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Nature and Nurture in Atherosclerosis: The Roles of Acylcarnitine and Cell Membrane-Fatty Acid Intermediates

Harry C Blair^{1,2,*}, Jorge Sepulveda³, and Dionysios J. Papachristou^{2,5,*}

¹Pittsburgh VA Medical Center, Pittsburgh, PA 15261

²Department of Pathology, University of Pittsburgh, Pittsburgh, PA 15261

³Department of Pathology & Cell Biology, Columbia University College of Physicians and Surgeons, New York, NY 10032

⁵Department of Anatomy-Histology-Embryology, and the Unit of Bone and Soft Tissue Studies, University of Patras Medical School, Patras, Greece

Abstract

Macrophages recycle components of dead cells, including cell membranes. When quantities of lipids from cell membranes of dead cells exceed processing capacity, phospholipid and cholesterol debris accumulate as atheromas. Plasma lipid profiles, particularly HDL and LDL cholesterol, are important tools to monitor atherosclerosis risk. Membrane lipids are exported, as triglycerides or phospholipids, or as cholesterol or cholesterol esters, via lipoproteins for disposal, for re-use in cell membranes, or for fat storage. Alternative assays evaluate other aspects of lipid pathology. A key process underlying atherosclerosis is backup of macrophage fatty acid catabolism. This can be quantified by accumulation of acylcarnitine intermediates in extracellular fluid, a direct assay of adequacy of β -oxidation to deal with membrane fatty acid recycling. Further, membranes of somatic cells, such as red blood cells (RBC), incorporate fatty acids that reflect dietary intake. Changes in RBC lipid composition occur within days of ingesting modified fats. Since diets with high saturated fat content or artificial trans-fatty acids promote atherosclerosis, RBC lipid content shifts occur with atherosclerosis, and can show cellular adaptation to pathologically stiff membranes by increased long-chain doubly unsaturated fatty acid production. Additional metabolic changes with atherosclerosis of potential utility include inflammatory cytokine production, modified macrophage signaling pathways, and altered lipid-handling enzymes. Even after atherosclerotic lesions appear, approaches to minimize macrophage overload by reducing rate of fat metabolism are promising. These include preventative measures, and drugs including statins and the newer PCSK9 inhibitors. New cell-based biochemical and cytokine assays provide data to prevent or monitor atherosclerosis progression.

*To whom correspondence should be addressed: Harry C. Blair, Department of Pathology, S705 Scaife Hall, 3550 Terrace St., Pittsburgh, PA 15261, Tel: (412) 383-9616; FAX: (412) 647-8567; hclair@pitt.edu, or to Dionysios J. Papachristou, University Campus, Building of Preclinical Studies, 2nd Floor, Room B48, 26504 Rion-Patras, Greece, Tel.: (0030) 2610-969188; Fax: (0030) 2610-969178; papachristoudj@med.upatras.gr or dip13@pitt.edu.

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Graphical abstract

Keywords

Atherogenesis; carnitine; PCSK9; statin; coenzyme A; cholesterol synthesis; HDL; LDL; Fc γ

Introduction

Inherited lipoprotein receptor defects cause severe atherosclerosis, but in most people environmental factors are important promoters of atherosclerosis. The pioneering work of Brown and Goldstein followed LDL receptor defects that have devastating consequences, with early and very severe atherosclerosis [1]. However, most people with atherosclerosis do not have receptor or carrier defects, or at least not such serious abnormalities as would lead to atherosclerotic disease without other factors to promote the development of atherosclerosis. This is clear from historical data. Stroke and heart attack were uncommon causes of death before 1920, even when data are corrected for age, and increased ~ seven fold from 1900 to 1950 [2]. There are many consistent studies, such as a landmark study of deaths attributed to coronary atherosclerosis in a series of autopsies in London for over a century from 1865 [3]; this key report has an abundance of data that can be presented in a variety of ways: Figure 1 shows the occurrence of deaths from ischemic heart disease per over age 40 as percent, and dissecting aortic aneurysms. While the data are biased by the increase in age at death, by about 10 years from 1920–1950, the size of the effect is similar to that noted by Morris [2], and so large that there can be no doubt that death from atherosclerosis many fold from 1900 to 1950.

