J Pharmacol Sci **108**, 393 – 398 (2008)

Journal of Pharmacological Sciences

Current Perspective

Aortic Aging in the Fischer 344 / NNiaHSd × Brown Norway / BiNia Rat

Kevin M. Rice[1,2,3], Miaozong Wu[1,3], and Eric R. Blough[1,2,3,4,*]

[1]Department of Biological Sciences, College of Science; [2]Department of Pharmacology, Physiology, and Toxicology, Joan C. Edwards School of Medicine; [3]Cell Differentiation and Development Center; [4]Department of Exercise Science, Sport and Recreation, College of Education and Human Services; Marshall University, Huntington, WV 25755, USA

Received September 4, 2008

Abstract. Aging is now recognized as one of major risk factors for cardiovascular disease (CVD). It is well documented that elderly populations show increased incidence of CVD symptomology but whether these changes are directly related to aging is not well understood since the possibility exists that other age-associated pathologies in different organ systems could impact on cardiovascular function. Hence, the development of an aging model with reduced systemic illness could invigorate efforts to understand the direct role of aging in CVD progression. The Fischer 344 / NNIaHSD × Brown Norway / BiNia rat (F344BN) has been proposed as a potential model for aging that exhibits reduced systemic pathology and increased longevity compared to other models. Here we examine the current literature regarding the F344BN, focusing on age-associated changes in aortic structure and function.

Keywords: aging, Fischer 344 / NNIaHSD × Brown Norway / BiNia rat (F344BN or FBN), aorta, morphological alteration, molecular signaling

Introduction

Cardiovascular disease (CVD) is the leading cause of death the world over and is responsible for approximately 30% of all deaths (1). Changes in the structure and function of the large arteries have been shown to significantly influence the development of complications in CVD. The aorta experiences severe mechanical demands during the cardiac cycle. At the peak of ventricular systole, the aorta is subject to the highest luminal pressure of any blood vessel in the body. The elastic nature of the aorta dampens the pulsatile pressure, protecting the more vulnerable downstream peripheral vessels. This function translates into a repetitive insult to the structure of these large compliance vessels equivalent to a sustained force of 1.88 lbf/in^2 (13.0 kPa) and a repetitive pulse of 0.75 lbf/in^2 (5.17 kPa). Given the nature of this hemodynamic loading, it is not surprising that the contractile-elastic unit (elastin) begins to fatigue by the sixth decade of life, having experienced the accumulative cyclic stress of more than 2 billion aortic expansions (2) This continual assault eventually leads to fracturing of the elastin and structural changes of the extracellular matrix, which includes proliferation of collagen and deposition of calcium (2, 3). As a result, aging of large arteries in humans and animals leads to elongation, torsion, enlarged lumens, and thickened vascular walls, particularly in the intima and media (4 – 6). This deteriorative process, termed arteriosclerosis, culminates in increased elastic arterial stiffness and an increase of pulse pressure (2 – 5). Here, we address whether the Fischer 344/NNiaHSd × Brown Norway /BiNia Rat (F344BN) may serve as a model to study the effects of aging on the aortae. Attention will be given to the mechanisms responsible for age-related morphological alterations as well as molecular and signaling changes.

*Corresponding author (affiliation #1). blough@marshall.edu
Published online in J-STAGE
doi: 10.1254/jphs.08R02CP

Invited article

Fischer 344/NNiaHSd × Brown Norway/BiNia: a good model of vascular aging?

Mammalian species share remarkable similarities in cardiovascular structure and function and the exploitation of these similarities in animal models has proven extremely helpful in developing treatment strategies for

CVD in humans. Nonetheless, it is important to recognize that conclusions drawn from the differences observed in a gerontologic investigation can be compromised due to the differential development of disease states in young and old animals. Data gleaned from the study of an aging model that dies at an early age may be problematic if it is unclear whether that animal's truncated longevity is due to a specific disease as opposed to the process of aging. Indeed, some diseases are themselves considered as models of accelerated aging. If death occurs prior to the physiological changes normally associated with advanced chronological age for a given species, the animal may reach the end of their life before experiencing significant aging. Therefore, it is advantageous to use cohorts of animals that are relatively lesion free for a longer period of time because they provide a larger window to compare changes associated with age without the complication of disease (7, 8).

