysterols increased influx of ⁴⁵Ca²⁺ to the same degree as plasma from CABG patients (**Table 2**) [11]. These results showed that the greater concentration of oxysterols in the plasma of CABG patients was responsible for the greater ⁴⁵Ca²⁺ influx, and suggested that oxysterols contribute to arterial calcification in humans *in vivo*.

Phospholipid, oxysterols, atherosclerosis and sudden death

Table 3. Phospholipid composition in plaque and non-plaque tissue from the carotid arteries of the same coronary artery bypass grafting surgery patients

Phospholipid (%)	non-plaque	plaque
Lysophosphatidylcholine	8.33 ± 2.6 ^a	8.78 ± 2.3 ^a
Sphingomyelin	50.93 ± 2.62 ^b	59.95 ± 4.35 ^b
Phosphatidylcholine	25.77 ± 4.37	23.40 ± 2.34
Phosphatidylethanolamine & Phosphatidylserine	14.03 ± 4.36	7.28 ± 1.73

Results are expressed as mean ± S.D. from three patients. Mean values with a common letter are significantly different at a level of $p < 0.05$. "a" is significant at 1% level, "b" is significant at a 5% level.

Table 4. Phospholipid composition in the hard and the surrounding soft tissue from the coronary arteries of the same non-coronary artery bypass grafting surgery patients

Phospholipid (%)	soft	hard
Lysophosphatidylcholine	6.58 ± 2.40 ^a	10.64 ± 2.26 ^a
Sphingomyelin	38.3 ± 3.96 ^b	49.10 ± 3.38 ^b
Phosphatidylcholine	34.2 ± 3.78	30.70 ± 3.57
Phosphatidylethanolamine & Phosphatidylserine	20.4 ± 5.4	9.48 ± 3.2

Results are expressed as mean ± S.D. from ten patients. Mean values with a common letter are significantly different at a level of $p < 0.05$. "a" is significant at 1% level, "b" is significant at a 5% level.

The concentration of cholesterol and lipid oxidation products in the plasma of cardiac catheterized patients

The concentration of cholesterol, lipid oxidation products, and total antioxidant capacity in the plasma of 2,000 cardiac catheterized patients with 0, 10–69, and 70–100% stenosis of their arteries were analyzed [20]. The results showed that lipid oxidation products increased with the severity of 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. Therefore, the concentration of oxidation products rather than the concentration of cholesterol in the plasma increased with the severity of coronary heart disease. All of the women and men, in all age groups, with cardiovascular coronary heart disease also had increased individual and total oxysterol levels in their plasma compared with the controls.

Lipid composition and calcium concentration in plaque tissue of the carotid and coronary arteries

The lipid composition and calcium concentration in plaque tissue of the carotid and coronary arteries were analyzed. The total phospho-

lipid concentration in the plaque of the carotid arteries of the CABG surgery patients did not differ from that in the non-plaque area from the same patient (**Table 3**). However, the percentages of individual phospholipids were changed. The percentage of sphingomyelin in the plaque was more than 20% higher than in the non-plaque tissues from carotid arteries. In coronary arteries, an almost 20% increase of sphingomyelin was also observed in the hard areas which would form a plaque later. In these areas, the calcium concentration also significantly increased to 23.6 ± 12.1 mg/g tissue, compared to 5.0 ± 1.02 mg/g tissue in the surrounding soft areas (**Table 4**).

An increased lysophosphatidylcholine percentage in the hard tissue was supported by a report that expression of lysophosphatidylcholine and lipoprotein-associated phospholipase A₂ was higher in carotid plaques of patients with than without cardiac events [21]. This increase may also indicate more oxidation of LDL trapped within the arterial tissue, which leads to more lysophosphatidylcholine and oxysterol formation. The increased lysophosphatidylcholine would accelerate the atherogenesis because lysophosphatidylcholine was involved in the formation of calcified nodules in the atherosclerotic plaques [22].

