

Postprandial Lipid Oxidation and Cardiovascular Disease Risk

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A mild pro-oxidative state accompanies meal ingestion, which results in an increase in biomarkers of inflammation, adhesion, and endothelial dysfunction, all of which are factors in the development of cardiovascular disease.

Both fat and carbohydrate can cause the effect, which is additive and exacerbated by diabetes. The presence of lipid, glucose, and cholesterol oxidation products of dietary or endogenous origin may contribute to postprandial oxidative stress. However, the generation of excess superoxide due to abundant energy substrate after the meal may be a predominate factor resulting in oxidative stress and a decrease in nitric oxide, which is important to endothelial function. Remediation of postprandial oxidative stress through direct reduction of superoxide generation and simultaneous consumption of antioxidants with each meal should be a focus of future research.

Introduction

Cardiovascular disease (CVD) is an umbrella term that includes conditions like coronary heart disease (CHD), atherosclerosis, angina pectoris, arrhythmia, congestive heart failure, congenital and rheumatic heart disease, and stroke. Established risk factors for CVD include modifiable risk factors (eg, hypercholesterolemia, low high-density lipoprotein cholesterol, hypertension, cigarette smoking, diabetes mellitus, overweight, and diet) and nonmodifiable risk factors (eg, family or personal history of early-onset CVD, ethnicity, age, and gender). These factors predict only some of the observed clinical events. In addition to the classic risk factors, several other factors have been identified as contributors or predictors of CVD. They are obesity, lack of exercise, infectious agents, indicators of inflammation and oxidative stress, as well as increased circulating levels of fibrinogen, triglycerides, homocysteine, and lipoprotein(a). This has led to an interest

in other nutritional and physiologic factors that might contribute to the underlying pathophysiology of CVD. Many of these risk factors are associated with increased oxidative stress in the vascular wall, which may contribute to CVD by several mechanisms. Low-density lipoprotein (LDL) modification due to oxidative damage by free radicals is an established cause of CVD, especially coronary artery disease. There is also a growing body of evidence that lipid peroxidation plays a pivotal role in developing atherosclerosis and that some of the risk factors for CVD may be partially mediated by increased concentrations of fatty acid, cholesterol, and glucose oxidation products or the pro-oxidative events that generate them. In 1979, Zilversmit [1] proposed that atherogenesis was a postprandial phenomenon, with the remnants of postprandial lipoproteins directly infiltrating the arterial wall leading to accumulation of atheromatous plaques. Endothelial dysfunction, an early feature of the atherosclerotic disease process, is evident during the first few hours following fat consumption [2]. Additionally, postprandial lipemia raises blood coagulability with the elevation of Factor VII in plasma [3]. Many of these postprandial changes are transient in nature. One of the most enjoyable and frequent activities in which people engage is eating multiple meals during the day, with the contents of the new meal coming into circulation before the metabolic disturbances associated with the previous meal have subsided. Thus, there is a dynamic postprandial state prevailing for about two thirds of the day. These repeated metabolic challenges represent the usual physiologic state of a free-living individual.

The postprandial state normally includes hyperglycemia and hypertriglyceridemia, which is exaggerated in type 2 diabetes patients. Both of these conditions, if evident in the fasting state, are CVD risk factors and are associated with oxidative stress. Therefore, it is important to discuss in detail the impact of the meal on the generation of oxidative stress because most foods undergo cooking- and storage-related oxidative modifications even before they are consumed. This review discusses postprandial lipid oxidation, oxidative stress, and the interplay between high-fat and high-carbohydrate meals on the postprandial modulation of inflammatory and endothelial risk factors for CVD.

Oxidative Stress and Cardiovascular Disease

Oxidative stress is defined as an imbalance between free radical production and antioxidant defense. Oxidative stress occurs when reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as lipid hydroperoxides (LOOH), superoxide anions (O_2^-), singlet oxygen, nitric oxide (NO), and peroxynitrite (ONOO⁻), overwhelm the body's defensive mechanisms due to poor antioxidant capacity. ROS are produced by several intracellular systems, such as cyclooxygenases, lipoxygenases, cytochrome P450, mitochondrial respiration, NADPH oxidase, and xanthine oxidase, and are generated under various physiologic and pathologic conditions such as inflammation, ischemia, reperfusion, sepsis, and ionizing irradiation [4]. NO is produced by numerous cell types. When produced by endothelial cells, it normally functions as a potent vasodilator, opposing leukocyte-endothelial cell adhesion and the aggregation of platelets. It is also a cytotoxic agent when produced by macrophages as part of the immune and inflammatory response. It is accompanied by the co-release of superoxide radical, hydrogen peroxide, fas ligand, tumor necrosis factor (TNF), and inflammatory cytokines [5].