Although researchers have utilized various rat models to investigate the aging process, many of these models have been show to demonstrate the development of various complications that could compromise the observations of age-associated cardiovascular dysfunction. For example, the Fischer 344 (F344) and Wistar rats exhibit glomerulonephritis (7), which could affect blood volume regulation and hence blood pressure; the F344 develops spontaneous leukemia and prostate cancers (7); the Sprague-Dawley rat suffers from a very high incidence of pneumonia (9); the Brown Norway rat exhibits spontaneous rupture of the internal elastic lamina in various arteries as early as 5 weeks of age (10); and the spontaneously hypertensive rat (SHR) exhibits impaired arterial function due to long-term hypertension (11).

In the 1970's, the National Institute of Aging (NIA) selected two rat strains for the development as aging models for biomedical research. These two strains were the Sprague-Dawley and the Fischer 344 (9). The Sprague-Dawley rat proved to be a poor selection due to high incidence of pneumonia early in life and tumor pathology susceptibility that appeared at 12 – 13 months of age (9). Due to this fact the Sprague-Dawley rat model was dropped by the NIA by the late 1970's. In the early 1980s, NIA developed five F1 hybrid crosses with the Fischer 344 to minimize the influence of age-associated pathologies in the F344. Extensive pathological assessment of these F1 strains revealed that the Fischer 344/NNiaHSd × Brown Norway/BiNia F1 hybrid (F344BN) was the superior animal, which resulted in this model being recommended by the National Center for Toxicological Research and the NIA as the preferred model for aging rat research (7, 9, 12).

The Fischer 344/NNiaHSd × Brown Norway/BiNia demonstrates longer maximal life span, normal growth curve, normal distribution of age-related pathology occurring relatively late in life, and a greater mean age for 50% mortality when compared to the F344 (7, 12). In addition, this aging model has a distinct advantage over the F344 rat in that the F344BN present less age-related pathologies than those observed in the Fischer 344, such as age-associated renal dysfunction, glomerulonephritis, increased incidence of leukemia, and spontaneous prostate cancer (7, 9), thus meeting NIA goals to provide an animal model that exhibits age-associated changes as a result of "normal aging processes," rather than the simple reflection of ongoing disease (9). Based on our experience, it is common for the F344BN to appear robust at 36 months of age, roughly equivalent to the eighth decade of life in humans (6, 13 – 19). To date, the F344BN has been utilized as a rodent model in many areas of aging research, including age-associated sarcopenia (13, 18, 19), myocardial senescence (20), along with age-related neuronal (21), retinal (22), and renal (23) changes.

Morphological changes in the aging aorta of F344BN

Recent studies using the F344BN rat (6, 16, 17) has found no evidence of age-associated pathology such as intimal fibrosis, fibrolipid plaques, or ulceration in histological cross-sections of the male 6-, 30-, or 36-month age-groups, suggesting that these animals do not exhibit age-associated atherosclerosis (Table 1). Nonetheless, and like that observed in aging humans, aging in the F344BN is characterized by an increase in tunica media thickness (3, 6, 24). Similar findings have been demonstrated in the aging female F344BN. For example, Gaballa et al. (3) found that medial thickness was increased in aortae of 23-month-old compared to 6-month-old animals. Vessels from aging female F344BN also demonstrated increased collagen content, collagen /elastin ratio, and smooth muscle area (3). In conjunction with these changes, elastin density and nuclei number decreased with age (3). However no changes in nuclear cross-sectional area, collagen density, or elastin content were observed with aging (3). Figure 1 depicts a Van Gieson trichrome staining of a male F344BN aorta at 6 and 36 months of age. Similar to that seen in aged humans, the elastin rings apparent in the 6-month aorta appear fragmented and discontinuous in the 36-month. Also, the appearance of a large area of collagen staining is present at the adventitia (unpublished data from ER Blough and colleagues). Von Kossa's calcium staining further demonstrates areas of calcium deposition with advanced aging that is consistent with arteriosclerosis.

Table 1. Changes associated with atherosclerosis, arteriosclerosis, and the F344BN model

	Atherosclerosis	Arteriosclerosis	36 months F344BN
Fibrosis	○	○	○
Collagen deposition	○	○	○
Calcium deposition	○	○	○
Elastin fracturing	○	○	○
Intima thickening	○	○	○
Fibrolipid plaques	○	—	—
Intracellular lipid accumulation	○	—	—
Fatty streak	○	—	—
Fibroatheroma	○	—	—
Ulceration	○	—	—

Comparisons between the various changes associated with atherosclerosis and arteriosclerosis and the pathology seen in the male F344BN aortae with age (○, present; —, not present).