There is a strong effort to arrest, pharmacologically, atherosclerosis in adults at high risk based on lipid profiles and other data. This complex topic, where important progress has been made both in management of serum lipid profiles, and in improved control of related co-morbidities including blood pressure and adult onset diabetes, is very well reviewed elsewhere and is not addressed here [4]. On the other hand, there are new approaches that might, by different mechanisms, interrupt or reverse plaque development; these include limiting availability of the lipid carrier carnitine to prevent overload of macrophages with

fat, and thus interrupting the vicious cycle of cell death and atherosclerosis, as reported in this issue in an atherogenic mouse model [5, in press].

We conclude from the historical data that atherosclerotic disease is to a large extent environmentally promoted. Hardly a shocking assertion, although for the generations born after World War II, the change in incidence may be surprising. The environmental factors include smoking, insufficient exercise, and diet. Smoking is approximately an equal risk factor to elevated LDL cholesterol for early lesions in young people [6]; an encouraging trend toward declining heart attack and stroke death since 2000 may reflect in part a decline in smoking, in one example smoking rates in Men in Spain declined from 60% in 1960 to 30% in 2005 [7]; many other examples show similar changes over this period. Regular moderate exercise is a strong factor for resistance to heart attacks and strokes [8]. In exercise, much less progress has been made than in smoking, although some segments of the population are exceptions. Neither smoking nor exercise will be considered further.

Diet is a third major environmental factor. We will focus on this, and its biochemical mechanisms in lipid-driven pathology. Dietary changes in cell membrane lipids, lipid catabolism, and the effect of cell membrane lipids on atherosclerosis progression, are an evolving subject. We will not discuss obesity although obesity is related to dietary risk and to development of type 2 diabetes.

Lipid components of the diet and atherosclerotic risk

It is accepted that diets with excess saturated fat are unhealthy, as are diets with any significant amount of artificial trans-unsaturated fatty acids. On the other hand, how these dietary effects translate into atherosclerosis is less clear, and the literature is opaque due to odd and inconsistent nomenclature. Fatty acids are organic acids with a tail of carbons, including a variable number of double bonds. Almost all cell membrane phospholipids contain fatty acids of 14 to 22 carbons. All mid-chain natural double bonds are *cis* (Fig 2), *Z* isomers in formal IUPAC nomenclature; trans-bonds are *E* isomers. Trans bonds have lower free energy and are strongly favored in non-enzymatic synthesis. Natural (*cis*) double bonds are enzymatically produced by dehydrogenation at 3-carbon increments; the double bonds are not conjugated. *Exceptions* include trans bonds between carbons 2 and 3 from the parent carboxyl chain in specialized derivatives including sphingosine, an important amino-alcohol synthesized from serine and palmitoyl CoA, and 2,3 trans bonds specifically synthesized during catabolism of double bonds (Fig 2A). Double bonds at odd numbered carbons are out of phase with the 2–3 reductases, and require an additional step in catabolism, mediated by enoyl CoA isomerases (Fig 2). These enzymes vary in efficiency depending on substrate, which can cause a backup in β oxidation with important consequences [9]. Serum acylcarnitine analysis can quantify the state of β oxidation, considered further below

Linoleic acid, C18:2 9,12 *cis, cis*, is an essential nutrient, alternatively “vitamin F”. Linoleic acid is required for synthesis of arachidonic acid, which comprises ~ 20% of RBC membrane fatty acids. Arachidonic acid, 5Z,8Z,11Z,14Z-5,8,11,14-eicosatetraenoic acid, is also the substrate for prostaglandin synthesis. Omega, ω , names refer to double bond positions from the *distal* end of fatty acids. This is often used to identify unsaturation 3 or 6

carbons from the end. These bonds cannot be synthesized in mammals. They occur in vegetable or fish oils. Linoleic acid is ω 6, ω 9-octadecadienoic acid. A common abbreviation replaces “-ic acid” with “-ate”. This will be used below: oleic acid is oleate.

Animal fats contain ~ 50% saturated fatty acids (Fig 2), of this about a quarter is C18:0, stearate, and there is typically twice as much C16:0, palmitate as stearate.

