

Cardiovascular Disease: C-Reactive Protein and the Inflammatory Disease Paradigm: HMG-CoA Reductase Inhibitors, alpha-Tocopherol, Red Yeast Rice, and Olive Oil Polyphenols. A Review of the Literature.

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Abstract

The current understanding of the origin of atherosclerosis is that of an inflammatory process that involves the acute phase response – an innate biological response to a disturbance in homeostasis – infection, inflammation, tissue injury, neoplasm, or immune disturbance. The activation of the acute phase response, signaled by interleukin-6, produces proteins (fibrinogen, C-reactive protein (CRP), serum amyloid A) that lead to inflammatory reactions. The tissues themselves contain elevated levels of acute phase proteins and cytokines resulting in a localized inflammatory effect. Localized inflammatory responses in the intimal layer of the arterial wall have been shown to be responsible for many of the aspects of intimal thickening and plaque disruption, leading to acute cardiovascular events. The predictive value of plasma C-reactive protein as a risk factor for cardiovascular events has led some researchers to support the use of CRP as a main cardiovascular risk assessment tool, along with total cholesterol:HDL ratios and homocysteine levels.

The ability of HMG-CoA reductase inhibitors to lower C-reactive protein levels has recently brought into question the mechanisms of action of the statin drugs. Because these medications lower incidences of acute cardiovascular events as well as decreasing morbidity and mortality well before the effects of lowered LDL cholesterol can be expected to occur, questions have been asked about whether they may work independently of LDL-lowering mechanisms. Red yeast rice contains a naturally-occurring statin (lovastatin) as well as other cholesterol-lowering compounds, some with antioxidant effects. Alpha-tocopherol also significantly lowers CRP levels in diabetics and nondiabetics, and minimizes other aspects of the acute phase response and inflammatory damage involved in atherosclerosis. This may account for alpha-tocopherol's positive effect on cardiovascular morbidity and mortality. Finally, polyphenolic compounds present in virgin olive oil also have anti-inflammatory and antioxidative effects in cardiovascular disease. The phenolic compounds in virgin olive oil may explain some of the protective effects found in epidemiological studies.

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Epidemiology of Cardiovascular Disease

Mortality from cardiovascular disease (CVD) is the leading cause of death in the industrialized world and the second leading cause of death worldwide; it is the cause of more lost years of potential life before the age of 75 than any other human condition.¹ U. S. death rates for stroke and heart disease have dropped significantly in the last 30 years (rates increased from 1950 to 1965 and started to drop in 1970), due to an increase in the aging population. Unfortunately, the absolute number of deaths from cardiovascular disease – 750,000 deaths per year – has not changed for the last 25 years,² and within the spectrum of cardiovascular disease, two separate epidemics are emerging. The first is a significantly increased incidence of heart failure – the result of those with hypertension who survive strokes and myocardial infarctions. The mortality from heart failure is now more than double what it was in 1986.³ And, the second – atrial fibrillation, which causes an increased risk of stroke and heart failure – is responsible for over a quarter million hospitalizations yearly and is increasing at epidemic proportions.⁴

It is estimated that by the year 2020 cardiovascular disease will be responsible for 36 percent of all deaths and the leading cause of death in the world. The rise in cardiovascular death might be due to a worldwide increase in smoking incidence; an estimated 21 million smokers in developed countries (with one-third of the world's population) have died as a result of tobacco use in the last decade. In these countries, this statistic is equivalent to one-third of all deaths in people aged 35-69, making smoking the largest single cause of premature death in the developed world.⁵

Obesity (30 percent of U.S. adults are obese) and diabetes (80 percent of diabetics die of CVD) are also prominent risk factors. Yet our knowledge about the etiology of coronary vessel disease is limited and incomplete. Over half of patients with atherosclerosis do not have what are known as accepted risk factors: hypercholesterolemia, hypertension, history of smoking, diabetes, significant obesity, or sedentary lifestyles.¹

New risk factors for CVD are emerging as the result of a growing understanding of the process of atherogenesis. These factors include levels of circulating homocysteine,⁶ fibrinogen,⁷ C-reactive protein (CRP),⁸ endogenous tissue plasminogen-activator, plasminogen-activator inhibitor type I, lipoprotein(a), factor VII,¹ and certain infections such as *Chlamydia pneumoniae*.⁹

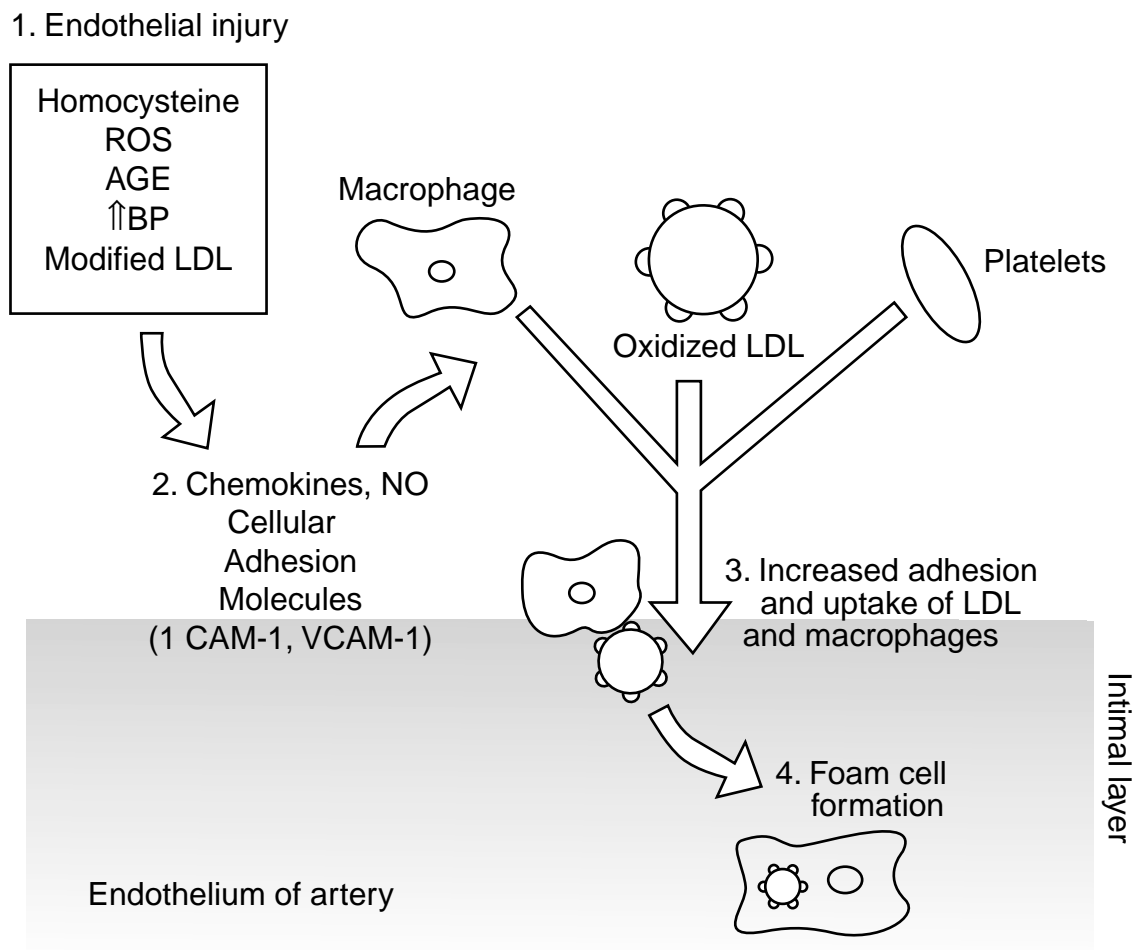
The Inflammatory Disease Theory

The process of inflammation is now believed to be the etiological event that precedes the development and the continual process of atherosclerosis.^{10,11} This process, beginning with an injury or change in the endothelial wall of the artery, causes an alteration in the intimal layer that increases leukocyte, low density lipoprotein (LDL), and platelet adhesion to the endothelium (Figure 1). Possible causes of “dysfunctional endothelium” include: free radical damage from environmental exposure; hypertension and its proinflammatory effects (smooth muscle lipoxygenase activity and oxide radical formation); direct toxic effects of homocysteine; infections with *Chlamydia pneumoniae* and herpes viruses; and advanced glycosylated end-products (the result of an oxidation reaction with glucose that results in a type of oxidant commonly found in the blood of diabetics).¹²