By comparison, staining of aortic sections with Oil Red O Stain failed to show evidence of intracellular lipid accumulation or fatty streaks within the intima (Fig. 1), suggesting the absence of atherosclerosis. Taken together, these histological data are consistent with the notion that aging in the F344BN rat is non-pathological in nature (7, 9).

Molecular and signaling changes in the aging aorta of F344BN

Similar to human non-pathological vascular aging, the F344BN model demonstrates reduced vascular compliance (24) and increased axial stiffness (3) (Table 2). There is evidence that the Rho cascade may play an important role in the reduction of passive vascular tissue compliance (25) and that a deficiency in endothelium-dependent inhibition of this mechanism may explain increased age-associated vessel stiffness in the F344BN (24). Differences in aortic stiffness with age were obviated, for the most part, with incubation of tissue with the NO donor sodium nitroprusside or by endothelial denuding (24, 26). In addition to these mechanical alterations, significant changes in the composition of aortic smooth muscle myosin heavy chain (MHC) variants have been observed. A well established effect of aging is a shift in the MHC isoform expression from smooth muscle (SM)-2MHC to slower cycling SM-1MHC. Aging in the F344BN results in an increased SM1:SM2 ratio, as well as a dramatic reduction of maximal force development in response to high K^+ (24).

Age-associated alterations in vascular tissue have been linked to increases in reactive oxygen species (ROS). The free radical theory of aging suggests that aging occurs due to gradual accumulation of free radical damage to biomolecules (21, 27). Recent evidence that supports this theory within the aging F344BN has shown that age-associated increases in ($\cdot O_2^-$) and oxidative protein damage occur in the aorta by the 30 month of age (6). The same investigation also revealed that Bax and Bcl-2, regulators of cell viability and apoptosis, were highly correlated with increases in ROS, suggesting a compensatory mechanism for maintaining cell viability in the presence of ROS accumulation (6). In addition, the expression levels of mitogen-activated protein kinase (MAPK) and c-SRC family proteins were also affected by the accumulation of ROS (6).

In light of the age-associated functional and mechanical changes associated with ROS increases, we have postulated that mechanically regulated pathways may be adversely affected by aging in the F344BN aorta. Two approaches of evaluating the regulation of mechanotrasduction were employed: multi-axial stretch, utilizing pressurization of *ex vivo* aortic vessel, and uni-axial stretch, utilizing caliper mounted aortic vessel segments. Using these types of analysis, it has recently been demonstrated that aging in the aging aorta is associated with alterations in the regulation of focal adhesion kinase (FAK) and is FAK-associated (14, 16, 17). For example, pressure-induced Rho translocation appeared unaffected with aging while FRNK (p41) translocation increased (14). In adult F344BN aortae, both increased aortic intraluminal pressure and uni-axial stretch caused increased activation of ERK1/2-, p38-, and JNK-MAPK (15, 16). By comparison, aging diminished the response of p38- and JNK-MAPK to increased aortic pressure but had no effect on the response to uni-axial stretch (15, 16). With aging the contents of Akt, SHP-2, and PTEN were significantly increased, while those of p70s6k and GSK-3β were unchanged (17). Additionally, the pressure-induced phosphorylation of p70s6k in aged F344BN aorta was