Vegetable oils, for example soy, are largely unsaturated fatty acids. Most of these occur as doubly or triply (poly) unsaturated fatty acids, with variable ω -3 and ω -6 content. This high proportion of unsaturated fatty acids is 100% *cis* in unmodified vegetable oil. Typically 90% of fatty acids in vegetable oils are C18; animal fat has some C14 and a lot of C16 fatty acid.

Trans bonds occur in some natural fatty acids, but in central regions of long chain fatty acids they are not natural; these result from partial hydrogenation. Unsaturated fatty acids with trans-bonds are stiff and have high melting points; the singly-unsaturated *cis*-fatty acid oleate is a liquid at room temperature; its *trans*-isomer, elaidate, is a hard wax (Fig 2). Elaidate, (E)-octadec- 9-enoic acid, comprises the major proportion of *trans* fatty acid in partially hydrogenated oil. It is very stable. Its *trans* bond, at C9–C10, is in the middle of the chain *and* an odd number of carbons from the carboxy group, thus requiring enoyl CoA isomerase for oxidation (Fig 2).

Hydrogenated oils were estimated to account for in excess of 7% of fatty acid calories in the US in 1999 [10]; this has dropped to approximately 2.5% in 2015, and may go to near zero if use of partially hydrogenated oils is phased out. Industrial partial hydrogenation produces artificial *trans* fatty acids, particularly elaidate (Fig 2B). Hydrogenation, bubbling hydrogen gas through oil in a closed container with a catalyst, was developed by Paul Sabatier in the late 1800s. He received the Nobel Prize for direct hydrogenation [11], which when applied to vegetable oils, produces stable solid shortening that has much longer shelf life than the oils. Hydrogenation, *if complete*, produces 100% saturated fatty acids, mainly stearate from vegetable oils. *Partial* hydrogenation converts, in unreduced bonds, *cis*-unsaturated bonds almost entirely to *trans*. This reflects the lower free energy of the *trans* isomers. Double bonds in the carbon chain form isomers with double bonds at positions from $\otimes$ C4 and $\otimes$ C14, but predominately $\otimes$ C9. Bonds at even numbered positions are directly oxidized, but unfortunately are uncommon. During β -oxidation, these are in phase with *trans* 2,3 CoA reductase (Fig 2A). Hydrogenated oils have similar characteristics to saturated fatty acids in food, and were proposed to be preferable to saturated fat. With lipid profiles and metabolic screening, this conclusion was revised [12]. Consumption of partially hydrogenated oils results in insertion of the *trans*-fatty acids, within days, into newly synthesized cell membranes, including RBC membranes, in a similar distribution to that consumed [13]. The consequences of this are discussed further below.

Dietary fatty acids and health: Complex issues

Increasing evidence indicates that both *trans*-unsaturated and large amounts of saturated fatty acids, long chain in particular, have damaging effects on the human health. Part of this is indirect, in contributions to obesity and diabetes, as well as to heart disease [14]. Diets including of high amounts of long-chain saturated fatty acids or small amounts of *trans* fatty

acids promote atherosclerotic lesions [15]. The major saturated fatty acids in human diets from animal fats are palmitate (C16:0) and stearate (C18:0), together 90% of the saturated fatty acids eaten. Two thirds of this is palmitate; ~80% of tissue cell membrane saturated fatty acid is palmitate [15,16].

Natural fatty acids with trans-double bonds occur, which generally are not toxic, or at least much less so than elaidate. Naturally occurring trans fatty acids include conjugated linoleate (C18:2, 9 *cis*, 11 *trans*) and vaccenic acid, C18:1 11 *trans* (or ω -7 *trans*). Conjugated linoleic acid and vaccenic acids are produced in ruminant cattle and occur in meat and dairy products. These trans fatty acids are degraded with fewer problems. This may reflect that the efficiency of the enzymes required vary with substrate chain length, and that the quantitative loads are lower.