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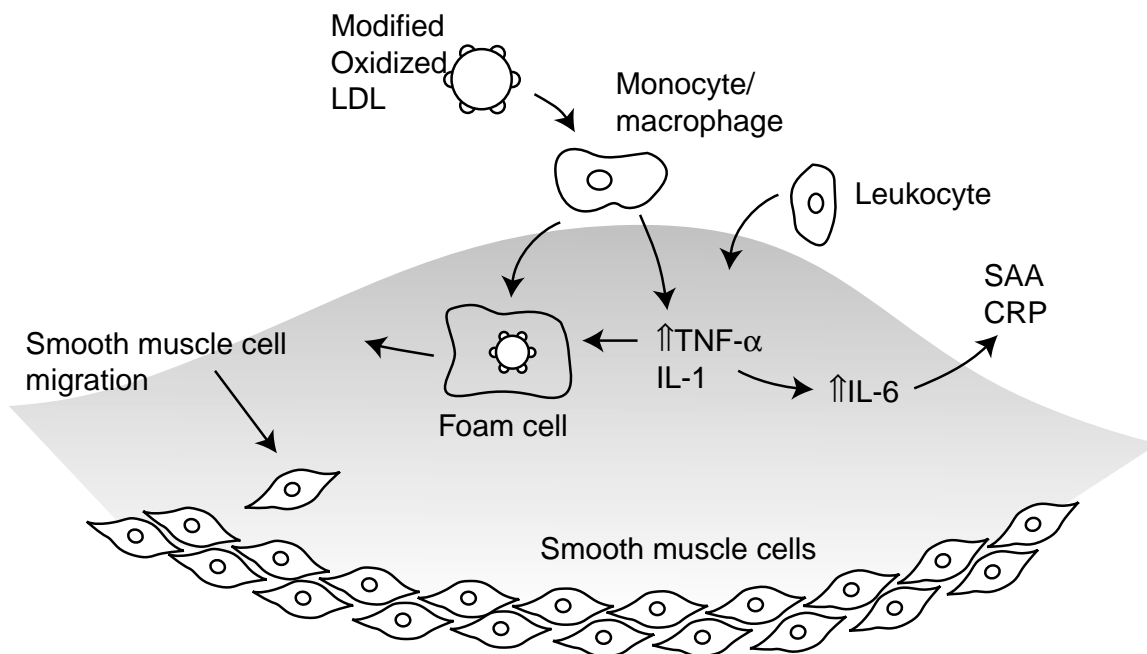
Figure 1. Endothelial Dysfunction Caused by Injury and Subsequent Attraction and Adhesion of Damaged LDL and Macrophages.



KEY:
 LDL= low density lipoprotein, BP=Hypertension, AGE=advanced glycosylated end products
 ICAM-1= intracellular adhesion molecule 1, NO= Nitric oxide
 VCAM-1= Vascular adhesion molecule 1

Oxidized or modified LDL is a recognized source of damage to the endothelial wall. LDL can damage the endothelium when it becomes oxidized by free radicals, when it becomes immunogenic (autoantibodies have been isolated against oxidized LDL), when LDL aggregates form, or when LDL undergoes glycation.¹³⁻¹⁵ Oxidized LDL becomes a

chemo-attractant for monocytes because it represents cell damage. Macrophages then bind to the altered LDL via scavenger receptors on the macrophage surface, a mechanism that is part of the innate immune system and is a rapid first line of defense designed to respond to tissue damage.¹⁶ Altered LDL particles continue to undergo oxidation in the lumen of

Figure 2. Formation of the Fatty Streak.

Inflammatory monocytes and leukocytes enter intima. Modified or oxidized LDL is engulfed by macrophages and forms a foam cell. Immune activation leads to increased production of pro-inflammatory cytokines (TNF- α =tumor necrosis factor alpha, IL-1=interleukin 1) These cytokines stimulate production of IL-6 (interleukin-6) that leads to hepatic production of acute phase proteins CRP (C-reactive protein) and SAA (serum amyloid A).

the artery and promote injury to the artery through multiple pathways. The presence of oxidized LDL promotes the expression of growth factors and chemotactic proteins, causing an expanding inflammatory response and up-regulating monocyte replication to increase macrophage populations. The mediators of the inflammatory response: interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF- α), interleukin-6 (IL-6), macrophage colony stimulating factor, etc., all increase the binding of LDL to the endothelium and smooth muscle to further up-regulate the inflammatory response.¹⁷

Present at every stage and in every lesion of atherosclerosis, the monocyte-turned-macrophage responds in an attempt to remove

oxidized LDL by binding to it, engulfing the modified LDL, and turning into a “foam cell” (Figure 2). This process signifies the attempt of the macrophage to remove an offending agent. Foam cells are fatty cells that together form the fatty streak – the first identifiable characteristic lesion of advanced atherosclerosis.¹⁸ At the same time, the increase in the “stickiness” of the endothelial wall also alters its permeability, making it easier for leukocytes, macrophages, and LDL to migrate into the wall. The adhesiveness and permeability of the endothelial wall is controlled by a variety of chemokines, prostacyclin, nitric oxide, angiotensin II, growth factors, and monocyte chemotactic proteins released as a complex interaction between monocytes, T-cells, and the

endothelium.¹² As endothelial permeability becomes increasingly altered, increasing amounts of modified LDL are absorbed along with increasing numbers of monocytes and T-cells. The resultant inflammation causes increasing numbers of macrophages and lymphocytes to be produced and to multiply inside the lesion, leading to the production of enzymes, cytokines, and growth factors. The inflammatory response initiates migration of smooth muscle cells into the fatty streak and eventually the inflammatory cells produce a lesion of necrotic cellular debris inside the artery wall. The lesion then becomes covered with a “fibrous cap” that protects the area. The artery dilates to accommodate this lesion initially and then the lumen begins to narrow.¹²