Nitric oxide reacts with O_2^- to produce ONOO⁻, a potent long-lived oxidant that inhibits mitochondrial electron transport, oxidizes sulfhydryl groups, and initiates lipid peroxidation independent of the presence of iron or copper ions. It reacts with tyrosine residues of proteins to produce nitrotyrosine, which is measured as a biomarker of the presence of ONOO⁻ and the loss of NO. NO loss results in the deterioration of endothelial elasticity [6•].

Vascular inflammation and endothelium activation may be additional mediators of CVD. The biomarkers of chronic inflammation include proinflammatory cytokines such as soluble E-selectin (sE-selectin), and acute-phase reactants produced by the liver such as C-reactive protein (CRP). CRP is now recognized as an independent predictor of CHD [7]. Granger *et al.* [8••] reviewed in detail the role of inflammation in CVD and suggested that oxidative stress is a characteristic feature of the inflammatory response. During inflammation, ROS can promote the adhesion of blood cells to vascular endothelium by 1) eliciting the production of inflammatory mediators; 2) activating nuclear transcription factors such as nuclear factor κ B and activator protein-1, which bind to genes encoding endothelial cell adhesion molecules or cytokines; or 3) mobilizing preformed adhesion molecules in leukocytes (CD11b/CD18) and endothelial cells (P-selectin) to the endothelial cell surface.

Isoprostanes are prostaglandin-like compounds produced by peroxidation of arachidonic acid. Plasma and urinary F_2 -isoprostanes are established biomarkers of in vivo lipid peroxidation [9]. Keaney *et al.* [10] used the Framingham Offspring Cohort to assess the CVD risk factors and urinary concentrations of 8-iso-prostaglandin $F_{2\alpha}$ in more than 2800 men and women and found an independent association with both obesity and oxidative

stress. Obesity promotes clusters of CVD risk factors and the rate of morbidity and mortality is greater in obese people. The inflammatory cytokines interleukin 6 (IL-6) and TNF- α are expressed in increased concentrations with adiposity. Plasma CRP concentrations, significantly regulated by IL-6, are elevated with obesity and are consistently associated with increased incidence of CVD, suggesting an important role of the inflammatory state of obesity in cardiovascular pathology [11].

It has long been suggested that high levels of lipid oxidation products in the diet may accelerate the development of atherosclerosis both in animals and humans by increasing the proportion of oxidized lipid in the lipoprotein and making the LDL more susceptible to oxidation [12]. It is now believed that increased susceptibility of LDL to oxidation, associated with prolonged postprandial lipemia, contributes to the increased risk of coronary artery disease. First, aldehyde products of lipid peroxidation such as malondialdehyde (MDA) react with the amino groups of LDL lipoprotein to form MDA adducts recognized as antigens by scavenger cells. Second, oxidized phospholipid accumulated in various fractions of LDL may lead to inappropriate pathophysiologic responses within the cell types with which they come in contact. LDL contains up to 80% lipid, including polyunsaturated fatty acids and cholesterol, mostly as esters. Linoleic acid (LA), one of the most abundant fatty acids in LDL, produces a number of products when subjected to oxidative modification [13]. Hydroxy fatty acids formed from LA are hydroxyoctadecanoic acids (HODEs), and 9-HODE and 13-HODE are the major components of atherosclerotic plaque [14].

Hyperglycemia also stimulates ROS production by several pathways, such as the formation of glycation products, the auto-oxidation of glucose, and the stimulation of the polyol pathway. The rearrangement of these glycation products into advanced glycation end products (AGE) are recognized by cell-surface AGE-receptors. These mediate endocytosis and degradation of AGE-modified molecules, but they also stimulate ROS production and the release of cytokines and growth factors [15•]. AGE products stiffen arterial walls. The 2-hour postprandial plasma glucose concentration is an independent predictor of mortality and macrovascular disease and has been found to be the most important determinant of carotid intima media thickness (a surrogate marker of atherosclerosis), even more important than fasting plasma glucose or glycosylated hemoglobin (HbA_{1c}) concentrations [16].