A vicious cycle of cell death-induced cell death contributes to progressive atherosclerosis

The vascular intima amasses caseous lipid-rich cellular debris known as plaque in advanced atherosclerotic disease, with the plaque surrounded by reactive macrophages, many with lipid inclusions, called foam cells (modeled *in vitro* using human macrophages, Fig 3), surrounded by proliferating stromal cells. The healthy vascular intima has no cellular debris, no reactive macrophages, and stable stromal composition. In studies focused on the composition of arterial plaque, researchers have found that the caseous lipid debris is a mixture with large amounts of cholesterol and oxidized phospholipids [17], with fat-engorged macrophages [18], and proliferating smooth muscle cells, with complex biochemical interactions of macrophage and smooth muscle components [19]. The caseous debris includes dead cells, both macrophages and stromal cells. However, the normal role of the macrophages is phagocytosis with processing of cellular debris, including lipids. The completeness and speed of this process determines whether the vessel wall is restored to normal or whether a plaque will form. Ignoring partial oxidation effects, the profile of lipids in an atherosclerotic vascular sections and in RBC from the same subject is essentially the same, in fatty acid chain length, saturation, and percent cholesterol (30–40%). This reflects that membranes of dead cells are un-degraded lipids. In long-standing plaque, typically some regions are converted to scars with dense collagen and mineral nodules, while regions around caseous debris remain to show active, progressive disease.

Free fatty acids are toxic; inside of cells they are carried as thioesters or esters by coenzyme A or by L-carnitine respectively. Coenzyme A is the primary carrier. Carnitine is a secondary carrier, essential for translation of fatty acids across the inner and outer mitochondrial membranes, a process involving carnitine-carnitoylpalmitate transferases I and II respectively. The system is regulated by malonyl CoA, a gatekeeper for fatty acid uptake and β -oxidation versus fat synthesis and export [20]. Carnitine is synthesized primarily in the liver and kidney from lysine and methionine and also acquired from diet; on a Western diet, a large proportion of carnitine is absorbed, a vegan diet provides no exogenous carnitine [21]. The carnitine enantiomer D-carnitine is biologically inactive in this system and not discussed further. At very high doses, on the order of 5 grams, absorption drops to low levels, while in a diet containing meat with on the order of 50 mg,

absorption of functional carnitine may reach ~ 80% [21]. Carnitine is a rate-limiting carrier, and is used as “nutraceutical” that is used to promote mitochondrial enzyme complex function in endurance athletes, as a potential treatment for the metabolic syndrome, and as a potential therapy to reduce cardiovascular risk [21,22,23]. The most recent data suggest that cardiovascular risk may on the contrary be increased by oral carnitine, via secondary mechanisms including metabolism by gut flora [24]. Unsurprisingly, carnitine is also important in fatty acid transport from peroxisomes [25], where long chain fatty acids are partially degraded. Other recent findings related to metabolism of amines in food include that choline in dietary phosphatidylcholine promotes coronary artery disease through production by gut flora to trimethylamine, further converted to trimethylamine-N-oxide, TMAO, in the liver [26]. The relative importance of different amine sources is unclear, but dietary effects of meat and eggs are mediated, in significant part, by these mechanisms.

In vitro, fat loading of macrophages causes a backup of β -oxidation reflected in supernatant acylcarnitines. This varies significantly depending on the type of fat (Fig 3). This may model the vicious cycle *in vivo*, at least in part, in that in a setting with abundant lipid carrier carnitine, the macrophages from one cycle of reaction to effete phospholipid debris die attempting to clear the material, making the problem worse, particularly if the lipid being transported is resistant to degradation. Recent experimental work supports this hypothesis, suggesting that limiting carnitine used as a fatty acid carrier attenuates atherosclerosis progression in an atherogenic mouse model [5]. If confirmed, properly controlled regulation of macrophage lipid metabolism may be an important new method of preventing progressive atherosclerosis. On the other hand, the circumstances where limiting carnitine may prevent atherosclerosis may be limited and conditional; it remains for further work to resolve the issues.

Mechanism-specific effects of fatty acids and phospholipids on atherosclerosis: New disease indicators

The mechanisms of macrophage lipid accumulation and apoptosis due to artificial *trans* fats and due to excess dietary saturated fatty acids are, at least in large part, independent, and are reinforced by unfortunate antibody responses to oxidized phospholipids.