Platelets are also involved in the atherosclerotic process, as they can stick to the injured endothelium and release cytokines and growth factors. Platelet activation also initiates the production of thromboxane A2 and leukotrienes, which amplify the inflammatory response.¹⁹ As the lesion grows, a fibrous cap made of collagen and elastin walls off the lesion from the lumen of the artery to protect the arterial lumen from the atheroma. In areas where the fibrous cap is dense, lesions are usually stable. Thinning of the fibrous cap, however, occurs when enzymes called metalloproteinases are produced by macrophages in the lesions. On autopsy, evidence of inflammation – macrophage accumulation – occurs at the sites where plaque ruptures.²⁰ In areas where the fibrous cap is uneven or thinning and rupture occurs, the contents of the necrotic cellular debris are exposed to the lumen of the artery and a thrombus forms. If this thrombus is significant enough to cause a blockage in arterial flow, myocardial infarction is the result.²¹ The rupture of what is termed “vulnerable plaque” (plaque with a thinning fibrous cap and increased macrophage infiltration) and the resulting thrombosis is potentially responsible for at least 50 percent of all myocardial infarctions, even though

it is only seen in 10-20 percent of all lesions.²² Vulnerable plaque does not cause occlusion of the artery and is difficult to diagnose through angiography.²³ An accumulation of macrophages in vulnerable plaque has been associated with increased plasma concentrations of fibrinogen and CRP.^{24,25} These same inflammatory and fibroproliferative pathways are found elsewhere in the body: cirrhosis, rheumatoid arthritis, glomerulonephritis, chronic pancreatitis, and pulmonary fibrosis.¹⁰ At some point, components of the immune system (in the case of CVD, monocytes in particular) stop acting as a part of the protective immunity and begin to contribute to the pathology of the inflammatory response. Studies examining inflammatory pathways in CVD suggest novel therapies aimed at altering the inflammatory cytokines involved.²⁶

The Acute Phase Response in CVD

The acute phase response occurs prior to antibody-mediated immunological defense. It occurs in response to a homeostatic disturbance brought on by injury and trauma, neoplasm, or disordered immunological activity. A local reaction at the site of injury or infection leads to an activation of cytokines (specifically, IL-1, TNF- α , IL-6, and interferons) that trigger systemic responses: leukocytosis; increases in glucocorticoid production; increases in erythrocyte sedimentation rates, fever, activation of complement and clotting cascades; decreases in serum zinc and iron; and an increase in plasma levels of acute phase proteins, CRP, serum amyloid A, fibrinogen, and other proteins.²⁷ CRP is one of the two most abundant acute phase reactants in humans: levels rapidly increase in the circulation as a result of either trauma or infection. Manufactured in the liver and deposited in damaged tissue, CRP is found in high levels in inflammatory fluids and in both the intimal layer of the atherosclerotic aortic artery and the foam cells within the lesions of atherosclerotic plaque.²⁸ CRP has been found in the fatty

streak of the aortic artery and is key in many of the inflammatory sequences that promote the progression of atherosclerosis.²⁹⁻³⁰ CRP stimulates mononuclear cells to release tissue factor, an identified protein that is central to the initiation of coagulation reactions, complement activation, and the neutralizing of platelet-activation factor. Together, these reactions promote a thrombotic response.³¹

C-Reactive Protein as a Marker for Inflammation and Cardiovascular Disease

Elevated levels of cytokines involved in the acute phase response – TNF- α , IL-1, IL-6, and fibrinogen – have been shown to be elevated in cases of unstable angina related to aneurysm,³²⁻³⁴ and have been positively correlated with the risk of primary and recurrent myocardial infarction and death.³⁵⁻³⁷ Even though these markers can signal acute infection or inflammation, in the majority of patients elevated levels tend to remain stable over study periods as long as six years. The risk associated with these elevated levels remains constant even when the data is adjusted for other major risk factors: blood pressure, total and HDL cholesterol, body mass index, diabetes, alcohol use, family history, and exercise frequency.³⁶

The most convincing data concerning the inflammatory response and relative ability to predict vascular events and vascular disease is the relationship between highly sensitive C-reactive protein (hs-CRP) assays and cardiovascular events. Elevated levels of CRP have been related to increased risk of cardiovascular disease, myocardial infarction, and coronary artery disease (CAD) deaths among individuals with angina pectoris.^{11,38,39} Assayed levels of hs-CRP can increase 100 times over normal levels within 24-48 hours after an acute inflammatory stimulus. However, in long term prospective studies it is clear that inter-individual variations in

hs-CRP levels are significantly stable over long periods of time, in the absence of trauma or acute infection.⁴⁰ Elevated levels of hs-CRP have shown a doubling of risk both for ischemic stroke in hypertensive men and women^{43,44} and for peripheral artery disease.⁴³ Elevated hs-CRP levels have also been shown to be predictive of future risk for age-related cataract – three times more likely in those with the highest values.⁴⁴ Elevated levels of hs-CRP have been positively correlated with smoking, body mass index, chronic asymptomatic infections, and increasing age.⁴⁵ It is known that CRP levels are increased among smokers, although the data are conflicting. Several of the studies cited below show no change in the relative risk for cardiac disease and events when smoking is controlled for as a variable.

The Cardiovascular Health Study and Rural Health Promotion Project, a study of elderly men and women with subclinical CVD followed for an average of 2.4 years, revealed that those with elevated hs-CRP had a significantly elevated risk for “future coronary events” (myocardial infarction, death). The risk for women in the study was almost double that for men (2.7 vs. 4.5) and smoking had no effect on CRP as a risk factor.⁴⁶

The well-known Physician’s Health Study, which evaluated the effect of aspirin and beta-carotene in heart disease, also found that patients in the highest quintile for hs-CRP had a two-fold increased risk for future stroke, a three-fold increased risk for future myocardial infarction, and between a two- and four-fold increased risk for peripheral artery disease. Only the total cholesterol:HDL cholesterol ratio had as high a predictive value. Smoking had no effect on the predictive value of hs-CRP.^{24,44} In the Multiple Risk Factors Intervention Trial, the male smokers with elevated hs-CRP levels had a 2.8-fold increased risk of coronary vessel disease and a 4-fold increased risk for death from coronary vessel disease.⁴⁷ The smokers who died had been evaluated at baseline and followed for as long as 17 years.

Table 1. Relative Risks of Cardiovascular Events According to Baseline Plasma Levels of Markers of Inflammation and Lipids

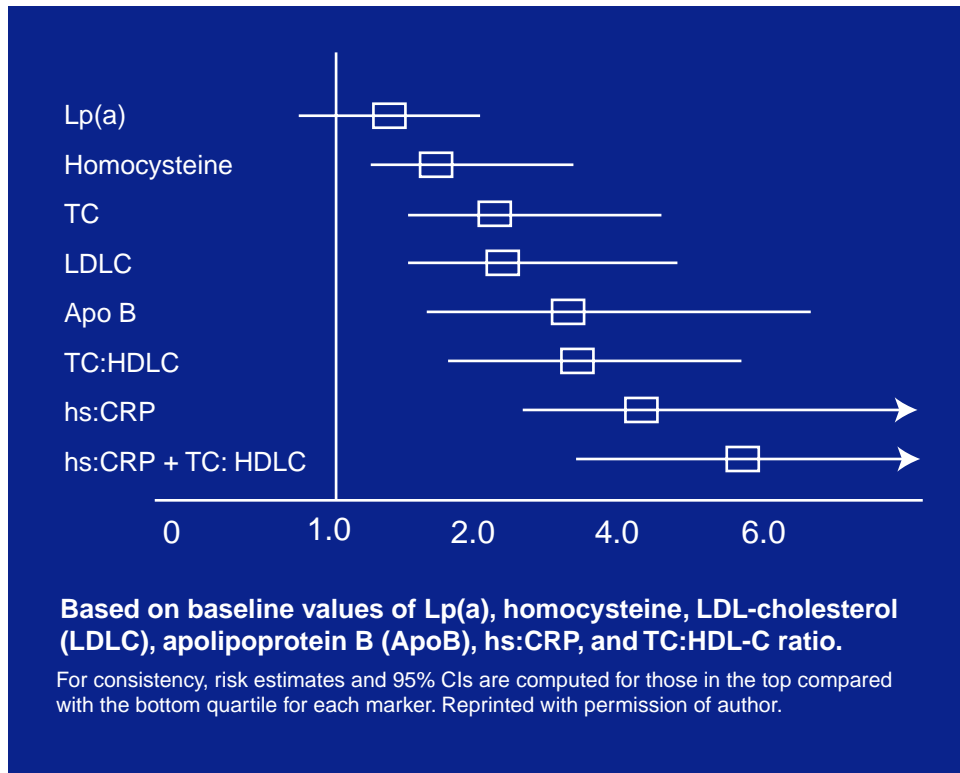
Marker	Relative Risk (95% CI) (comparing highest to lowest quartile)	P Value for Trend
Hs-CRP	4.4	<0.001
Serum amyloid A	3.0	0.002
Soluble intracellular adhesion molecule-1	2.6	0.004
IL-6	2.2	0.02
Total cholesterol	2.4	0.003
LDL cholesterol	2.4	0.001
HDL cholesterol	0.3	0.001
Apolipoprotein A-1	0.8	0.1
Apolipoprotein B	3.4	<0.001
Lp(a)	1.3	0.4
Ratio total cholesterol/HDL	3.4	<0.001
Homocysteine	2.0	0.02

From: Ridker PM, Hennekens CH, Buring JE, et al. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med* 2000;342:836-843.