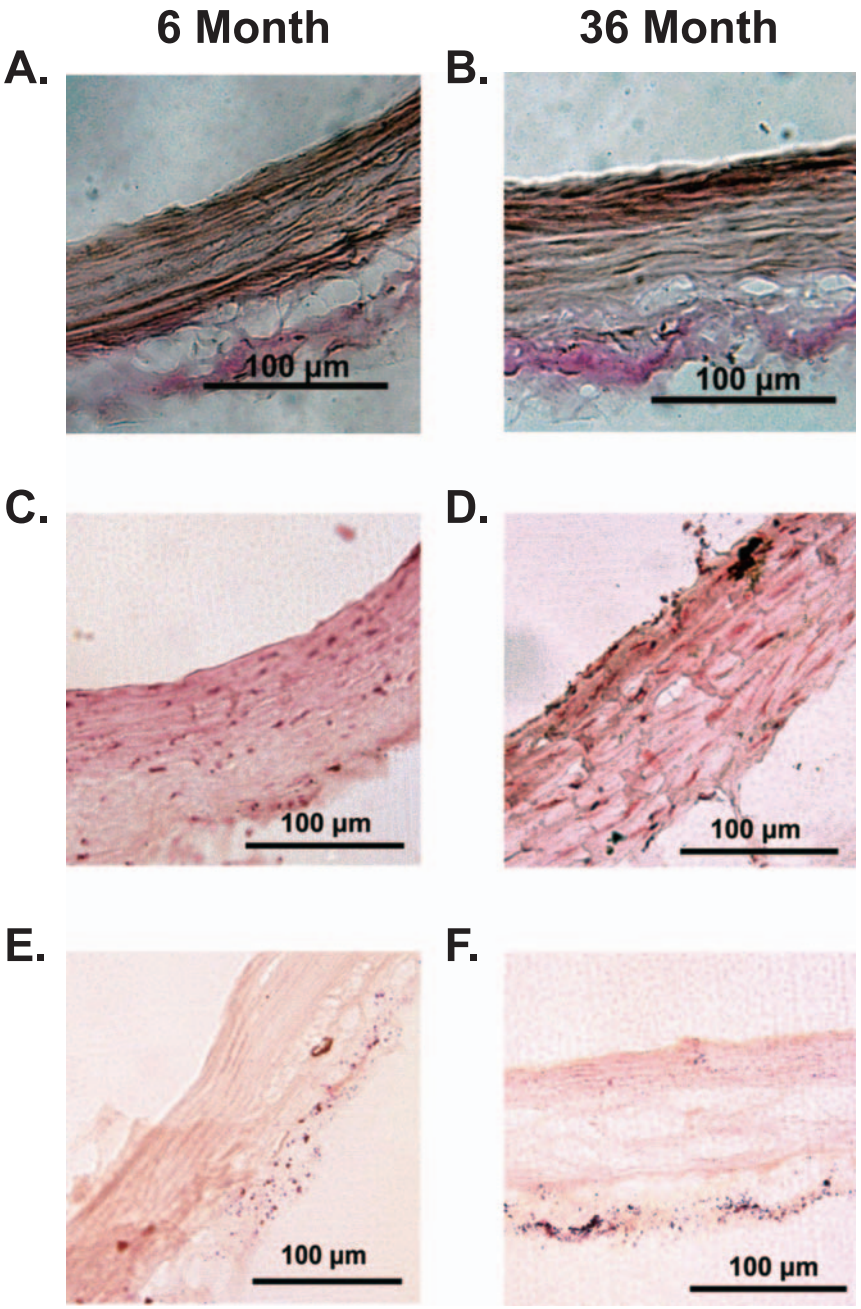

Fig. 1. Adult (6-month-old rat) and very aged (36-month-old rat) aortic sections depicting age-associated alterations in staining. Van Gieshen trichrome staining (A, B): elastin fibers appear as black bands and collagen appears as red staining. Von Kossa's calcium staining (C, D): calcium deposits appear as black and nuclei appears blue. Oil Red O Stain (E, F): lipid deposits appear as red droplets.

impaired compared to that observed in the adult F344BN aorta (17). Conversely, in adult F344BN, uni-axial stretch of aortae elicited a cascade leading to a decrease in the phosphorylation of p70s6k (15). These results suggest that smooth muscle, like skeletal muscle, can distinguish different mechanotransduction types and that the signaling response is altered with age.

Although the mechanisms underlying age-associated alterations in signaling are not fully understood, it is clear that aged vessel responds differently to stretch compared to vessels from younger animals. Because the process of aging is multifaceted and likely to exert debilitating influence on several physiological systems, consideration must be given to the effects of aging when evaluating systemic signaling pathways. The area of physiological age-associated signal transduction is

Table 2. Age-associated alterations in signaling in F344BN aging aortae

	vs 6 Months	
Arterial structure	30 months	36 months
Medial thickening	N.T.	↑
Stiffness	N.T.	↑
Stress relaxation	N.T.	↓
Multi-axial load–induced signaling		
MAPK	↓	↓
p70 s6k	↓	↓
Uni-axial load–induced signaling		
MAPK	↔	↔
p70 s6k	↔	↓

This table displays changes associated with uniaxial versus multaxial strain and the signaling changes associated with aging. (N.T., not tested; ↔, no change; ↑, increase; ↓, decrease).

slowly moving into the forefront in many areas of gerontological research. Nonetheless, the full impact of age-associated alteration in signal transduction has yet to be elucidated, especially with respect to pharmacological interventions. Hence, age must be considered with the development of pharmacological therapies and understanding the limitations of pharmaceuticals designed from data derived from young adult populations.