Some artificial *trans* fats, particularly elaidate, are macrophage metabolic inhibitors. Even when elaidate comprises only a few percent of dietary fat it has profound effects on human macrophage fatty acid catabolism, which may be monitored by serum acylcarnitines (Fig 3). In rats elaidate stalls β -oxidation at the C14 stage [27]. In humans, a similar incomplete block in elaidic acid β -oxidation occurs, but mainly at C12 [8]. When human macrophages are exposed to elaidate, together with accumulation of intermediates from incomplete β -oxidation (Fig 3), there are profound changes in metabolism that include increases in the ATP-binding cassette sub-family G-1, ABCG1, steryl CoA desaturase-1 (SCD), and hydroxymethylglutaryl CoA synthase-1 (HMGCS1) [28]. ABCG1 is a central regulator of lipid homeostasis in cells, mediating cholesterol and phospholipid transport. It is believed to play a role also in regulating inflammation with atherosclerosis [29]. The steryl CoA desaturase-1 creates 18:1 from 18:0 [30]. Downstream to this is doubly unsaturated C18, 18:2. The ratio of 18:1/18:2 is a marker of atherosclerotic disease progression (Fig 4) [31].

The HMG reductase, one step distal to HMGCS1, is also highly expressed in macrophages; it is the statin target, the rate-limiting cholesterol synthesis enzyme [32]. What role statins play in inhibition peripheral macrophage metabolism is not fully studied but probably important: *In vitro*, statins reduce macrophage proliferation, downregulate ABCG1 mediated cholesterol efflux, and suppress F_c receptor-mediated phagocytosis via cholesterol synthesis reduction [33,34,35]. Both SCD and HMGCS1 are regulators of inflammation and in lipid-related metabolism and pathology [29,30]. How these lipid pathway enzymes are induced by *trans*-fats [28] is not known. Recently, a new class of LDL cholesterol-lowering drugs based on inhibiting the proprotein convertase subtilisin/kexin type 9 (PCSK9), which binds to an EGF-like repeat domain of the LDLR, preventing LDLR recycling to the cell surface; in the liver this has the effect of dramatically lowering LDL cholesterol [36]. In plaque, secreted PCSK9 might reduce LDLR and thus processing of oxidized lipids in macrophages, potentially reducing damaging inflammation and macrophage death [37]. This promising additional tool to disrupt the vicious cycle of plaque formation is in process of study for significant off-target effects and for efficacy in atherosclerotic disease outcomes [36].

In contrast, saturated fatty acids cause receptor-mediated response via toll-like receptor 4 (TLR4), with consequences including release of TNF [28] and of macrophage chemotactic protein-1 (MCP-1) [38]. These are likely to contribute proliferation and cell recruitment in atherosclerosis. Several studies have documented that excess lipid accumulation is linked to apoptosis [39,40]. It is likely that excess saturated fatty acids are compensated by ω -3 fatty acids. This has been linked to peroxisome proliferator-activated receptor (PPAR γ) expression induced by saturated fatty acids [41].

Lipoproteins have other functions, including promoting antibody response to oxidized phospholipids. It is commonly assumed that low density lipoprotein exists to move cholesterol from the diet or the liver to peripheral tissues, and that peripheral tissues use high-density lipoprotein to solely to transport cholesterol for central disposal or to deliver lipids and cholesterol to other organs. These functions certainly exist and they are important. And cholesterol is an essential membrane constituent, comprising up to a third of cell membrane lipid. However, by no means is cholesterol from LDL off-loaded into atherosclerotic debris; transport is multi-directional and entirely cell mediated. Both LDL and HDL carry, in addition to cholesterol and cholesterol ester, significant amounts of phospholipid. Phospholipid is quantitatively about half as much as the cholesterol content in LDL, and significant triglyceride is also bound [42]. Additional specific roles of lipoproteins include of that HDL delivers cholesterol to the adrenal gland [43], and there is an important role of HDL in bone synthesis [44]. Fatty acid-promoted atherosclerosis is to a major extent driven by Fc γ receptor activation [45,46]. The antibody-mediated pro-atherogenic effect is, at least in large part, driven by antibodies to oxidized lipids bound by LDL [47,48]. This strong association supports the drive to reduce LDL to reduce atherosclerotic progression, in addition to suppressing LDL cholesterol *per se*.