The MONICA Augsburg study in Germany followed 936 men for eight years and found those with high hs-CRP levels were 2.6 times more likely to experience an acute myocardial infarction or sudden cardiac death.⁴⁸

The Helsinki Heart Study evaluated the effect of gemfibrozil versus placebo in hypercholesterolemic men who were otherwise healthy.⁴⁹ Non-smoking men in this study who had elevated hs-CRP had 2.3 times the risk for myocardial infarction or coronary death

Figure 3. Relative Risk for Future Cardiovascular Events Among Apparently Healthy Women in the Women's Health Study



likely to experience an event if they were in the highest quartile of hs-CRP levels. When all markers were simultaneously controlled for, the only markers able to independently predict coronary events were hs-CRP and total cholesterol:HDL ratio. The combined predictive effects of these two measurements were greater than either one alone and could predict women who were six times more likely to experience a future cardiovascular event (Figure 3). This predictive effect held true in women who were matched for smoking status, age, body mass

when compared to those without elevated hs-CRP. Unlike the Physician's Health Study, where adjusting for smoking did not alter the relative risk, smoking men in the Helsinki Heart Study who had elevated hs-CRP levels had 8.67 times the risk for the same coronary events.

Some of the most convincing data on hs-CRP has come from the Women's Health Study, a prevention trial using both vitamin E and aspirin.⁵⁰ Of 12 markers studied (Table 1), hs-CRP was the most sensitive predictor of a cardiovascular event: death from heart disease, myocardial infarction, or surgical re-vascularization. Women who were in the highest quartile of hs-CRP were 4.4 times more likely to experience an event than women in the lowest quartile. Even in women with low cholesterol (LDL below 130 mg), hs-CRP still had a predictive effect: women were 4.1 times more

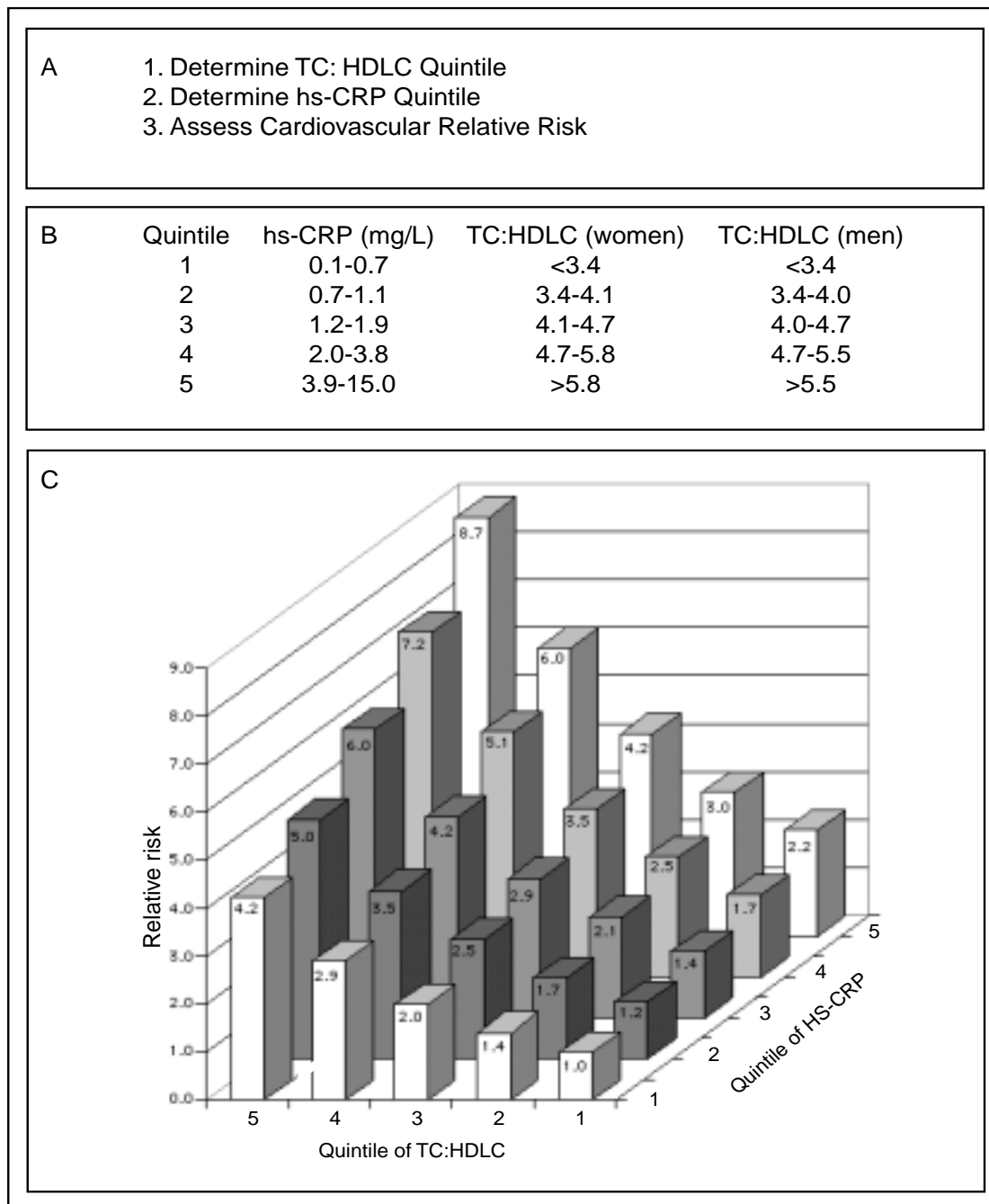
index, hypertension, diabetes, and parental history. In this study smoking did not effect the predictive value of hs-CRP.

As a result of this study and others, Ridker^{8,51} has proposed a predictive algorithm using the specific risk factors total cholesterol:HDL ratio and hs-CRP. Those in the top quintiles of both measurements have an 8.7-fold increased risk for cardiac events (Figure 4).

C-Reactive Protein and Hormone Replacement Therapy (HRT)

The recent Heart and Estrogen/Progestin Replacement Study (HERS) results indicate that hormone replacement may actually increase risk for cardiovascular events (a 1.5-times increased risk was found for women on estrogen/progestin replacement).⁵² Two large

Figure 4. Proposed Cardiovascular Risk Assessment Tool Using hs-CRP and Lipid Screening⁸



(A), steps in risk assessment. (B), hs-CRP and TC:HDL-C quintiles used for the risk assessment. (C), three-dimensional column plot showing RR for future cardiovascular events based on quintile of hs-CRP and quintile of TC:HDL-C ratio. The distribution of hs-CRP was derived from ongoing population-based surveys. The lipid cutpoints and risk estimates for incident cardiovascular disease were derived from studies by Ridker and co-workers. Reprinted with permission of the author.

trials have investigated the effect of hormone replacement therapy on C-reactive protein levels and may help to explain the mechanism for the negative effect of HRT on cardiovascular risk.

