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Antioxidant studies need a change of direction

AS HASNAIN AND MOORADIAN POINT OUT in this issue of the *Cleveland Clinic Journal of Medicine*,¹ most trials of antioxidants to date^{2,3} failed to show any clinical benefit from taking antioxidant supplements for diseases thought to be due to oxidative damage. Some studies even showed that they may be harmful.

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Given the lack of evidence of benefit, is it time to close the book on the “oxidative hypothesis,”⁴ the concept that antioxidants can be used to prevent or heal oxidative damage?

No, but it is certainly time to try a different approach. There may be patients who could benefit from antioxidant therapy but who have not yet been identified. Further, there is considerable evidence that so-called antioxidants such as vitamin E do not effectively block relevant oxidation pathways. Thus, a major flaw of the studies to date is that they failed to measure changes in levels of oxidative stress. We propose a number of markers of oxidative stress that could be used in future studies to identify patients who might benefit from antioxidant therapy, as well as to serve as indexes to gauge the effectiveness of antioxidant interventions.

■ OXIDATION MODIFICATION HYPOTHESES

Oxidation is thought to underlie a number of degenerative diseases of aging, particularly

atherosclerosis, cancer, and arthritis. Certain oxidation pathways have been shown to promote protein, lipid, and DNA oxidation in vivo, processes implicated in artery wall damage (contributing to atherosclerosis) and DNA damage (leading to cancer).

The link to atherosclerosis has perhaps been the most extensively explored. More than 20 years ago, Brown, Goldstein, and colleagues^{5,6} recognized that modification of low-density lipoprotein (LDL) cholesterol was necessary to give it atherogenic potential, permitting scavenger receptor recognition, cholesterol deposition, and formation of foam cells, the earliest cellular hallmark of atherosclerosis.^{5,6} Concomitant but separate research by Chisolm and Steinberg⁷ revealed that oxidative modification of LDL converts the native lipoprotein into a cytotoxic, proatherogenic form.

We now have an enormous body of evidence for mechanistic links between oxidation and atherosclerosis. However, although early observational studies showed a potential link between antioxidant consumption and decreased cardiovascular mortality, prospective randomized trials in humans have failed to show that giving antioxidant supplements confers any significant clinical benefit.

■ WHAT WE SHOULD BE MEASURING

Clinical trials have attempted to measure the effectiveness of antioxidants in terms of clinical end points. Remarkably, however, no trial has attempted to monitor markers of oxidation. This is an alarming flaw in the design of antioxidant studies so far. It is analogous to testing a lipid-lowering drug with-

Studies so far have tried to gauge antioxidant effect without measuring the effect on oxidation levels

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out measuring lipid levels, or testing an antihypertensive drug without measuring blood pressure.

This flaw is all the more relevant given that recent studies, such as that by Meagher and colleagues,⁸ revealed no suppression in systemic indices of oxidant stress despite massive doses of vitamin E.⁸ A likely explanation is that vitamin E—the antioxidant most often used in studies to date—does not easily inhibit the relevant oxidation pathways in the artery wall. In other words, vitamin E has little effect on oxidants derived from nitric oxide^{9–11} or on pathways catalyzed by myeloperoxidase.^{12,13} Moreover, studies have shown mechanisms through which “antioxidants” such as vitamin E and vitamin C actually promote rather than inhibit oxidation.^{14,15}

■ WHICH MARKER IS BEST?

As Hasnain and Mooradian suggest, future clinical trials that attempt to prove benefits of antioxidant therapy need to employ specific measures of oxidant stress to ensure that the intended actions of the antioxidants used are observed. This of course raises the question of which oxidation marker is best suited for monitoring in intervention studies (FIGURE 1).

F2-isoprostanes

F2-isoprostanes—derived from the free radical oxidation of arachidonic acid—are the most widely studied molecular markers of oxidant stress.^{16–20} Levels are increased in people with coronary artery disease, as well as in those with cardiovascular disease risk factors.^{21–24}

Nitrotyrosine

Specific molecular markers of distinct oxidation pathways known to be enriched within human atherosclerotic plaque have also been suggested as protein oxidation markers for monitoring cardiovascular risk and response to therapeutic interventions.^{11,12} For example, nitrotyrosine, an inflammatory product generated by protein modification by oxidants derived from nitric oxide, is associated with coronary artery disease and is modulated by statin therapy.¹¹

Myeloperoxidase

Myeloperoxidase, a leukocyte enzyme that plays a role in host defenses by generating reactive oxidants, is enriched in human atheroma and in the circulation of people at risk for cardiovascular disease.^{25–28}

As noted above, vitamin E does not block many of the oxidative processes at work within human atheroma, and supplementation with vitamin E failed to promote systemic antioxidant effects in humans, as monitored by urinary F2-isoprostanes.⁸ It is remarkable that numerous by-products of myeloperoxidase-catalyzed pathways (eg, chlorotyrosine, nitrotyrosine, dityrosine) are stable, detectable in plasma or serum, and able to be modulated by interventions that reduce cardiovascular risk (eg, statins).¹²

■ APPROPRIATE DESIGN OF ANTIOXIDANT TRIALS

The “cholesterol hypothesis” failed to gain widespread acceptance until trials of lipid-lowering drugs showed clinical benefit in pre-selected patients with high cholesterol levels. Future antioxidant trials should measure markers of oxidation in people with documented high levels of oxidative stress. Markers of oxidative stress must be monitored to show a direct effect on oxidation by the antioxidant being studied.