It would appear that treatment with exogenous antioxidants might reduce ROS and RNS production and ameliorate CVD risk factors and disease expression. Indeed, CVD-related epidemiologic studies have shown that dietary antioxidant status is often correlated with lower mortality rates and CVD [17]. Many studies have demonstrated a beneficial effect of antioxidants on surrogate markers of CVD, such as endothelial function and lipoprotein oxidation [18]. Studies in animal models have suggested that

natural and synthetic antioxidants can prevent the development of clinical symptoms of CVD [19]. However, major intervention trials using massive doses of vitamins E and C (800 IU and 1000 mg, respectively) and sometimes beta-carotene, have been disappointing because they show no effect on CVD mortality, CHD-related events, stenosis progression, atherosclerosis progression, myocardial infarction, or stroke. The angiotensin-converting enzyme (ACE) inhibitor ramipril and the statin simvastatin appeared to offer additional protection beyond the lowering of blood pressure and lipidemia, respectively [20–24]. Although there are numerous small studies with specialized populations that have shown some beneficial results of antioxidant supplementation, one study is notable. A group of 520 smoking and nonsmoking Finnish men and women with high serum cholesterol received in two daily portions 136 IU/d of α -tocopherol (eightfold lower than cited studies), 250 mg of slow-release vitamin C (fourfold lower than cited studies), combination of the two, or placebo for 3 years. The combination reduced the rate of progression of intimal wall thickening of the carotid artery and the proportion of men with progression was reduced by 74% [25]. The dosage schedule in this study approximated the delivery of antioxidants from food sources in meals, and study participants were likely in the early stages of the disease process.

Ceriello [26••] suggests that the reason why the classic dietary antioxidants such as vitamins C and E have less than expected effectiveness in clinical trials is that they act on already formed oxidation products, whereas the real problem starts with O_2^- overproduction by way of the mitochondrial electron-transport system, xanthine oxidase, and nicotinamide adenine phosphate oxidase due to *luxus* glucose and fatty acid substrates in endothelial cells, which are exposed to the postprandial flooding of these substrates. O_2^- overproduction is accompanied by increased NO generation due to an endothelial NO synthase and inducible NO synthase uncoupled state. O_2^- and NO form ONOO⁻, a phenomenon that may be independent of vitamin C or E quenching. ONOO⁻ damages endothelial cell DNA, activating the nuclear enzyme poly(ADP-ribose) polymerase, which depletes intracellular nicotinamide adenine dinucleotide (NAD⁺), slowing the rate of glycolysis, electron transport, and adenosine triphosphate formation. Severely damaged endothelial cells may undergo apoptosis. These processes result in acute endothelial cell dysfunction. Statins increase NO bioavailability and decrease O_2^- production, and ACE inhibitors also act at a very early stage to suppress O_2^- production (Fig. 1).

Dietary Lipid Peroxides

Given the role of ROS and RNS in the development of CVD, are ROS, RNS, and ROS-generated oxidation products present in the foods we eat and do they get absorbed? Free radical attack on food lipids initiates a series of autocatalytic

free radical reactions. These reactions lead to the formation of a wide variety of oxidation products. The nature and proportions of these products can vary widely among foods and depend on the composition of the foods and numerous environmental factors. Secondary lipid oxidation products can react with other food components with the formation of toxic products that limit animal growth if fed in large quantities. LOOHs are present in small amounts in dietary fats and oils, and their formation rate increases with exposure to heat and light. Deep-fat frying, a popular method for food preparation (especially in the fast-food industry), produces a complex series of chemical reactions, characterized by a decrease in unsaturated fatty acids and an increase in free fatty acids, cyclic fatty acids, primary lipid hydroperoxides, and secondary oxidation products such as aldehydes, ketones, polymers, hydroxy fatty acids, hydroperoxyalkenals, hydroperoxy epoxides, and volatile carbonyl compounds, which are formed by the decomposition of primary hydroperoxides. Many oils used for deep-fat frying in fast-food outlets have been found to exceed the upper limit of 25% for modified lipid products [27]. A number of studies have shown that both fatty acid and cholesterol oxidation products are readily absorbed by humans and animals, with substantial rises in postprandial lipid peroxides when thermally treated oils are fed. The effect is exaggerated in subjects with glucose intolerance [28••]. Oxidized fatty acids are readily incorporated into mixed micelles and taken up into CaCo₂ cells (model enterocytes) where they are reesterified into triglyceride, but they are not taken up by smooth muscle cells, endothelial cells, or macrophages [29]. Not all the oxidation products are absorbed. The hydroxy fatty acids (*ie*, HODEs), which are found in high concentrations in atherosclerotic plaques, are reduced, detoxified, and eliminated by gastrointestinal glutathione peroxidase. These hydroperoxides may disturb intestinal lipid metabolism by being esterified to complex lipids. The lipoproteins synthesized from such complex lipids are susceptible to further oxidative modifications [30].