The first trial, using a cross-section of 493 women participating in the Women's Health Study, revealed that CRP levels were double in women on HRT (both estrogen alone and estrogen plus progestogen) than those not taking HRT.⁵³ This difference held for all groups evaluated, even those with none of the traditional risk factors (hypertension, hyperlipidemia, diabetes, obesity, smoking, or family history of premature coronary artery disease). The women on HRT had higher hs-CRP levels than a study of 291 men taken randomly from the Physician's Health Study. The rise in C-reactive protein in the group, from a median of 0.14 mg/dL in the no-HRT group to 0.27 mg/dL, was reflected in higher levels of hs-CRP in all quartiles. That is, when the ranges of hs-CRP were divided equally into

four groups, ranging from lowest to highest, the women on HRT had the highest starting and ending points. This held true even when they were compared to the men's group.

When 365 women in the PEPI (Postmenopausal Estrogen/Progestin Interventions) Trial had several inflammation-sensitive factors measured, C-reactive protein levels were elevated 85-percent more in women on HRT compared with those on placebo.⁵⁴ Treatment in this study was conjugated estrogen alone or in combination with progestin or micronized progesterone. The other factors measured – von Willebrand factor and factor VIIIc – were not effected. Soluble E-selectin, a protein involved in the dysfunction of the arterial endothelial wall, decreased along with LDL cholesterol, confirming other studies with HRT that show LDL cholesterol-lowering effects.⁵⁴ The only measurement that correlated with rising C-reactive protein level was fibrinogen, underscoring the possible inflammatory effects of HRT.

Table 2. Strength of the Evidence Associating Infections with Coronary Artery Disease and Atherosclerosis⁶⁰

Condition	Strength of Evidence
Peptic ulcers	strong
Gastritis	strong
Non-ulcer Dyspepsia	weak
Gastric Cancer	moderate
Hypochlorhydria	strong
Malabsorption (esp. iron, B12)	moderate
Coronary Heart Disease	weak

Evidence for an Infectious Etiology of Atherosclerosis?

One of the initiators of the acute phase response is infection. Reports dating back 100 years or more have suggested a possible association between infectious agents and both atherosclerosis and myocardial infarction.⁵⁵ More recently, evidence has emerged that chronic infections may be associated with CAD, specifically those due to *Chlamydia pneumoniae*, *Helicobacter pylori*, cytomegalovirus, *Herpes simplex* virus, and periodontitis.⁵⁶⁻⁵⁹ One proposed mechanism suggests that persistent infection of a microorganism leads to chronic stimulation of the inflammatory response. Despite a number of compelling reports, the research is conflicting.

Positive epidemiological, clinical, pathological, animal, and *in vitro* evidence exists, but only evidence for *Chlamydia pneumoniae* has been firmly established (Table 2).⁶⁰ *C. pneumoniae* has been detected in a majority of studies examining atherosclerotic lesions and not often in normal vessels.^{61,62} In addition, rabbits fed a high cholesterol diet then infected with *C. pneumoniae* apparently developed atherosclerosis at an accelerated rate.⁶³

The majority of epidemiological research supporting an etiologic role for *C. pneumoniae* in CVD has been retrospective and cross-sectional. While associations found in these studies often appear impressive, these designs are prone to bias and other confounding factors. For example, only half of the retrospective studies of *C. pneumoniae* and CAD published before 1998 reported adjustment for cigarette smoking.⁶⁴ Even if design was not an issue, for every study demonstrating a positive association between *C. pneumoniae* antibodies and CAD, there is a study showing no correlation.⁶⁵

Prospective studies, on the other hand, are less susceptible to selection bias, reducing the influence of disease on the variable in question, making the study less prone to

confounding data. Results from this type of study are generally considered more reliable and meaningful. Danesh et al⁶⁴ identified 15 large prospective studies looking at *C. pneumoniae* IgG titers and coronary heart disease – including their own research. Typically, these studies looked at antibody titers years before cardiovascular events. Controls were matched for risk factors such as smoking, lipid profiles, age, and socioeconomic status and came from an initial random sample (nested controls) rather than being selected after the fact. Comparison of IgG (sometimes IgA and IgM) titers was made between those who developed CAD and those who did not. A meta-analysis of these studies was performed, totaling 3,169 cases of non-fatal MI or death from CAD with a weighted mean follow-up of 10 years. The combined analysis yielded a non-significant odds ratio of 1.15 for IgG titers and CVD events.

The Search for Evidence of Chlamydial Infection and Intervention Trials with Antibiotics

If antibiotic treatment of *Chlamydia pneumoniae* prevented CVD, it would lend substantial support for an infectious etiology. Several studies have looked at the effects of antibiotics on CVD. Two large retrospective studies examining the effect of antibiotics in the prevention of myocardial infarction have yielded mixed results. Patients with first-time acute MI were compared with controls for prior use of antibiotics. The larger of the two studies (3,315 cases) found that in MI cases there was significantly less use of tetracycline antibiotics and quinolones. No association was found for erythromycin, which is active against chlamydia species.⁶⁶ The second study found no alteration in the risk of first-time MI with the use of erythromycin, tetracycline, or doxycycline in 1,796 cases compared with controls. However, this later study did not control for patients with other known risk factors for MI.⁶⁷

Prospective intervention trials using antibiotics to prevent secondary cardiovascular events have generated considerable excitement over the role of infectious agents in CHD and the potential for treatment. However, this enthusiasm may be due more to the way results were reported than to the actual findings. In three such trials, patients with previous MI or heart disease were assessed for *C. pneumoniae* antibodies and then treated with antibiotics or placebo. Additional coronary events were compared between the two groups.

One trial showed a four-fold decrease in cardiovascular events in patients treated with azithromycin.⁶⁸ However, according to Danesh, the study actually “yielded a non-significant result” and the investigators reported a four-fold reduction in CHD on the basis of an inappropriate non-randomized comparison.⁶⁴ Another larger trial of 302 CHD patients with Chlamydial IgG titers $\geq 1:16$ showed no significant difference in cardiovascular events between the treatment group on azithromycin and placebo groups. Although the inflammatory markers CRP, IL-1, IL-6 and TNF- α were similar at three months, they had decreased by six months.⁶⁹

A less well-known property of macrolide antibiotics may explain the reduction in inflammatory markers. This class of antibiotics possesses potent anti-inflammatory properties which are independent of their antimicrobial actions. Therefore, if these drugs are found to have any benefit in the treatment of CAD, their effect may be the result of an arrest of inflammatory rather than infectious processes.⁷⁰

In the ROXIS trial, 202 patients with unstable angina were treated with roxithromycin or placebo for 30 days and followed for six months. A 30-day preliminary report was published in the *Lancet*, which, as in Gupta et al, showed a four-fold decrease in MI and death from MI.⁹ However, the six-month follow-up failed to show any reduction in secondary events in the treatment group.⁷¹

Whether this microbe is a causative agent, an opportunist, or an innocent bystander trapped during the formation of plaque remains unclear. Regardless of their conclusions, the authors of all three studies agree that treatment with antibiotics in high-risk patients would be unwarranted before the completion of several larger prospective studies currently underway.

