Aging-Related Nicotinamide Adenine Dinucleotide Oxidase Response to Dietary Supplementation: The French Paradox Revisited

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Abstract

Aging-related cell-surface NADH oxidase (arNOX)-specific activities increase with age between age 30 and ages 50–65. The protein is shed and circulates. Activity correlates with a number of aging-related disorders including low-density lipoprotein (LDL) oxidation as a precondition to atherosclerosis as well as oxidation of collagen and elastin as a major contributor to skin aging. arNOX inhibitors formulated for sustained release are capable of maintaining circulating arNOX at low levels with regular use as food supplements formulated with natural compounds. Among the best sources are certain culinary seasonings, all of which are ingredients used extensively in the French kitchen. Their regular use may contribute to an understanding of the nutritional basis for the French Paradox.

Introduction

Age and oxidative stress are major risk factors for heart disease. A large body of evidence supports the notion that reactive oxygen species (ROS) provide a causal link in the appearance of oxidized circulating lipoproteins such as oxidized low-density lipoproteins (LDLs) and their subsequent clearance by macrophages and delivery to the arterial wall. It now appears likely that oxidized LDL is a major contributor to progressive atherogenesis by enhancing endothelial injury, by inducing foam cell (lipoprotein-engorged macrophages) generation and associated smooth muscle proliferation. Macrophages clear the circulation of oxidized lipoprotein particles by internalizing them and in so doing they are transformed into foam cells. The foam cells deliver their cargo of oxidized fats and cholesterol where they are deposited beneath the arterial wall. Such progressive delivery of oxidatively damaged lipoprotein particles eventually leads to atherosclerotic plaques and advanced heart disease.

However, the basis for LDL oxidation has been little studied. Levels of common antioxidants, including α-tocopherol, β-carotene, and ascorbate, decline with age, but there is no apparent correlation between ingestion of these common antioxidants and amelioration of the aging process or decreased mortality. The implication is that the oxidative damage leading to aging and increased atherogenic risk is the result of a much more specific causation. Why does LDL oxidation increase in the elderly, and why is it greater in some individuals than in others? Our findings suggest that LDL oxidation in the elderly and in individuals at high risk for heart disease correlates with levels of circulating aging-related cell-surface NADH oxidase (arNOX). The arNOX proteins are shed into the milieu surrounding the cells. In aged individuals, the amount of superoxide generated by the shed arNOX proteins has been measured to be quite substantial reaching a maximum at age 65–75 in males and age 55–65 in females. Of those who die of a heart attack, 85% are 65 or older. Women surviving beyond age 65 usually have diminished arNOX levels compared to men and a lower risk of cardiovascular disease compared to men, further suggesting some causal relationship between arNOX levels and atherogenic risk.

Methods

arNOX activity was assayed from measurements of superoxide production based on superoxide dismutase (SOD)-inhibited reduction of ferricytochrome c determined from absorbance at 550 nm with reference at 540 nm. Protein amounts were determined by the bicinchoninic acid procedure.

Levels of oxidized LDL in serum were measured by using a malondialdehyde protocol modified from Smith et al. Briefly, the sera was combined with a mixture of 20% (wt/vol) trichloroacetic acid and 0.6 M HCl containing 0.06 M thiobarbiturate and heated for 15 min at 100°C. Absorbance was measured at 532 nm from spectra obtained between 300 and 600 nm with malondialdehyde equivalents calculated for 1,1,3,3-tetramethoxypropane (Aldrich) standards.

Lipoproteins were isolated by flotation ultracentrifugation. Sera of healthy volunteers were collected using Institutional Review Board (IRB)-approved protocols. Informed consent was used. Plasma membranes were isolated from a plant source enriched in arNOX and compared to plasma membranes from a comparable plant source not enriched in arNOX. Plasma membranes were isolated by aqueous two-phase partitioning as described.9

Results

arNOX proteins are unique among the ECTO-NOX (ENOX) proteins in that they result in the generation of superoxide at the cell surface and, as shed proteins, appear in the circulation and other body fluids (saliva, urine, perspiration, and interstitial fluids).10–12 The superoxide generated affords an opportunity to form hydrogen peroxide (H$_2$O$_2$) and other ROS for propagation to adjacent cells and tissues and for direct oxidation of serum lipoprotein particles. Because arNOX is shed, the ROS generated from the superoxide becomes accessible to lipoproteins in the circulation, resulting in their oxidation and increased atherogenic risk as well as damage to adjacent cells and extracellular supporting matrices that are important to skin health.