Cholesterol oxidation products (COP) in foods are known as compounds with adverse biologic effects and have been the subjects of extended research. 7-Ketocholesterol and 7-hydroxycholesterol are the main cholesterol oxidation products [31]. COPs can be formed *in vivo* and can also be absorbed from the diet. The mean COP absorption rate is about 30%, although it depends on the site of oxidation of cholesterol [32]. Cooked meat and meat products are major sources of COPs. Cooking methods such as grilling, roasting, and microwaving significantly increase cholesterol oxidation, increasing the total amount of COP as much as 4.5-fold [31,33].

Establishing a connection between oxidized dietary fat and CVD is severely hampered by a lack of valid measures for the level of oxidized lipids in the test fat. Most studies have reported the level of thiobarbituric acid-reactive substances, which is only a crude approximation of the wide variety of oxidation products that might be present.

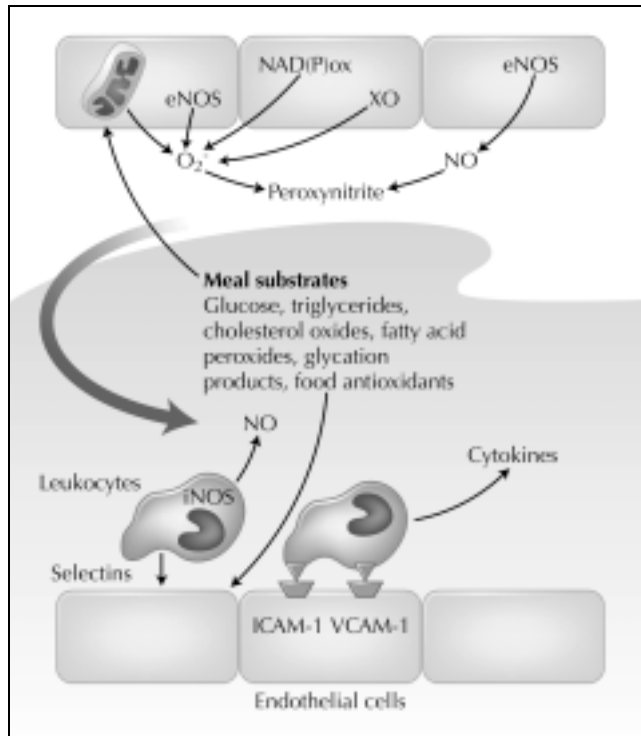


Figure 1. Meal substrates produce a mild inflammatory and leukocyte proadhesion response that leads to temporary endothelial dysfunction. This is due to overproduction of reactive oxygen species and reactive nitrogen species and a loss of nitric oxide (NO). The overproduction is due to a flooding of energy substrates into endothelial cell mitochondria and the activation of xanthine oxidase (XO) and nicotinamide adenine phosphate oxidase (NAD(P)ox), increasing the production of superoxide (O_2^-). Endothelial cell nitric oxide synthase (eNOS) and leukocyte inducible nitric oxide synthase (iNOS) produce NO, but the excess of O_2^- combines with NO to form peroxynitrite. Peroxynitrite, O_2^- , and meal lipid and glucose oxidation products stimulate inflammatory cytokines and adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular cellular adhesion molecule-1 (VCAM-1) that slow, then attract, leukocytes to the endothelial cell wall.

Studies with thermally treated oils have shown that both oxidized fatty acids and cholesterol can produce atherosclerosis in rabbits and gene knockout mice [34].