Reducing Inflammation: The Effects of HMG-CoA Reductase Inhibitors, Alpha-Tocopherol, and Polyphenols

HMG-CoA Reductase Inhibitors (Statins)

HMG-CoA reductase inhibitors (pravastatin, lovastatin, simvastatin, fluvastatin, atorvastatin, cerivastatin) have been shown to reduce cardiovascular-related disease and deaths in multiple clinical trials in the last six years. Cholesterol reduction of 15-60 percent and relative risk reduction for coronary events of 30-35 percent have validated the use of statin drugs in risk reduction for those at increased risk of coronary events and mortality from coronary heart disease.⁷²⁻⁷⁴ Cost-effectiveness studies, however, suggest that statins should only be used in primary prevention of myocardial infarction in high risk patients after using other more cost-effective interventions, including aspirin, smoking cessation, dietary change, exercise, and antihypertensive therapy.⁷⁵

Although it is known these medications decrease the production and circulation of LDL, their clinical effects appear to be greater than those associated with changes in LDL alone. Statin trials have shown that levels of LDL cholesterol after statin treatment are only weakly associated with the degree of arterial obstruction or the actual risk for acute myocardial infarction.⁷⁶ The effects of statin medications appear earlier in clinical trials (less than two years) than can be proven to occur from cholesterol lowering alone.

Pravastatin, which has been shown to lower CRP, has also been shown to significantly decrease overall risk for non-hemorrhagic stroke when other forms of cholesterol-lowering agents have not.^{77,78}

Statins have been demonstrated to have significant effects on several other pathways believed to be involved in the pathology of CVD. Specific statin drugs have been shown to suppress the production of cholesterol by macrophages in the arterial wall and the amount of inflammatory cells in plaque.⁷⁹ This class of drugs has also been shown to protect arterial endothelial dysfunction, reduce levels of serum fibrinogen, and possibly act as antithrombotic agents.⁷⁷ Statins have been shown in animal models to act as antioxidants by stabilizing vulnerable plaque, decreasing LDL oxidation, and reducing platelet aggregation and thromboxane production.⁷⁶ Statins have also been shown to decrease plasma C-reactive protein levels, a possible mechanism for their anti-inflammatory and clinical effects on morbidity and mortality. Specific trials with pravastatin, simvastatin, and atorvastatin have demonstrated a CRP-lowering capacity: 21.6 percent after five years with pravastatin versus placebo, and 35 percent after 12 months on atorvastatin or simvastatin.^{40,80} In the pravastatin trial there was no correlation between CRP levels and changes in LDL, HDL, or total cholesterol.⁸⁰ Changes in LDL levels as a result of statin use over the five-year period were not predictive of changes in CRP levels, evidence the anti-inflammatory effect of the statin was not simply a result of LDL lowering.⁴⁰ The simvastatin/atorvastatin trial also found that changes in CRP did not correlate with changes in LDL cholesterol or triglycerides but did correlate with changes in HDL.⁸⁰ Studies from the CARE (Cholesterol and Recurrent Events) trial found that in those randomized to receive pravastatin, the protective effect of the statin was much greater in those with elevated CRP, regardless of baseline lipid levels.⁴⁰

Unfortunately, statins are not without side effects. Gastrointestinal side effects (nausea, dyspepsia, constipation, diarrhea and flatulence) headache, dizziness, and rash are the most commonly reported. Sleep disturbance may occur with the lipophilic statins (simvastatin, atorvastatin).⁸¹ The most serious, although infrequent, adverse events encountered with statins are hepatotoxicity⁸² and myositis.⁸³ Elevated serum transaminases (AST, ALT) are a recognized effect of all statin drugs and often occur asymptotically. Most cases of significant liver function test elevations occur within the first 2-5 months of treatment and if they exceed three times the upper limit of normal, drug withdrawal is warranted.⁸⁴ All HMG-CoA reductase inhibitors can induce a coenzyme Q10 deficiency, thought to be responsible for myalgia and fatigue.⁸⁵ In addition, there have been reported cases of patients who have experienced peripheral neuropathy,⁸⁶ cataract progression, and a progressively decreased therapeutic response after long-term treatment.⁸⁷ Myopathy and frank rhabdomyolysis occur rarely and usually in combination with other pharmacological agents. The risk of muscle damage is higher if the patient is hypothyroid or has hepatic or renal impairment.⁸⁴ Finally, there is evidence that simvastatin, at clinical blood concentrations, is immunosuppressive.⁸⁸ Statins, as a class, have been recognized as “immunosuppressors” due to their ability to inhibit major histocompatibility complex class II(MHC-II)-induced T-lymphocyte activation.⁸⁹

Red Yeast Rice: A “Kinder, Gentler” Statin?

The recent legal battle between Merck & Co., pharmaceutical manufacturer of Mevacor (lovastatin), the U.S. Food and Drug Administration (FDA), and Pharmanex, Inc., the manufacturer of a red yeast rice product (which contains naturally-occurring lovastatin), has shed light on the possibility

that naturally-occurring forms of HMG-CoA reductase inhibitors may have clinical utility. Red yeast rice is a fermented rice product that has been used in Chinese cuisine and as a medicinal food to promote “blood circulation” for centuries.⁹⁰ The HMG-CoA reductase activity of the food comes from a family of naturally-occurring substances called monacolins. Monacolin K, also known as mevinolin or lovastatin, is the ingredient in red yeast rice that Merck asserted as a patent violation because it was sold in the United States as a food that promoted normal cholesterol levels. Red yeast rice contains a family of nine different monacolins, however, that all have the ability to inhibit HMG-CoA reductase. Other active ingredients in red yeast rice include sterols (beta-sitosterol, campesterol, stigmasterol, sapogenin), isoflavones, and monounsaturated fatty acids.⁹¹ In studies cited below, the daily lovastatin content of red yeast rice was calculated to be 0.2 percent of total product.⁹¹ At a daily dosage of 2.4 grams of red yeast rice, the lovastatin dosage is 4.8 mg. The dosages used in clinical efficacy trials with lovastatin were 20-40 mg.⁹² It is unlikely that the effects achieved with red yeast rice are solely a result of the lovastatin content of the supplement, and more likely that other monacolins, sterols, and isoflavones contribute to the cholesterol-lowering effect the studies achieved.

The first human study, done in China, evaluated the effect of 1.2 g/day red yeast rice on 324 hypercholesterolemic adults (total cholesterol above 230 mg/dL) who also had elevated LDL (over 130 mg/dL) and low HDL (under 40 mg/dL) versus controls for eight weeks.⁹³ Total cholesterol dropped by 23 percent, LDL cholesterol by 31 percent, and triglycerides by 34 percent. Serum HDL levels increased by 20 percent. The second study included 65 hypercholesterolemic adults on 2.4 g red yeast rice daily or placebo.⁹¹ They were asked to maintain a diet of 30-percent fat, 10-percent saturated fat, and a maximum of 300 mg cholesterol daily. After eight weeks

the treatment group had an 18-percent lower mean total cholesterol level compared to placebo and a 17-percent drop in total cholesterol from baseline. There was also a 23-percent difference in LDL between the treatment group and the placebo group and a 23-percent drop in the treatment group, evident at eight weeks. Triglycerides also dropped 16 percent in the treatment population. The drops in total cholesterol and LDL were consistent at 8 and 12 weeks. There were no changes in HDL levels.

Toxicity evaluations of red yeast rice in animals for as long as four months have shown no toxicity.⁹⁰ Human trials have not shown elevations of liver enzymes or renal impairment.^{91,93} Side effects have been limited to headaches and gastrointestinal discomfort. The contraindications for lovastatin are probably prudent: pregnancy, nursing, hepatic or renal impairment, co-administration with niacin, gemfibrozil, cyclosporin, azole antifungals, erythromycin, clarithromycin, nefazodone, or protease inhibitors.

