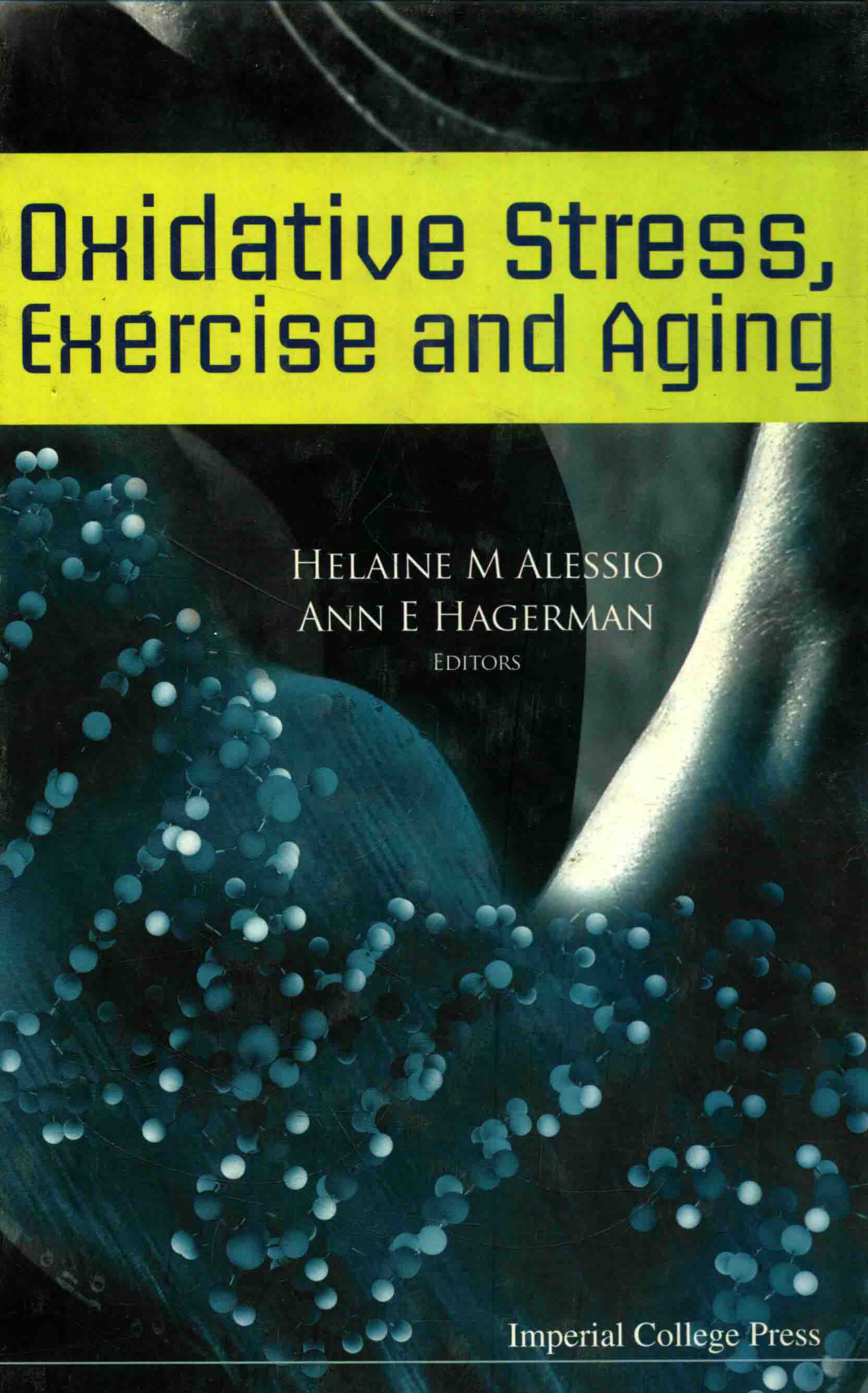
Oxidative Stress, Exercise and Aging



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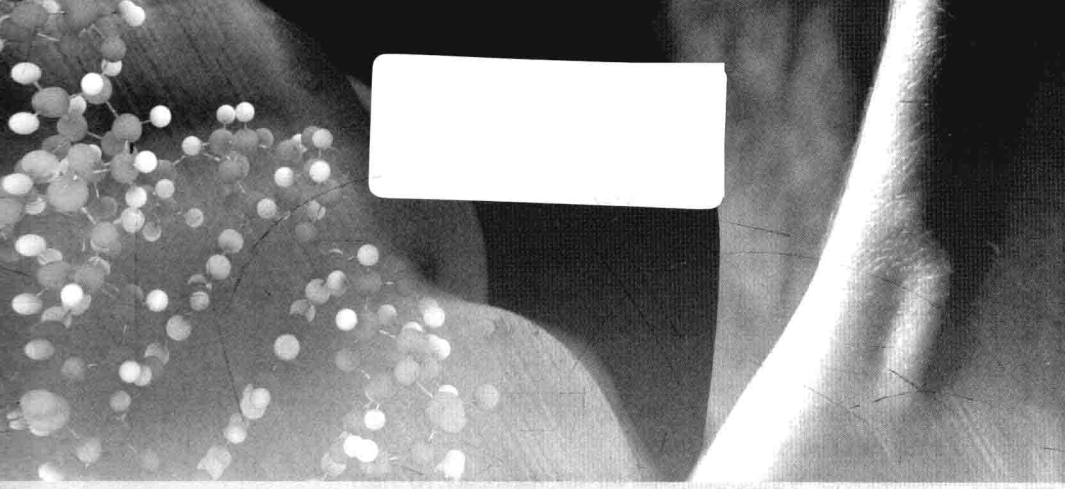

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Oxidative Stress, Exercise and Aging

We dedicate this book to our families for their support and inspiration.

Helaine M. Alessio
Ann E. Hagerman

PREFACE

This book is designed for individuals interested in learning about exercise-induced oxidative stress and its effects as we age. It covers concepts that have been developed and debated since 1956 when Denham Harman first introduced the Free Radical Theory of Aging. Our understanding of radicals, antioxidants, and oxidative stress has improved due to advances in technology as we have changed from relying on by-products of radical-induced cell damage to capturing radical signals *in vivo* to measuring specific gene expressions known to be activated by radical-induced cell signals. Furthermore, our appreciation of the pleiotropic (radicals can be helpful and harmful) and hormetic (a low level of radicals benefits proper cell functioning) properties of reactive oxygen species (ROS) has been enhanced by exercise and aging research. Topics chosen for this book reflect a focus on exercise and its impact on oxidative stress, which is known to influence aging processes.

The first chapter explains reactive oxygen species (ROS) so that the reader can appreciate the biochemical details and understand the importance of redox cycling in biological systems. Endogenous defense against ROS and the actions of major antioxidants are also covered. Chapter 2 presents interesting, new, and provocative information about similar ROS pathways and consequences that plants and animals experience, including environment, temperature, nutrition, oxygen availability, and disease.

Chapter 3 addresses the exercise continuum with isometric contractions on one extreme and dynamic contractions on the other. Since most exercise movement fall someplace along the continuum, muscles are usually exposed to varying levels of metabolic and mechanical stress. Oxidative stress, in particular, can occur during muscle actions throughout the exercise continuum. The different oxidative stress mechanisms during isometric and dynamic muscle contractions ultimately yield ROS. The study of exercise, aging, and oxidative stress usually includes basic research that relies on animal models and applied research that uses human models. In fact, plants experience oxidative stress in similar ways as animals do. In Chapter 4,

ROS produced by skeletal muscle are explored from different perspectives: natural selection, animal size, animal species, muscle fiber type, and aging, Exercise can produce ROS across the exercise continuum and Chapter 5 explores mechanisms and specific types of muscle contractions, exercises, and sports in which ROS and antioxidant activities change. Over time, oxidative stress in skeletal muscle changes due to up and down regulation of antioxidants. Age-related changes in skeletal muscle antioxidant activities and their roles as cell signalers and in cell damage and repair are elucidated in Chapter 6. This is followed by Chapter 7, which focuses on age-related changes in ROS and its role in muscle dysfunction and sarcopenia.