Endogenously Produced Lipid Oxidation Products

Dietary LOOH, after absorption, may first alter arachidonic acid, which is a primary fatty acid in membrane phospholipids. The organic free radicals thus formed may stimulate a cascade of damage to endogenous lipids. LOOH incorporates into the lipid bilayer, decreasing membrane fluidity and initiating lipid peroxidation in the lipid phase. Peroxidation of the membrane bilayer lipids initiates their autocatalytic breakdown, resulting in various byproducts such as hydroxynonenal, MDA, and other types of alkanals [35]. Udilova *et al.* [36] reported that heated dietary oils containing high amounts of LOOH were found to be a prerequisite for the initiation of membrane peroxi-

dation in artificial or isolated membranes. Oxidative membrane degradation entails a Fenton-type reaction (requiring iron or copper ions) with formation of alkoxy radicals. The LOOH, incorporated in the lipid phase of the membrane, also interacts with iron and ascorbic acid from the aqueous phase. However, alkoxy radicals formed directly in the lipid phase seem to possess a stronger damaging effect than those formed in the aqueous phase. Insertion of hydroperoxides in the membrane or loss of unsaturated fatty acids by oxidation alters membrane fluidity and may modify the activity of integral membrane proteins involved in cell signaling, transport, ion channels, and others [36].

In contrast, oxidation of arachidonic acid produces compounds such as F_2 -isoprostanes and hydroxyeicosatetraenoic acids (HETEs), both of which are found in atherosclerotic plaques. The hydroxy fatty acids in the plaque may represent markers of *in vivo* oxidative stress and may also have a role in the progression of plaque pathogenesis and instability. They are shown to upregulate the peroxisome proliferator activated receptor α (PPAR α) gene in macrophages, which differentiates the monocytes to macrophages and hence uptake of oxidized LDL to form foam cells [37]. Another important compound found in atherosclerotic arteries is cholesterol oxide. High cholesterol oxide concentrations are found in the lipoprotein of hypercholesterolemic patients. Lipid alkoxy and peroxy radicals remove hydrogen atoms at positions 7 and 8 of cholesterol, forming cholesterol oxide (oxysterol). This formation is regulated by both free radical and nonradical reactive species, such as $ONOO^-$. Cholesterol oxidized at carbon 7 may reduce endothelium-dependent relaxation and NO production by endothelial cells. The lower production of NO was related to either reduced availability of NO synthase or inactivation of NO by the reaction of superoxide radical forming the peroxynitrite ($ONOO^-$), which is a strong oxidizing agent. The peroxynitrite and its decomposition products induce peroxidation of the membrane lipids, causing endothelial lesions and an increase in vascular permeability [39]. Kanazawa *et al.* [40] demonstrated that dietary LOOH generated alkylperoxy radical (LOO^\cdot) after its reaction with various heme compounds such as myoglobin, cytochrome c, hemin, and hematin. The LOO^\cdot exhibited cytotoxicity and caused DNA damage, including strand breakage and basic site damage. Blair [41] recently reviewed the role of LOOH-derived endogenous DNA adducts as mediators of CVD, cancer, and neurodegeneration, the three most prevalent diseases. The mechanisms discussed involved the formation of LOOH by two pathways: 1) ROS-mediated pathway and enzyme-mediated (lipoxygenase and cyclooxygenase) pathway, and 2) decomposition of LOOH. Homolytic decomposition of LOOH to the α , ϵ -unsaturated aldehyde genotoxins, 4-oxo-2-nonenal, 4,5-epoxy-2 (E)-decenal, and 4-hydroxy-2-nonenal occurs through two distinct pathways. One pathway involves a complex rearrangement of the alkoxy

radical and the other pathway involves the intermediate formation of another potential genotoxin, 4-hydroperoxy-2-nonenal. 4,5-Epoxy-2(E)-decenal forms the etheno-2-deoxyadenosine adduct with DNA. 4-hydroxy-2-nonenal forms propane adducts with 2'-deoxyguanosine and upregulates cyclooxygenase-2 expression. Because cyclooxygenase-2 converts linoleic acid into LOOH, this provides a potential mechanism for increased production of genotoxic bifunctional electrophiles. MDA, another genotoxic electrophile, is formed during decomposition of LOOH. Other sources of MDA include hydroxyl radical-mediated decomposition of the 2'-deoxyribose DNA backbone and its formation as a side-product during the biosynthesis of thromboxane A₂. We see that the lipid constituents of the meal can give rise to a variety of lipid oxidation products that have been shown to have real effects on cardiovascular physiology.