Phingomyelin, oxysterols, atherosclerosis and sudden death

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

Years	1912		1950		1999		2011	
Items	Total*	Per cap	Total*	Per cap	Total*	Per cap.	Total*	Per cap
Corn oil	53.0	0.6	223.0	1.47	1416.9	5.2	1620.0	5.2
Cottonseed oil	950.0	10.0	1445.0	9.51	832.8	3.1	620.0	2.0
Olive oil	43.0	0.5	76.0	0.50	329.8	1.2	650.1	2.1
Palm oil	0.0	0.0	26.0	0.17	416	1.5	2525.2	8.1
Palm kernel oil	0.0	0.0	26.0	0.17	233.2	0.9	778.0	2.5
Peanut oil	8.0	0.1	103.0	0.68	1524.7	5.6	202.7	0.6
Canola oil	0.0	0.0	0.0	0.00	111.2	0.4	4249.0	13.6
Safflower oil	0.0	0.0	5.1	0.03	15.8	0.1	60.9	0.2
Sesame oil	0.0	0.0	5.0	0.03	15.8	0.1	27.2	0.1
Soybean oil	16.0	0.2	1446.0	9.51	8029.6	29.4	9000.0	28.8
Sunflower oil	0.0	0.0	0.5	0.00	393.7	1.4	395	1.3
Total unsaturated oils	1070.0	11.3	3355.6	22.1	13319.5	48.8	20128.1	64.5
Lard	1069.0	11.2	1891.0	12.60	202.0	0.7	480.0	1.5
Butter	1579.0	16.6	1648.0	10.70	1307.0	4.8	1510.0	4.8
Tallow	22.0	0.2	69.0	0.45	996.0	3.6	1050.0	3.4
Coconut	0.0	0.0	69.0	0.45	927.0	3.4	1155.1	3.7
Total saturated fats	2670.0	28.0	3677.0	24.2	3432.0	12.6	4195.1	13.4
US Population in millions	95		152		273		312	

(*) Totals in millions of pounds. Courtesy Mark Ash, of U.S. Department of Agriculture. http://factfinder2.census.gov/faces/tableservices/jsf/pages/productview.xhtml?pid=ACS_11_1YR_DP05&prodType=table. http://www.npg.org/facts/us_historical_pops.htm. <http://www.census.gov/population/estimates/state/st-99-1.txt>.

The influence of thromboxane and prostacyclin in blood flow

The presence of thromboxane, a potent inducer of vasoconstriction and platelet adhesion, in the arteries is responsible for the interruption of blood flow, causing the clogging of the arteries and therefore sudden death [23]. Mahfouz and Kummerow showed that the components of oxidized low-density lipoprotein (OxLDL) were responsible for platelet sensitization to thrombin and the increase of thromboxane release. More significant, we found that OxLDL at low concentrations is more potent in enhancing the platelet response to thrombin and to increasing thromboxane release than native LDL [24]. In contrast, prostacyclin is vasoprotective and keeps blood flowing. It is a dominant prostaglandin produced by endothelial cells and is a potent vasodilator and inhibitor of platelet aggregation and leukocyte adhesion [25]. However, smoking cigarettes and consuming *trans* fatty acids in partially hydrogenated fats in the diet inhibits prostacyclin generation by the coronary arteries [26, 27].

Cigarette smoke is a major risk factor for atherosclerosis and is associated with coronary, cerebral, and peripheral vascular disease. Cigarette smoke is a mixture of gases and particulate matter. Each puff has been reported to contain 10^{14} free radicals in each phase (soluble and particulate) [28]. Both phases contain high concentrations of reactive oxygen species, nitrogen oxide, peroxyrate, and free radicals

of organic compounds which cause two major processes: the oxidation of LDL and the inhibition of prostacyclin synthesis, both dangerous for life [25]. According to Maddox, sudden cardiac death is the largest cause of natural death in the U.S., causing about 325,000 adult deaths in the United States each year [29].

Antioxidants

Feeding rabbits a high-cholesterol diet supplemented with antioxidants prevented the intimal thickening of aortas, even though their blood continued to have a cholesterol level 40 times higher than control rabbits, but their plasma oxysterol levels decreased significantly compared to the rabbits fed without antioxidants [30]. The same results occurred in hyperlipidemic chickens fed vitamin E, where lipid peroxidation and coronary heart disease lesions significantly decreased while hyperlipidemia remained high [31].