Oxidative stress, exercise, and aging in cardiac muscle are described in Chapter 8. Heart and skeletal muscle share common oxidative stress mechanisms such as ischemia-reperfusion and calcium overload. Heart muscle has some unique protective courses of action, including heat shock proteins, for protection against ROS, and these are highlighted.

The final chapter explains how exercise and oxidative stress affect genetic expressions that regulate health and aging. The interaction between genes and environment is unfolding in greater detail with advances in microarray technology that allows for the simultaneous analyses of tens of thousands of genes at once.

H.M.A

CONTENTS

CHAPTER 1

CHEMISTRY OF REACTIVE OXYGEN SPECIES AND ANTIOXIDANTS

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1.1 What are Reactive Oxygen Species?

Reactive oxygen species (ROS) are compounds derived from molecular oxygen, O_2, by partial chemical reduction. ROS thus have "extra" electrons[a]. ROS include the familiar oxygen compound hydrogen peroxide, H_2O_2, produced when O_2 is reduced with two electrons, and reactive forms of oxygen including superoxide, $O_2^{\cdot-}$, and hydroxyl radical, $OH^{\cdot}$. Complete reduction of O_2 by addition of four electrons yields 2 molecules of water, H_2O, a stable compound that is not an ROS (Simic, 1988).

When a species has an uneven number of electrons it is called a free radical. The unpaired electron which makes the radical unstable is indicated by the superscript ($^{\cdot}$). Molecular oxygen can be reduced with one electron to yield superoxide ($O_2^{\cdot-}$), the first species produced in many biological oxidative cascades. Hydrogen peroxide can undergo homolytic cleavage to yield two hydroxyl radicals ($OH^{\cdot}$). Free radicals are considered ROS even when they are not directly derived from oxygen. For example, oxidation of an unsaturated lipid by $OH^{\cdot}$ yields the lipid radical and water [Eq. (1.1)].

[a] Reduction, the addition of electrons to one species, is always at the expense of oxidation, or removal of electrons, from another species.

(1.1)

Ground state O_2 is unusual in that it is a biradical, with two unpaired electrons. The relative stability of the ground state oxygen radical is because the unpaired electrons have identical spins (triplet state), making them unreactive with ordinary singlet state (electron pairs with opposite spin) compounds. Oxygen is reactive enough to be a useful substrate for the oxidative metabolism essential to life, but unreactive enough to comprise around 20% of the earth's atmosphere. Input of energy can reverse the spin of one of the unpaired electrons to produce an excited state of oxygen known as singlet oxygen ($^1O_2$). Singlet oxygen is more reactive than ground state oxygen, and is an important ROS in photosynthetic organisms since chlorophylls can facilitate photochemical excitation of oxygen to the singlet state.

The superoxide radical is only moderately reactive with most biological compounds, but in aqueous solution it rapidly reacts to form hydrogen peroxide [Eq. (1.2)]:

$$2\,O_2^- + 2\,H^+ \longrightarrow H_2O_2 + O_2 \qquad (1.2)$$

H_2O_2 is moderately reactive, has a relatively long half-life and can diffuse some distance from its site of production and across cell membranes (Vranová *et al.*, 2002).

Hydrogen peroxide reacts with reduced metal ions such as iron or copper to form the highly reactive hydroxyl radical (Fenton chemistry) [Eq. (1.3)].

$$H_2O_2 + Fe^{2+} \longrightarrow OH^{\cdot} + OH^- + Fe^{3+} \qquad (1.3)$$

In biological systems, the metal ion can be reduced by superoxide, ascorbic acid, or a variety of other reducing agents. The redox cycle involving repeated reduction of the metal ion, and continued production of hydroxyl radical, ensures that only catalytic amounts of metal are

required to produce hydroxyl radical. Unlike superoxide and hydrogen peroxide, hydroxyl radical reacts rapidly with organic compounds.