Postprandial Rise in Lipid Oxidation Products and Cardiovascular Risk Factors

Although a theoretical case can be made that the postprandial period is important for the development of oxidized lipids, either of dietary origin or endogenously generated, are there studies that demonstrate a postprandial rise of lipid oxidation products? Furthermore, are these excursions of clinical significance? Several researchers have found a 20% to 30% increase in a number of parameters of oxidative stress, such as LOOH [42,43], MDA [44], free radicals [45], and nitrotyrosine [6•] in diabetic subjects 2 hours after meals. These increases were small or nonexistent in nondiabetic subjects fed normal-sized meals [42,46] except for nitrotyrosine, which increased about 30% in response to a meal containing 75 g of fat or 75 g of glucose. An exception was a preliminary study of nine healthy men who experienced a mean 153% increase in lipid peroxides measured using single photon counting technology in response to a meal containing 73 g of fat [43]. In addition, 2 hours after a meal, *ex vivo* LDL oxidation is accelerated and circulating antioxidant status is depressed [44]. Gradek *et al.* [47] determined that meal polyunsaturated fatty acids were the major source of oxidized lipids in patients with atherosclerosis as measured by postprandial suppression of antibodies to MDA-modified LDL.

Both dietary glucose and fat were capable of producing the increase in oxidation products, and when fed together they have an additive effect on nitrotyrosine generation. When simvastatin was given acutely it depressed nitrotyrosine generation independent of postprandial increases in triglyceride, indicating that the statins may have an additional antioxidant effect [48•]. Acute rises in glucose by way of glucose clamp produced a 150% increase in plasma nitrotyrosine levels that was accompanied by a 5% increase in mean blood pressure in healthy subjects. There was a correlation ($r = 0.42$; $P = 0.02$) between blood pressure and plasma nitrotyrosine levels [49].

In addition to blood pressure, flow-mediated endothelial vasodilation (FMD), which is a measure of endothelial function, deteriorated with a high-fat meal in diabetic subjects and was improved by prior treatment with ciprofibrate, a lipid-lowering drug. The improvement was also accompanied by a decrease in postprandial free radical generation [45]. Both fat and glucose meals depressed FMD in diabetic and nondiabetic subjects, and the effect was additive when the two were combined [50]. The meal-induced modulation of endothelial cell function was accompanied by concomitant increases in cytokines and adhesion factors. Interleukin 6 (IL-6) was increased by 150% 2 hours after a high-fat meal but not after a high-glucose meal, whereas IL-8 was increased 200% after both a high-fat meal and a high-glucose meal and increased 500% by 8 hours postprandially when both were fed together in healthy men [42]. Both of these are proinflammatory cytokines. The effect on IL-8 was not evident at 2 and 4 hours after either high-fat or high-carbohydrate meals in both diabetic and nondiabetic subjects, but high-fat meals increased IL-18 at both time points [51]. Both high-fat and high-glucose meals also produce modest increases (8% to 20%) in the adhesion molecules intercellular adhesion molecule-1 (ICAM-1) and E-selectin at 2 hours in both diabetic and nondiabetic subjects, but vascular cellular adhesion molecule-1 (VCAM-1), another adhesion molecule, was only elevated in diabetic subjects because it appeared only responsive to elevated glucose levels [48•].

We see that both dietary fat and carbohydrate produce oxidative stress expressed as a rise in lipid and tyrosine oxidation products and a decrease in circulating antioxidant capacity. The rise is accompanied by increases in biomarkers of inflammation and atherosclerotic plaque formation (adhesion molecules), and a decrease in endothelial cell function. The effect is more dramatic in diabetic subjects (with greater postprandial triglyceride and glucose excursions) but is also evident in nondiabetic subjects. The effect of dietary carbohydrate and triglyceride appear to be additive, with the fat effect slightly delayed and mirroring the time course of triglyceride entry into circulation.