Data obtained from the US. department of agriculture and U.S. census bureau

Data from the U.S. Department of Agriculture indicated that from 1912 through 2011, the consumption of polyunsaturated fat has increased from 11.3 pound per capita to 64.5 pounds per capita and saturated fat has decreased from 28 pounds per capita to 13.4 pounds per capita in the same period (Table 5). [32] <http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1290>,

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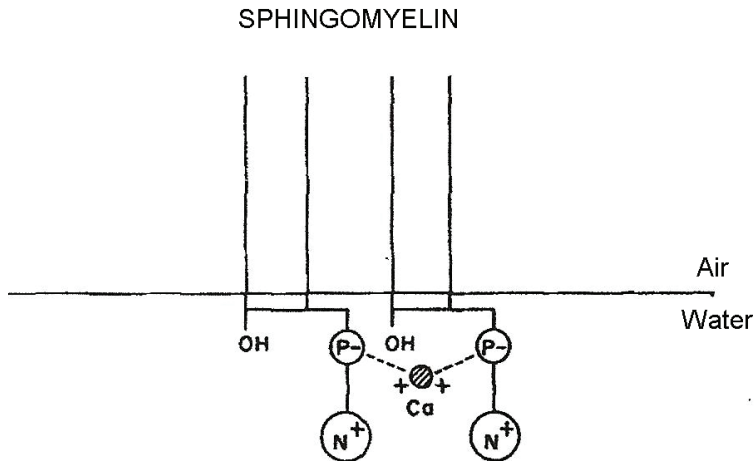


Figure 4. Schematic representation of the interaction of calcium ion with sphingomyelin monolayer. By Shah and Schulman with permission [37].

A report from the Inter-Society Commission for Heart Disease Resources [33] stated: "Use salad and cooking oils, new soft margarines and shortenings low in saturated fat" and "avoid butter, margarine and shortenings high in saturated fats;" which I did not agree with, because the consumption of polyunsaturated fats increase the production of oxysterols [34-36].

Conclusion

My hypothesis for the cause of heart disease and sudden coronary death is based on the composition, structure, and biochemistry of the coronary arteries. The composition of the cellular membrane has five different phospholipids which change in composition during the life time regardless of whether you have heart disease or not. One of these phospholipids, sphingomyelin, increases from 10% at birth to 48% in someone who had a bypass surgery CABG and to 60% in patients who have plaques in their coronary arteries.

The sphingomyelin and calcium that occluded the veins and arteries of these patients was possible because of the binding of calcium to sphingomyelin. According to Shah and Schulman [37], the binding of Ca^{2+} to monolayers of phospholipids such as sphingomyelin happens because of the hydrogenation of sphingomyelin in the presence of water. This hydrogenation turns a normally neutral sphingomyelin into an anion with two negative charg-

es and therefore Ca^{2+} is attracted to sphingomyelin and binding occurs **Figure 4**. Direct evidence showing that metal ions do bind to fatty acid monolayers has been given by Langmuir and Schaefer [38] as well as by Schulman and Dogan [39], who removed the monolayers from the interface and subjected them to chemical analysis. In the case of phospholipids the interaction of Ca^{2+} can be indicated as $2\text{PO}_4^- + \text{Ca}^{2+} \rightleftharpoons \text{PO}_4^- \cdots \text{Ca}^{2+} \cdots \text{PO}_4^-$. At high salt concentration the amount of bound Ca^{2+} indicates an interaction between metal ions and the monolayer. The main generator of atherosclerosis

and sudden death are the oxysterols in excess. Normal levels of oxysterols in the plasma will not cause phosphatidylcholine to convert into sphingomyelin and therefore less calcium will bind to it resulting in less artery blockage.

Data from the U.S. Center of Disease Control, http://www.cdc.gov/nchs/data/dvs/lead-1900_98.pdf, http://www.cdc.gov/nchs/data/nvsr/nvsr60/nvsr60_04, shows that death due to heart disease decreased from 1970 to 2010 by 19%. It also shows that smoking habits decreased significantly by 43% in the same period. Since cigarette smoking also causes heart disease, the 19% death decrease due to heart disease in the last 40 years may be because of the major decrease in smoking habits. In 2010 the total number of deaths was 600,000, with half caused by sudden death. Therefore the death rate due to coronary heart disease and sudden death remains high. According to the U.S. Department of Agriculture data [32] we have switched from the consumption of saturated fats to polyunsaturated fats, which now are in almost everything that is consumed. Vegetables oils, partially hydrogenated fats, and fried foods are responsible for the persistently high rate of heart disease. The most effective way to prevent coronary heart disease and sudden death according to these conclusions is to eat fewer commercially fried foods, fewer polyunsaturated fats and to avoid partially hydrogenated fats. Conversely, we should eat more vegetables and fruit as a source of antioxidants.

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Address correspondence to: Dr. Fred A Kummerow, Burnsidess Research Laboratory, 1208 W. Pennsylvania Avenue, Urbana, IL 61801, USA. Tel: 217-344-6380; Fax: 217-333-7370; E-mail: fkummerow@illinois.edu

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