Although larger, long-term trials will be helpful in understanding the efficacy and potential long-term effects of red yeast rice, the apparent lack of statin-like side effects in these short-term studies warrants further investigation of this hypolipidemic agent. Because HMG-CoA reductase inhibitors reduce production of coenzyme Q10 (CoQ10),⁸⁵ supplementation of CoQ10 with long-term use of monacolin K in red yeast rice extract may be prudent.

Vitamin E

The LDL particle contains 2700 fatty acid molecules of which about 50 percent are oxidation-sensitive polyunsaturated fatty acids. Between five and nine molecules in any LDL are alpha-tocopherol and less than one molecule contains the remaining antioxidants (beta-carotene, lycopene, coenzyme Q and gamma-tocopherol).⁹⁴ According to the inflammatory disease model of atherosclerosis, the oxidation of these fatty acids in the LDL

Table 3. Anti-inflammatory Functions of Tocopherol and HMG-CoA Reductase Inhibitors

Function	Vitamin E	HMG CoA-reductase inhibitor
Preserves endothelial function	+	+
Decreases LDL oxidation	+	+
Prevents plaque rupture	+	+
Inhibits platelet adhesion and aggregation	+	+
Inhibits smooth muscle cell proliferation	+	+
Decreases endothelial adhesion by monocytes	+	+
Decreases cytokine expression	+	+
Reduces leukotriene synthesis	+	?
Represses MHC-II mediated T-cell activation	—	+
Decreases plasma C-reactive protein levels	+	+

Data from: Pryor W. Vitamin E and heart disease: basic science to clinical intervention trials. *Free Rad Biol Med* 2000;28:141-164. Vaughan CJ, Murphy M, Buckley BM. Statins do more than just lower cholesterol. *Lancet* 1996;348:0179-1082. Rosenson RS, Tangney C. Antiatherothrombotic properties of statins. *Clin Cardiol* 1998;279:1643-1650.

particle initiates changes that induce macrophage activation and internalization of LDL in the intimal wall.

In animal studies antioxidants have been able to actually reduce the size of the fatty streak and the size of the atherosclerotic lesion.⁹⁵ In multiple studies in cell culture and

in humans, vitamin E has been shown to significantly reduce the vulnerability of LDL to oxidation, directly in proportion to the levels of plasma tocopherol.^{96,97} Vitamin E (specifically alpha-tocopherol) has also been shown to limit atheroma progression, theoretically by stabilizing vulnerable plaque.⁹⁸ In fact, many

of the mechanisms outlined above for the anti-inflammatory effects of statin drugs are duplicated by tocopherol (Table 3). In cell culture and in human studies 1200 IU of alpha-tocopherol has been shown to stabilize and protect endothelial tissue, decrease monocyte-induced inflammation, inhibit smooth muscle cell proliferation, act as an antithrombotic by inhibiting platelet aggregation, and decrease the expression of inflammatory cytokines.⁹⁹⁻¹⁰⁴

There has been recent controversy about the effect of supplemental tocopherol in its ability to reduce risk of cardiovascular events in randomized trials. The recent HOPE (Heart Outcomes Prevention Evaluation) trial evaluated 4,761 men and women over age 55 who were given 400 IU of natural source vitamin E and followed for 4.5 years. The results proved discouraging: vitamin E had no protective effect on the occurrence of myocardial infarction, stroke, or death from CVD.¹⁰⁵

However, in the CHAOS trial (Cambridge Heart Antioxidant Study), 400 or 800 IU alpha-tocopherol significantly reduced risk of a non-fatal myocardial infarction.¹⁰⁶ In a trial looking at risk for myocardial infarction or death from CVD in 100 individuals who had prior transient ischemic attacks or minor strokes, the additive effect of 400 IU vitamin E was significantly greater than aspirin alone.¹⁰⁷ In the GISSI-Prevenzione trial, 11,324 recent myocardial infarction survivors were randomized to 1 g fish oil daily, 450 IU vitamin E, both, or neither and followed for 3.5 years.¹⁰⁸ While the fish oil capsules decreased the risk of death from cardiovascular disease by 30 percent when compared to all the other groups, the authors reported vitamin E had no benefit. A re-analysis by another group of researchers, however, pointed out that when the vitamin E results were compared to the other three groups, there was a 20-percent reduction in deaths due to cardiovascular disease and a 35-percent reduction in sudden death due to CVD.¹⁰⁹

Some of the discrepancies in trial outcomes may result from the doses of vitamin E used in the trials, as the dosing protocols of vitamin E in these and other trials have been questioned.¹¹⁰ Animal studies looking at dose-response curves for tocopherol found higher doses of tocopherol resulted in greater benefit in improving immune response and in preventing hemolysis.¹¹¹ In human studies looking at the effect of different dosages of alpha-tocopherol on LDL oxidation there is a direct dose-response effect up to 1200 IU for anti-inflammatory effects and inhibition of C-reactive protein.¹¹² Supplementation with 1200 IU alpha-tocopherol was able to reduce elevated levels of C-reactive protein by 33 percent in non-diabetic controls and approximately 25 percent in type 2 diabetics after three months.¹¹³

In a similar trial, dosages of 800 IU resulted in a 48-percent reduction in C-reactive protein in patients with type 2 diabetes after only four weeks.¹¹⁴ Type 2 diabetics are known to have higher C-reactive protein levels than non-diabetics due to higher body mass index, and increased levels of glycosylated (therefore, more easily oxidized) LDL, IL-6, and hemoglobin.^{115,116} The dose-response curve for cytokine modification by alpha-tocopherol needs further study in larger long-term trials in both diabetics and non-diabetics. It appears, however, that doses of 1200 IU may be necessary to alter the inflammatory processes that lead to atherosclerosis. Alpha-tocopherol at 1200 IU is considered a therapeutically safe dosage.¹¹⁷

It is also apparent that using alpha-tocopherol alone is inadequate for complete protection from LDL oxidation.¹¹⁸ Tocopherol is recycled by ascorbate and, although ascorbate is not lipophilic and is unable to enter the LDL particle, it has the ability to prevent LDL oxidation in human cell lines.¹¹⁹ Levels of ascorbate have been found to be lower in the aorta of patients with atherosclerotic disease compared to disease-free controls.¹²⁰ There are

also epidemiological correlations between low plasma ascorbate and the incidence of ischemic heart disease.¹²¹ If vitamin E is accepted in anti-inflammatory protocols for CVD, the role of ascorbate will also need to be assessed.