Ceriello [26••] has effectively argued that the underlying unifying factor for the modulation of the variety of cardiovascular risk factors in response to carbohydrate meals is the generation of ROS and RNS, and Heine and Dekker [52] argue that postprandial hypertriglyceridemia-related oxidation products may be equally important to the development of CVD. A number of studies have explored whether the co-administration of a variety of antioxidants might ameliorate the rise in postprandial oxidative stress, inflammatory cytokines, adhesion molecules, and the deterioration of endothelial function. Vitamins C (1000 mg) plus E (800 IU) given with high-fat meals completely prevented the rise in VCAM-1, ICAM-1, IL-6, and TNF- α in both healthy and diabetic subjects. High-carbohydrate meals produce raises in these inflammatory

and adhesion molecules only in the diabetic subjects, which again were prevented with vitamin supplementation [53]. Vitamin C and E supplementation at these same high levels was more effective in completely lowering the rise in CRP (another inflammatory cytokine and predictor of cardiovascular disease) when given with a high-fat, high-carbohydrate dinner resembling a McDonald's Big Mac® meal (1314 kcal) compared with supplementation at breakfast the day of the evening test meal in diabetic subjects. The postprandial rise in plasminogen activator inhibitor-1, a clot-lysis prevention factor and predictor of cardiovascular disease, was only prevented when the vitamins were administered at breakfast. The postprandial rise in plasma MDA concentration was only decreased when vitamins were given with the test meal, but these investigators [54] measured free MDA even though most MDA is bound to protein and must also be measured. The same high doses of vitamins C and E also prevented the deterioration of FMD that occurs when glucose tolerance tests are given to healthy young men and women [55]. A large glass of red wine (300 mL) had a similar effect and also depressed lipid peroxides [43] and improved antioxidant status (measured by ex vivo LDL oxidation and by a telomerase repeat amplification protocol assay) and the clotting factor F-VII [56].

Simultaneous administration of antioxidants was able to prevent the rise of ROS, RNS, inflammatory cytokines, clotting factors, and deterioration of endothelial function. These findings point to a foundational role of ROS and RNS in the generation of inflammation and CVD risk factors during the postprandial period.

Conclusions

Studies with single meals demonstrate that the meal itself produces a mild inflammation, a leukocyte proadhesive environment, and a loss of endothelial reactivity. The effect is maximal 2 to 4 hours after meal ingestion. Both dietary glucose and triglyceride are equally effective in producing the response, and when fed together their effect is additive. Diabetic subjects have an exaggerated response to both dietary fat and glucose and suffer from greater postprandial excursions of these energy substrates. The inflammation is either preceded or accompanied by an increase in ROS and RNS and nitrotyrosine, which is a marker of peroxynitrite production. Current hypotheses propose that glucose and fatty acids temporarily flood the mitochondrial capacity for their metabolism, and excess superoxide is produced via a variety of mechanisms. Furthermore both xanthine oxidase (XO) and nicotinamide adenine dinucleotide phosphate oxidase are also induced and also produce superoxide. At the same time, endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) are stimulated to produce NO to modulate vascular tone. eNOS also produces superoxide as a side product. The excess superoxide

combines with NO to produce peroxynitrite, a very reactive RNS, which results in reduced concentrations of NO, which decreases endothelial responsiveness. ROS and RNS appear to be the stimulators of cytokine and adhesion molecule production in endothelium and leukocytes (Fig. 1).

Other lines of research implicate fatty acid peroxidation products and cholesterol oxides, which are produced in food fats via cooking and storage, and endogenously produced lipid oxidation products, which all may directly stimulate an inflammatory response. Glucose can also be auto-oxidized and produces AGE products that have a direct effect on the endothelial lining. These products are well absorbed and animal studies have demonstrated increased CVD when these products are fed as part of the diet.

Reactive oxygen species and RNS are likely the prime movers in postprandial inflammation because classic antioxidants such as vitamins C and E and glutathione have all been shown to ameliorate the postprandial rise in cytokines, adhesion molecules, and the deterioration of endothelial responsiveness. It is likely they are most effective when included at each eating occasion. This may explain part of the failure of classic antioxidants to affect CVD outcomes in clinical trials, but it is also likely that they do not diminish the production of peroxynitrite and the consequent reduction of NO. A more effective approach may be to reduce the production of superoxide at its sources by 1) reducing the postprandial flood of glucose and triglyceride by the consumption of smaller meals and improving insulin sensitivity through exercise and a reduction of adiposity; 2) improving mitochondrial efficiency through the provision of cofactors such as lipoic acid and carnitine; and 3) by the use of statins and ACE inhibitors that block superoxide via other mechanisms than direct attack. The postprandial period, which extends during a major portion of the day, is clearly relevant to the development of CVD and should be a focus of research to ameliorate postprandial oxidative stress and inflammation.

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