Pathways of *trans* fat toxicity

It is clear that artificial *trans* fat consumption increases the threat of atherosclerosis [49]. There are many studies with findings supporting this viewpoint; *trans* fatty acid

consumption increases total serum cholesterol and low density lipoprotein cholesterol, and decreases high density lipoprotein-cholesterol [50]. Consuming a large portion of dietary calories as saturated fat is also unhealthy, as discussed above, but mechanisms differ significantly from *trans*-fat effects. In comparing *trans* fat and saturated fat in young healthy volunteers with analysis of RBC lipids [49], the dietary fatty acids appeared within three weeks in RBC membranes in close proportion to the diet consumed. This is important in that it shows that *trans*-fatty acids are directly used in membrane production and thus are placed in position to inhibit β -oxidation when the cells die. Why this should affect mainly the vascular wall is unclear, although much higher amounts of enoyl CoA isomerase in the liver [8] make it clear why this central organ appears to be completely resistant. At the same time, it was shown, interestingly, that saturated fat largely increased low density lipoprotein-cholesterol, while *trans* fat also *reduced* high-density lipoprotein cholesterol [50]; the mechanisms for the HDL effect are unknown. Many other studies have produced consistent results [51].

Further, transport of debris into macrophages is associated with inflammatory response. Macrophages express both HDL and LDL receptors, and import cholesterol, oxidized or native, from lipoproteins. This process involves several binding and transport proteins [52]; when macrophages are presented with more cholesterol and lipoproteins than can be exported, this causes metabolic back-up and cell death (Fig 3). This transformation reflects too much lipid as well as response to cellular debris by receptor-mediated responses including antibodies to oxidized phospholipids carried by Fc γ [53]. Immune-related diseases with antibodies to oxidized lipids, complexed with LDL, accelerate atherosclerotic disease [44]. The Fc γ signaling contributes to inflammatory cytokine expression that drives macrophage recruitment with the vicious cycle of further recruitment to the site of cell death.

Additional pathways altered by fatty acid exposure in toxic concentrations

Macrophages are primary, but not the sole, cellular agents that respond to changes in membrane fatty acids in catabolic pathways.

Lipid overload leads to production of inflammatory cytokine in macrophages and in the liver. Saturated fatty acids cause serum release of TNF and MCP-1, discussed above [28,38], IL-6, IL-1, and C-reactive protein, the last a secondary response of the liver [54,55]. Many additional chemokines and growth factors are reported, as reviewed elsewhere [56].

Palmitate (C16:0) or oleate (C18:1 *cis*) at 30 μ M elicits stress responses in many cell types including cardiac myocytes, with increased intracellular calcium at pre-apoptotic levels [57]. Cardiac myocytes regulate metabolism including of fatty acids during pathological changes including cardiac hypertrophy, with enzymes induced including HMGCoA reductase [58], highlighting the integrated nature of fatty acid response in many organs.

Outward transport of lipid degradation products from macrophages

In addition to mechanisms causing lipid accumulation and toxicity, it is important to consider that in health macrophages may remove excess lipid, in major part as cholesterol,

thus interrupting the process of atheroma growth. While this subject has a literature too large to discuss, key apolipoproteins involved in reverse transport of cholesterol include apolipoprotein E, largely found in intermediate density lipoproteins, which is produced by macrophages and liver cells [59] and apolipoprotein A1, which comprises about 80% of high density lipoprotein carrier proteins. Over-expression, of ApoA1 disperses lipid from foam cells *in vivo* [60]. Two sterol membrane transporters in macrophages ATP-binding cassette proteins ABCA1, also known as cholesterol-export regulatory protein, and ABCG1, are important in cholesterol export [61]; macrophage ABCG1-mediated transport is suppressed by statins via limiting cholesterol transport [34].

Conclusion

Further progress in suppressing atherosclerosis-mediated diseases will strongly be supported by further efforts to reduce cigarette smoking, promote atherosclerosis, and improve diet to reduce excess saturated fat consumption as well as eliminate artificial trans fats from the diet. The latter will also support efforts to limit obesity and related diseases. More specific work aimed at pharmacological mediators of atherosclerosis includes study of the specific mechanisms by which cell membranes are modified to reflect dietary fatty acids, and cellular responses to these diet-mediated changes. Assays include cell membrane fatty acid content, and serum acylcarnitine profiles. These may allow better prediction of patients at high risk for progressive disease than solely indicated by serum lipid profile, and might allow more direct monitoring of therapeutic effect on macrophage-mediated progressive atherosclerosis.