Olive Oil and the Antioxidant Effect of Polyphenols

Interest in dietary fats and their role in the development of heart disease has spurred a considerable amount of research the past three decades. The Mediterranean diet, rich in olive oil, has been associated with significantly lower mortality from CAD.¹²² The Lyon Diet Heart Study compared a Mediterranean diet with the standard post-infarction “prudent Western diet” in patients who had suffered a first MI. Its Scientific and Ethics Committee terminated the original five-year trial after finding a 76-percent reduction in cardiac events after 27 months in the experimental group.¹²² Although the trial was stopped and participants informed of the study results, most continued to follow the diet they had been assigned. The final report showed that after a mean follow-up of 46 months the initial dramatic effects had persisted.¹²³ These results are impressive since the trial was conducted in France, a country with one of the lowest rates of heart disease. It is noteworthy that this well-controlled study of 605 post-infarct patients achieved greater reductions in coronary mortality using simple dietary changes than any cholesterol-lowering study to date.¹²⁴

Dietary factors are difficult to study because it is difficult to isolate individual ingredients. However, research on the monounsaturated fatty acid (MUFA) olive oil and its components suggest it plays an important role in the prevention of heart disease. There are multiple mechanisms by which olive oil might impact the development of atherosclerosis: reduction of hypertension and LDL oxidation, beneficial changes in lipid

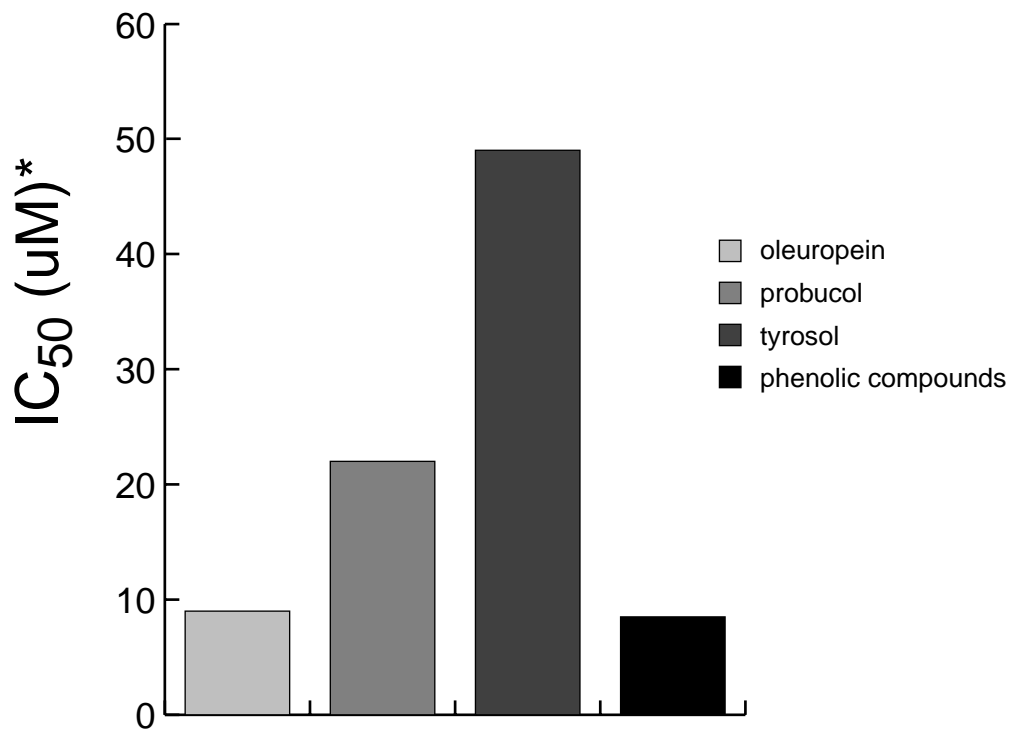
ratios, and reduction of macrophage uptake of LDL cholesterol.¹²⁵⁻¹²⁸

In a well-controlled trial of 23 patients with mild to moderate hypertension, extra-virgin olive oil was compared to sunflower oil, a polyunsaturated fatty acid (PUFA), for effects on blood pressure.¹²⁵ Patient diets were carefully controlled and differed only in the type of oil supplemented. After random assignment to the MUFA or PUFA diet for six months, patients crossed over to the alternate diet for an additional six months. Oils were added after foods were cooked, with men receiving 40 g (~ 4 tablespoons) and woman 30 g (~ 3 tablespoons) each day. Systolic (127 vs 135 mm Hg) and diastolic (84 vs 90 mm Hg) blood pressures were significantly lower at the end of the MUFA diet when compared to the PUFA diet. At the start of the trial, 21 of 23 patients required one or more medications for hypertension. A 48-percent reduction in daily medication dosage was observed after the MUFA diet, but no significant difference after the PUFA diet. At the end of the trial, all patients on the sunflower oil diet required medication while eight patients receiving the olive oil diet had no need for drug therapy.

The mechanism behind the effect of olive oil on blood pressure is not clear since serum lipid profiles were similar in both groups as well as confounding variables such as weight loss and potassium levels. The authors suggested that polyphenols found in olive oil may be responsible. The content of phenols and lignans is significantly reduced in refined olive oil¹²⁹ and is completely absent in sunflower oil. The authors’ explanation is that 15-20 mg/day of polyphenols obtained from olive oil is similar to the flavonoid intake found to be associated with decreased mortality from CVD in the Zutphen Elderly Study.¹³⁰

Several studies have shown phenolic compounds (PCs) extracted from extra-virgin olive oil significantly inhibited the oxidation of LDL cholesterol.^{129,131,132} Other polyphenolic compounds from *Camellia sinensis* (green

Figure 5. Individual Phenolic Components of Olive Oil versus Probucol versus Mixture of Phenolic Compounds from Olive Oil. Ability to Inhibit Oxysterol Formation.¹³¹



*IC₅₀=concentration at 50% inhibition oxysterol formation. Values represent mean \pm SEM of duplicate measurements of at least 4 experiments.

tea), red wine, *Glycyrrhiza glabra*, and Zingiber have also been found to inhibit the oxidation of LDL cholesterol.¹³³

In one study PCs from virgin olive oil were compared with single PC components such as tyrosol and oleuropein (polyphenols found in olive leaf and olive oil).¹³¹ They were also compared with probucol, a synthetic antioxidant medication demonstrated to prevent restenosis after balloon angioplasty, as a result of its antioxidant activity.¹³⁴ Probucol (a dimer of hydroxytoluene) also inhibited LDL oxidation when compared to alpha-tocopherol and inhibited LDL uptake into atherosclerotic lesions.¹³⁵ PCs were a more potent inhibitor of oxysterol (oxidized LDL) formation than single PC components or probucol. The IC₅₀

values for oxysterols were approximately 48, 21, 8, and 7.5 mM for tyrosol, probucol, oleuropein, and PCs, respectively (Figure 5).

In another study, healthy men were supplemented daily with 50 g olive oil for two weeks. The susceptibility of LDL to oxidation was reduced 73 percent.¹²⁸ In addition, macrophage uptake of LDL was reduced 61 percent. Significant reductions in both parameters were observed after only one week. Both oxidation and macrophage uptake of LDL are key factors initiating intimal cell injury, foam cell formation, and ultimately atherosclerosis.¹⁰ While the high ratio of unsaturated to saturated fats found in the Mediterranean diet may contribute to its substantial benefit in CHD, it appears olive oil may be working in other ways

as well. The antioxidant activities of its phenolic compounds, shown to inhibit key elements in the pathogenesis of heart disease, are very likely important.

Conclusion

The prevention and management of cardiovascular disease is undergoing radical change. World-wide availability of highly-sensitive C-reactive protein assays that have World Health Organization standardization may alter risk-screening for cardiovascular disease and increase targeted populations above and beyond those with hypercholesterolemia. Large-scale clinical prevention trials with antibiotics will seek to answer the question of whether or not CVD has an infectious etiology. Widespread use of statin drugs in the population with hypercholesterolemia will increase as the anti-inflammatory effects of these medications are more widely touted. Investigation of the hypocholesterolemic effects of red yeast rice by the newly-formed UCLA Center for Dietary Supplement Research on Botanicals will assess the action of monacolins compared to lovastatin and coenzyme Q inhibition.

Given the anti-inflammatory effects of inexpensive, side effect-free therapies like alpha-tocopherol and extra-virgin olive oil, it appears prudent to utilize these therapies initially in populations at risk for cardiovascular events.

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