A further unique feature of the arNOX proteins is their absence or presence at levels below the limit of detection for cells and sera of young individuals. They then increase with increasing age (>30 years) to about age 60–70.13 The distribution of arNOX activity with age correlates closely with the American Heart Association’s assessment of risk for coronary artery disease.

That the arNOX activity of plasma membranes is active in oxidizing lipoproteins was demonstrated from data showing malondialdehyde-like materials being formed during 2 h of incubation of lipoprotein particles isolated from human sera with plasma membranes expressing high levels of arNOX compared to plasma membranes lacking arNOX (Table 1). The amount of lipoprotein oxidation that occurred during the 2 h of incubation was enhanced 13-fold by the presence of the plasma membranes expressing arNOX compared to plasma membranes lacking arNOX. Similar results have been obtained subsequently using various soluble (or recombinant) arNOX sources.

This work has subsequently been advanced at NOX Technologies, Inc., to result in a nutritional supplement based on arNOX sources. Each herbal or phenolic arNOX inhibitor source exhibited its own characteristic time course of inhibition. The different kinetics exhibited by each of the various sources of arNOX inhibitors offers the opportunity of preparing combinations of inhibitor sources to achieve broad-spectrum inhibition. However, by formulating the herbal preparations as sustained-release preparations, 24 h of protection was attained with just two 400-mg capsules/day (one in the morning and one before bedtime). It is this aspect that makes possible a therapeutic utility of the technology to reduce aging-related arterial damage from oxidized circulating lipoproteins in individuals as they age beyond 30 years.

| Table 1. arNOX-mediated Oxidation of Serum Lipoproteins |
|--------------------------|--------------------------|--------------------------|--------------------------|
|                          | MDA-LM μmole/L           |                          |                           |
| Donor                    | Acceptor −SOD            | Lipoprotein +SOD         | Change due to arNOX      |
| Plasma membrane overexpressing arNOX | 0.93 ± 0.03               | 0.57 ± 0.03               | 0.40 ± 0.06             |
| Plasma membrane lacking arNOX      | 0.43 ± 0.06               | 0.43 ± 0.06               | 0.03 ± 0.95             |

MDA-LM formed 2 h of incubation with donor plasma membranes overexpressing high levels of arNOX compared to plasma membranes not overexpressing arNOX.

Abbreviations: arNOX, Aging-related cell-surface NADH oxidase; MDA-LM, malondialdehyde-like materials; SOD, superoxide dismutase.
The hypothesis under investigation is that arNOX is responsible for LDL oxidation, which correlates with atherogenesis. Our expectation is that arNOX inhibitors will ameliorate and possibly prevent progression of coronary artery disease. On this basis, work conducted at NOX Technologies, Inc., has led to the concept of anti-arNOX nutritional supplements based on the arNOX inhibitory properties of various French seasonings known collectively as Herbes de Provence.

Ingestion of such preparations results in a decrease in the generation of ROS by arNOX and provides a possible explanation for the French Paradox where the French diet or lifestyle leads to reduced atherogenic risk despite a cholesterol-rich diet high in cheese and butter. Previous studies attributed the reduction in risk to consumption of red wine as a natural source of the polyphenol resveratrol. However, the herbs listed in the text, which are staples of the French diet, offer a more compelling explanation. Certain of these herbs and phenolics may have benefit as well by inhibiting platelet adhesion and aggregation.

Author Disclosure Statement

All authors are affiliated directly or indirectly with NuSkin International, Provo, Utah, manufacturers and distributors of ageLOC®, skin care products based on arNOX inhibitors developed in part by NOX Technologies, Inc., West Lafayette, IN.

References


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