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Figure 1.

Percent of deaths from ischemic heart disease from 1865–1950 from a long series of autopsies at St Bart’s Hospital, London. Shown are deaths in the over age 40 population attributed to ischemic heart disease or dissecting aortic aneurysms, divided by the numbers of autopsies in the over 40 age groups in the time intervals shown. Data are from Table 2 in Finlayson [3], and are used by permission. Data for 1940–45 are assumed all to be over age 40; this is not specified for these years.

Figure 2. Degradation of unsaturated fatty acids, and structures of common fatty acids

A. Fatty acids unsaturated at odd-numbered bonds require enoyl Co-A isomerase for oxidation. The β -oxidation of unsaturated fatty acids at with unsaturation at odd carbons from the terminal carboxy is complex. Double bonds at odd numbered position are of phase with *trans*-2,3 CoA reductase. This is adjusted by enoyl CoA reductases (two isoforms) that move the double bond from 3,4 to 2,3-*trans*. Fatty acids with *cis* double bonds at even positions are degraded similarly but by *cis*-2,3 CoA reductase (not illustrated). The enoyl CoA reductases are mitochondrial NADPH-requiring enzymes. Either *cis*- or *trans*-unsaturation that is out of phase with the *trans*-2,3-CoA reductase reduces the efficiency of β -oxidation. The effect varies widely with the position and type of unsaturation. Oleate at 30 μ M suppresses β -oxidation measurably; elaidate at the same concentration has a strong inhibitory effect. Inefficient processing of C18:1 $\Delta 9$ *trans* by enoyl CoA lead to accumulation of extracellular *trans*-C12 and *trans* C-14 carnitine adducts; CoA is the mitochondrial carrier; carnitine is the cytoplasmic fatty acid carrier. **B. Key fatty acid types.** Animal fat is about 50% saturated. Of this, of this about a quarter is C18:0, stearate, the bottom drawing. There is typically twice as much C16:0, palmitate, as stearate. Unsaturated *cis* fatty acids melting points lower than saturated fatty acids of the same length. These increase membrane flexibility. Oleate, C18:1 $\Delta 9$ *cis*, is shown in the top diagram. The *cis* bond is in red. Oleate is a liquid and the most abundant monounsaturated fatty acid in mammalian cell membranes. The occurrence of *trans* double bonds creates stiff structures that have much higher melting points; elaidate, the middle diagram, is a hard wax. The *trans* bond is in red. This is the major *trans*-fatty acid in partially hydrogenated oils.

Figure 3. Foam cells in vitro and accumulation of acylcarnitines in the supernatant

Human macrophages in vitro were maintained in MEM with 10% fetal bovine serum (left) or with the same serum saturated with soy oil or partially hydrogenated soy oil (middle and right). At 10 days supernatants were collected as dried spots for MS/MS determination of acylcarnitine fatty acids as described [8]. The bottom row shows phase photographs of oil red O stained cells; note that in control cells lipid (red) occurs in dust-like vacuoles (red, carets) although the cells are physaliferous, or prominently vacuolated, (arrows). In the lipid saturated cells the large vacuoles contain lipid (arrows). This is pronounced in the partially hydrogenated oil saturated serum, where also reduced cell numbers reflect increased cell death (not shown). The graph at top shows that there is a slight, but significant, increase in C12-carnitine in the soy oil treated cells, and a much larger increase in C12-carnitine in the partially hydrogenated soy oil treated cells (right). To what extent this reflects release due to cell death is unknown. Since carnitine is required as a carrier for the free fatty acids entering the cells, this is a potential gate-keeper to restrict overload and death of “foam cells” hypothesized to lead to a vicious cycle of phagocytosis and toxicity causing growth of atheromata. Modified from [8]; used with permission.

Figure 4. Unsaturated fatty acids in RBC occur with development of atherosclerotic disease Fatty acid extraction and analysis by gas chromatography and mass spectroscopy shows clearly that red blood cells accumulate C18:2 in a manner reflecting development of cardiovascular disease. N=10–18, mean $\pm$ SEM. The data are from [27] and are used with permission.