Future perspectives

Although the current body of data, though limited, appears to support the hypotheses that age-associated changes in the F344BN are predominately non-pathogenic in nature, the field of age-associated vascular aging utilizing this model remains largely unexplored. Aging and atherosclerosis appear to share similar biochemical pathways, leading to the conclusion that vascular aging may represent a prodromal stage of atherosclerotic disease or, alternatively, that atherosclerosis may be a form of accelerated aging (4). Current data from the F344BN as well as the healthy elderly human population suggest that aging and atherosclerosis are not irrevocably linked and that delineating the non-pathological mode of vascular aging is required for a true understanding of these two processes. In particular, recent research has provided several potential molecular targets for restoring aortic mechanotransductive signaling (15, 16, 19). These findings further emphasize the need for specific knowledge of age-related changes in the design of treatments for hypertension in the elderly. Because aging is an organismic process, a systemic analysis of age-related mechanisms must be considered when examining the functionality of an aging model. Further research is needed to determine the age-associated changes of the response of the F344BN to currently used cardiovascular pharmacological agents, dietary effects on CVD prevalence, and effects of altered circulating lipid profiles on CVD risk. The other limitation of the current body of research on the F344BN is its emphasis on the use of male rats. Evaluation of female F344BN may be expected to provide interesting insight into gender differences in vascular aging. In particular, because of the cardioprotective nature of estrogen, employing the female F344BN model may be of great value in defining the mechanisms underlying the post-menopausal risks of CVD. Therefore, further evaluation of this aging model may prove of significant importance in unraveling the true nature of vascular aging.

Acknowledgments

The authors wish to express their gratitude to the staff and students working in the Laboratory of Molecular Physiology, at Marshall University for their tireless efforts in research. This work is supported by NIH Grant #027103-1 to E.R. Blough and NSF-EPSCoR #0314742 to Marshall University.

References

1 Pilote L, Dasgupta K, Guru V, Humphries KH, McGrath J, Norris C, et al. A comprehensive view of sex-specific issues related to cardiovascular disease. Cmaj. 2007;176:S1–S44.
2 Franklin SS. Hypertension in older people: part 1. J Clin Hypertens (Greenwich). 2006;8:444–449.
3 Gaballa MA, Jacob CT, Raya TE, Liu J, Simon B, Goldman S. Large artery remodeling during aging: biaxial passive and active stiffness. Hypertension. 1998;32:437–443.
4 Ferrari AU, Radaelli A, Centola M. Invited review: aging and the cardiovascular system. J Appl Physiol. 2003;95:2591–2597.
5 Virmani R, Avolio AP, Mergner WJ, Robinowitz M, Herderick EE, Cornhill JF, et al. Effect of aging on aortic morphology in populations with high and low prevalence of hypertension and atherosclerosis. Comparison between occidental and Chinese communities. Am J Pathol. 1991;139:1119–1129.
6 Rice KM, Preston DL, Walker EM, Blough ER. Aging influences multiple incidices of oxidative stress in the aortic media of the Fischer 344/NNia × Brown Norway/BiNia rat. Free Radic Res. 2006;40:185–197.
7 Lipman RD, Chrisp CE, Hazzard DG, Bronson RT. Pathologic characterization of brown Norway, brown Norway × Fischer 344, and Fischer 344 × brown Norway rats with relation to age. J Gerontol A Biol Sci Med Sci. 1996;51:B54–B59.
8 Lipman RD. Pathobiology of aging rodents: inbred and hybrid models. Exp Gerontol. 1997;32:215–228.
9 Sprott RL. Development of animal models of aging at the National Institute of Aging. Neurobiol Aging. 1991;12:635–638.