Rather than use conventional antioxidants, ie, agents that intercept reactive oxidants, scavenging them before they can damage targets *in vivo*, future studies should test drugs that induce systemic antioxidant biochemical profiles, such as that which was recently demonstrated with HMG-CoA reductase inhibitors (statins).¹²

■ OUR RECOMMENDATIONS

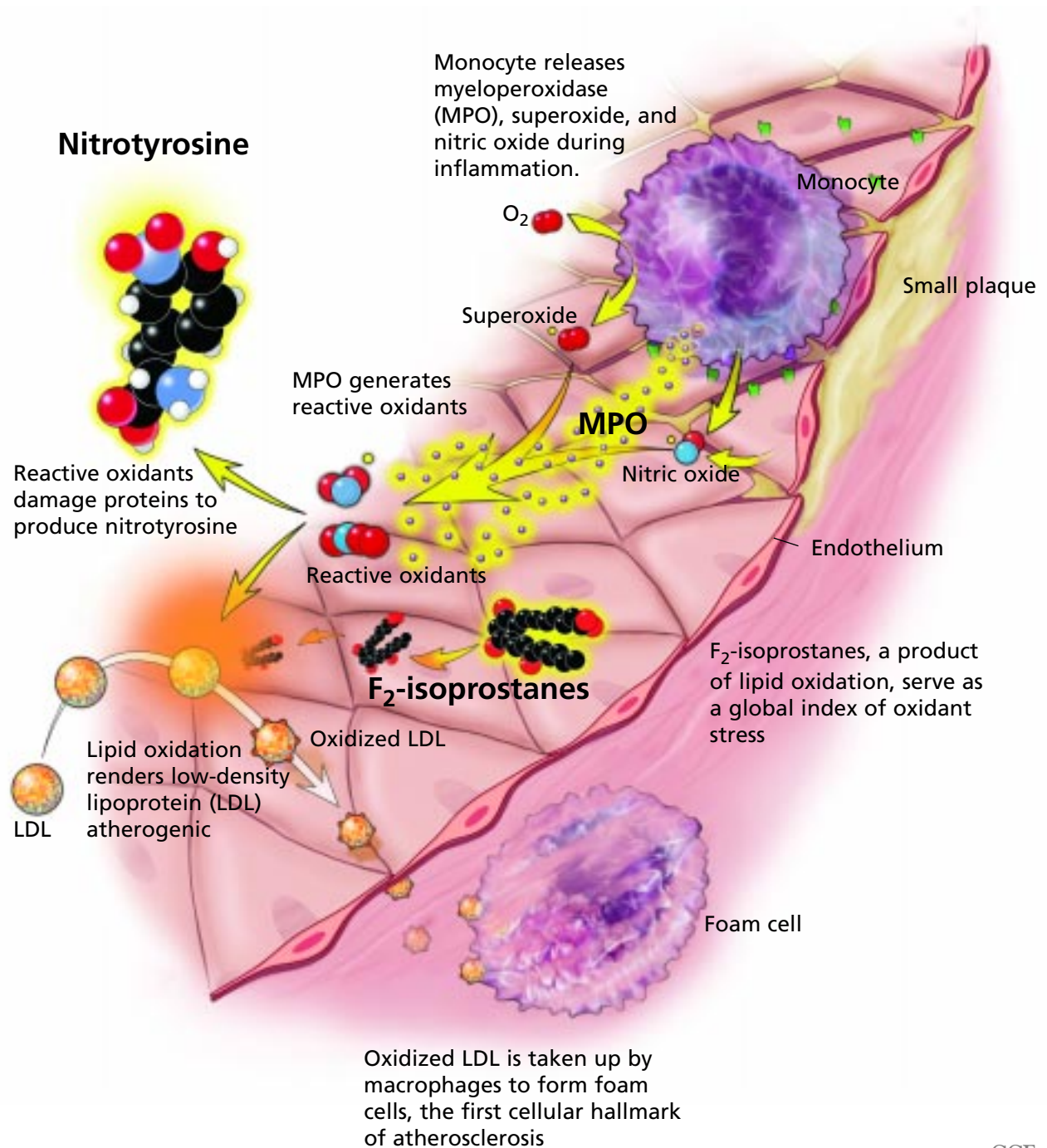
We agree with Hasnain and Mooradian that none of the so-called “antioxidants” has been shown to reduce cardiovascular morbidity, mortality, or cancer incidence, and therefore none of them can be recommended for routine primary or secondary prevention at this time. However, we believe that certain groups who may benefit may be identified in properly performed future clinical studies.

The best way to gauge antioxidants' effects is to monitor markers of oxidative stress



Formation of three markers of oxidative stress

Inflammatory pathways can produce several potential markers for measuring oxidative stress in people, including myeloperoxidase (MPO), nitrotyrosine, and F₂-isoprostanes.



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FIGURE 1



The only way studies of antioxidants or antioxidant-eliciting therapeutic agents can test the oxidation hypothesis is by monitoring antioxidant effects through measurement of specific oxidative products. The ideal marker of

oxidative stress would be one that has been linked to disease pathogenesis, that is elevated in people with atherosclerosis, and that is modulated by interventions shown to reduce cardiovascular risks (eg, nitrotyrosine and statins).¹²

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