All of the ROS (superoxide, hydrogen peroxide, and hydroxyl radical) are produced as a consequence of normal metabolism, and have roles as cell signaling molecules as well as in defense from invading micro-organisms. However, when any ROS is produced in uncontrolled amounts it can damage proteins, DNA, and lipids (Gutteridge and Halliwell, 1996). Reaction of hydroxyl radical with unsaturated lipids is the most familiar cascade of radical induced damage (Fig. 1.1). Reaction of radicals with proteins can lead to oxidation of reactive amino acid side chains, to protein crosslinking and denaturation, and to damage to other nearby proteins. Oxidation of DNA leads to strand breaks and release of oxidized bases, particularly 8-oxo-deoxy-guanidine.

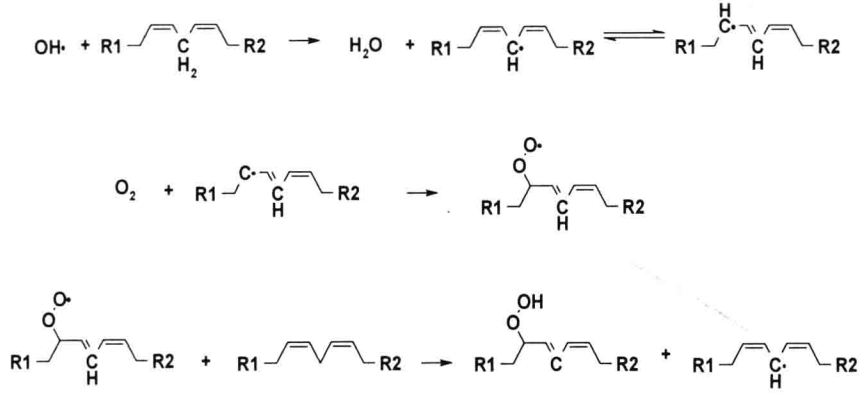

Fig. 1.1. Oxidation of unsaturated lipid to lipid hydroperoxide by $OH^{\cdot}$

Nitric oxide ($NO^{\cdot}$) is a biologically important radical produced by nitric oxide synthase (NOS) from arginine and oxygen. Nitric oxide is important as a cell signaling molecule, especially involved in vasoconstriction. As with other free radicals, uncontrolled production of nitric oxide can lead to oxidative damage.

1.2 What are Antioxidants?

Antioxidants minimize oxidative damage to biological systems either by preventing formation of ROS, or by quenching ROS before they can react with other biomolecules. Antioxidants can be either endogenous compounds, produced by the organism as part of its ROS defense, or can be exogenous compounds acquired from the diet. The endogenous system includes both enzymes and nonenzymatic antioxidants, and dietary antioxidants are small molecules.

All aerobic organisms contain the enzyme superoxide dismutase (SOD). Superoxide dismutase catalyzes the conversion of superoxide to hydrogen peroxide as shown above, providing about a 10,000-fold rate enhancement and ensuring that virtually no superoxide is found in the cell. In many tissues hydrogen peroxide is inactivated by catalase [Eq. (1.4)].

$$2\,H_2O_2 \xrightarrow[\text{catalase}]{} 2\,H_2O + O_2 \qquad (1.4)$$

Glutathione peroxidase (GPX) provides an alternative route for destruction of hydrogen peroxide at the expense of the small molecule antioxidant glutathione (GSH). The oxidized glutathione (GSSG) is reduced via NADPH and glutathione reductase (GR). Analogous ascorbate peroxidases also play a role in hydrogen peroxide destruction [Eq.(1.5)].

$$2GSH + H_2O_2 \xrightarrow[GPX]{} GSSG + 2\,H_2O$$

$$GSSG + NADPH + H^+ \xrightarrow[GR]{} 2GSH + NADP^+ \qquad (1.5)$$

Some antioxidants prevent ROS formation. For example, metal ion chelators such as transferrin and ceruloplasmin prevent metal ions from

participating in Fenton chemistry, and thus minimize hydroxyl radical formation.