10 Capdeville M, Coutard M, Osborne-Pellegrin MJ. Spontaneous rupture of the internal elastic lamina in the rat: the manifestation of a genetically determined factor which may be linked to vascular fragility. Blood Vessels. 1989;26:197–212.
11 Baumann M, Megens R, Bartholome R, Dolff S, van Zandvoort MA, Smits JF, et al. Prehypertensive renin-angiotensin-aldosterone system blockade in spontaneously hypertensive rats ameliorates the loss of long-term vascular function. Hypertens Res. 2997;30:853–861.
12 Turturro A, Witt WW, Lewis S, Hass BS, Lipman RD, Hart RW. Growth curves and survival characteristics of the animals used in the Biomarkers of Aging Program. J Gerontol A Biol Sci Med Sci. 1999;54:B492–B501.
13 Rice KM, Blough ER. Sarcopenia-related apoptosis is regulated differently in fast- and slow-twitch muscles of the aging F344/N × BN rat model. Mech Ageing Dev. 2006;127:670–679.
14 Rice KM, Desai DH, Kinnard RS, Harris R, Wright GL, Blough ER. Load-induced focal adhesion mechanotransduction is altered with aging in the Fischer 344/NNiaHSd × Brown Norway/BiNia rat aorta. Biogerontology. 2007;8:257–267
15 Rice KM, Desai DH, Preston DL, Wehner PS, Blough ER. Uniaxial stretch-induced regulation of MAP kinase, Akt and p70 S6 kinase in the aging Fischer 344 × Brown Norway rat aorta. Exp Physiol. 2007;92:963–970.
16 Rice KM, Kinnard RS, Harris R, Wright GL, Blough ER. Effects of aging on pressure-induced MAPK activation in the rat aorta. Pflugers Arch. 2005;450:192–199.
17 Rice KM, Kinnard RS, Wright GL, Blough ER. Aging alters vascular mechanotransduction: pressure-induced regulation of p70S6k in the rat aorta. Mech Ageing Dev. 2005;126:1213–1222.
18 Rice KM, Linderman JK, Kinnard RS, Blough ER. The Fischer 344/NNiaHSd × Brown Norway/BiNia is a better model of sarcopenia than the Fischer 344/NNiaHSd: a comparative analysis of muscle mass and contractile properties in aging male rat models. Biogerontology. 2005;6:335–343.
19 Rice KM, Preston DL, Neff D, Norton M, Blough ER. Age-related dystrophin-glycoprotein complex structure and function in the rat extensor digitorum longus and soleus muscle. J Gerontol A Biol Sci Med Sci. 2006;61:1119–1129.
20 Eklund KE, Hageman KS, Poole DC, Musch TI. Impact of aging on muscle blood flow in chronic heart failure. J Appl Physiol. 2005;99:505–514.
21 Gokulrangan G, Zaidi A, Michaelis ML, Schoneich C. Proteomic analysis of protein nitration in rat cerebellum: effect of biological aging. J Neurochem. 2007;100:1494–1504.
22 Kapphahn RJ, Giwa BM, Berg KM, Roehrich H, Feng X, Olsen TW, et a. Retinal proteins modified by 4-hydroxynonenal: identification of molecular targets. Exp Eye Res. 2006;83:165–175.
23 Tian Y, Riazi S, Khan O, Klein JD, Sugimura Y, Verbalis JG, et al. Renal ENaC subunit, Na-K-2Cl and Na-Cl cotransporter abundances in aged, water-restricted F344 x Brown Norway rats. Kidney Int. 2006;69:304–312.
24 Blough ER, Rice KM, Desai DH, Wehner P, Wright GL. Aging alters mechanical and contractile properties of the Fisher 344/Nnia × Norway/Binia rat aorta. Biogerontology. 2007;8:303–313.
25 Boer C, van Nieuw Amerongen GP, Groeneveld AB, Scheffer GJ, de Lange JJ, Westerhof N, et al. Smooth muscle F-actin disassembly and RhoA/Rho-kinase signaling during endotoxin-induced alterations in pulmonary arterial compliance. Am J Physiol Lung Cell Mol Physiol. 2004;287:L649–L655.
26 Paravicini TM, Touyz RM. Redox signaling in hypertension. Cardiovasc Res. 2006;71:247–258.
27 Harman D. The free radical theory of aging. Antioxid Redox Signal. 2003;5:557–561.