Small molecule antioxidants often act by scavenging, or quenching, free radicals. Glutathione and uric acid are important small molecule free radical quenchers found in plants and animals. In animals, the antioxidant vitamins (ascorbic acid, α-tocopherol and β-carotene) are radical scavengers. In plants ascorbic acid is endogenous, and is the most important small molecule antioxidant (Foyer, 1993). In the blood, non-specific radical quenching by albumins and other proteins contributes to total antioxidant capacity.

Glutathione is typical of several other thiol antioxidants including the amino acid cysteine and its derivatives, and thiol-containing proteins such as thioredoxins and peroxiredoxins. The thiol (sulfhydryl, R-SH) disulfide (R-S-S-R) pair provides a convenient redox couple used to destroy ROS; to regenerate other antioxidants; and to signal cellular redox status. Glutathione is a tripeptide (γ-Glu-Cys-Gly) with an unusual γ-glutamyl peptide bond, and a cysteine providing the thiol group (Fig. 1.2).

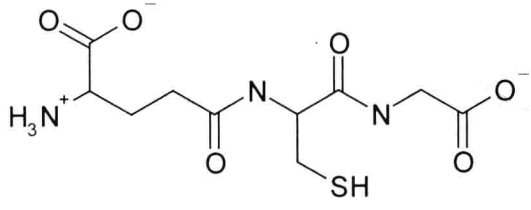

Fig. 1.2. Structure of glutathione.

Free radical scavenging is not always beneficial. For example, dietary polyphenols such as epigallocatechin gallate (EGCG), found in green tea react with radicals in a two step redox process. The EGCG is oxidized through the semiquinone radical to the quinone, while two radicals are reduced to nonradical forms. Both the EGCG semiquinone radical and the quinone are reactive species which can form covalent cross links to protein (Hagerman *et al.*, 2003). Although the original radical is destroyed by the "antioxidant" EGCG, oxidative damage may

be promulgated by the altered polyphenol. For an antioxidant to be effective, it must not only quench radical species, but it must form relatively unreactive products that are not more damaging than the original radical.

Small molecule antioxidants often react in a network, involving multiple steps of oxidation/reduction to destroy the ROS (Blokhina *et al.*, 2002). One of the best characterized networks uses the fat-soluble vitamin α-tocopherol to protect membranes from damage, and ascorbic acid/GSH to regenerate the tocopherol (Fig. 1.3). Tocopherol is synthesized only in plants, and is found mainly in the chloroplast where it protects membranes from lipid peroxidation and scavenges singlet oxygen (Hess, 1993). Carotenoids, also found within chloroplasts, also protect plants from singlet oxygen. The xanthophyll cycle is a redox cycle using specialized carotenoids to compensate for photo-oxidative stress encountered when plants are exposed to low temperatures and bright lights.

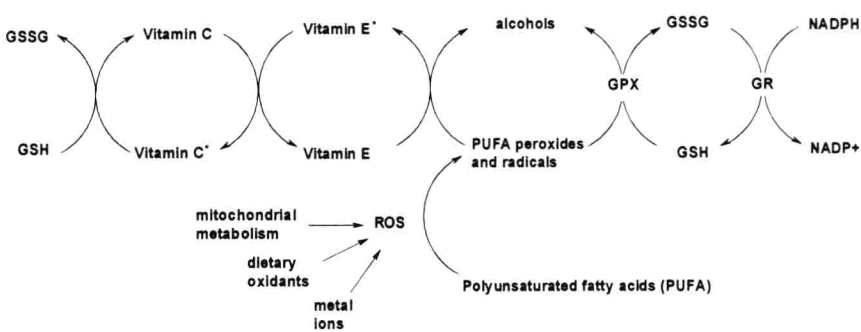

Fig. 1.3. Reduction of polyunsaturated fatty acid hydroperoxides (PUFA peroxides) and radicals by vitamins E, C, and GSH.

1.3 Oxidized Biomarkers

Biological molecules that are susceptible to oxidation damage include protein, lipids and nucleic acids. Selecting appropriate biomarkers to