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Aims and Scope

Heart and Metabolism is a quarterly journal focusing on the management of myocardial ischemia. Its aim is to inform cardiologists and other specialists about the newest findings of the role of metabolism in cardiac disease and to create awareness of its potential clinical implications. The management of patients with angina, as well as those with heart failure and hypertrophic or dilated cardiomyopathy, will also be discussed. Moreover, the effects of metabolic diseases such as diabetes mellitus on the heart will be highlighted. Each issue will include an editorial, followed by articles on a key topic. Experts in the field will explain the metabolic consequences of cardiac disease and the multiple potential targets for pharmacotherapy in ischemic and nonischemic heart disease.

The figure on the cover show PET images of a human heart in short-axis views obtained in corresponding midventricular levels before and after coronary artery bypass surgery (CABG). See page 19.

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Oxidative stress — an idiosyncrasy of nature

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The idiosyncrasies of nature are such that molecular oxygen, which is essential for maintaining cell viability, may also act as a trigger for tissue injury. This was originally highlighted by the observation of David Hearse and colleagues of an "oxygen paradox." The paradox was that, upon abrupt reoxygenation of an anoxic heart, myocardial injury was suddenly and markedly enhanced [1]. Since this initial observation, a large number of basic and clinical investigations have aimed at understanding the mechanisms involved and the functional consequences associated with oxidative stress. It has in particular become apparent that residual molecular oxygen in the ischemic myocardium, and more clearly the reintroduction of oxygen into the ischemic myocardium, results in the formation of oxygen-derived free radicals. Oxidative stress, which is usually associated with an increase in the formation of reactive oxygen species, modifies phospholipids and proteins, leading to lipid peroxidation and oxidation of thiol groups. These changes are thought to alter cell membrane permeability and configuration, in addition to producing functional modifications in various cellular proteins. This is summarized in two articles in this issue. Joël de Leiris provides a concise review of the biochemistry of free radicals, how they are produced or overproduced, and how they can be counteracted. It is the purpose of Sandeep Raha and Brian Robinson to focus mainly on how the mito-

chondria, a major source of cellular free radicals, contributes to the regulation of free radical metabolism.

Basic research is continually discovering new effects of excessive production of reactive oxygen species in the different aspects of cardiovascular disease. It is known that damage to mitochondria enhances the production of reactive oxygen species; and an increase in the formation of reactive oxygen species during ischemia-reperfusion has been reported using the electron paramagnetic resonance technique in both humans and animals [2, 3]. In addition, since investigations have reported a depletion of endogenous antioxidants in the ischemic heart upon reperfusion [4], it appears especially important to focus on antioxidant systems. Direct evidence, using genetically engineered animal models, has recently been presented to show the importance of several naturally occurring scavenging enzyme systems, such as catalase, superoxide dismutase, and glutathione peroxidase, in protecting the myocardium against ischemia-reperfusion injury [5–7]. During normal respiration, extensive oxidative damage is prevented by mitochondrial antioxidant enzyme systems [8]. Thus, manganese-containing mitochondrial superoxide dismutase, located in the mitochondrial matrix, eliminates superoxide radical $^{\circ}\text{O}_2^-$ by catalyzing dismutation to hydrogen peroxide H_2O_2 . H_2O_2 is then inactivated by either catalase or by the glutathione

redox system consisting of reduced glutathione as the cofactor for glutathione peroxidase and glutathione reductase. Although the relative contributions of catalase and glutathione peroxidase in H_2O_2 degradation remain unclear, these enzymes serve to minimize the accumulation of $^{\circ}O_2^-$ and H_2O_2 , which in the presence of the redox-active transition metals, copper and iron, form the very reactive and damaging hydroxyl radical $^{\circ}OH$, for which no antioxidant enzyme system exists [8]. Finally, when oxidative damage has occurred, mitochondria also possess enzyme systems that can repair. Phospholipid hydroperoxide glutathione peroxidase is a selenium-containing enzyme that directly reduces peroxidized acyl groups in phospholipids. Repair of oxidized protein sulfhydryl groups may also occur via thioredoxin and thiolutase enzymes. However, these repairing enzyme systems are likely to be damaged or ineffective in most conditions of oxidative stress unless their reserve can be boosted by antioxidant enzyme mimics.

Another fascinating aspect of the consequences of oxidative stress has emerged from very recent studies. It has been suggested that oxidative stress may trigger apoptosis (ie, programmed cell death) during ischemia-reperfusion. However, reactive oxygen species generated during ischemic preconditioning can upregulate the anti-death gene *Bcl-2* by activating a specific nuclear transcription factor (NF κ B), which in turn reduces apoptosis [9, 10]. These results indicate an additional antioxidant pathway for the protection of myocardium by ischemic preconditioning. Hopefully, this should lead to exciting developments for new antioxidant therapy, taking into account important intracellular signaling pathways for treating oxidative stress-associated injury. ■

REFERENCES

1. Hearse DJ, Humphrey SM, Chain EB. Abrupt reoxygenation of the anoxic potassium-arrested perfused rat heart: a study of myocardial enzyme release. *J Mol Cell Cardiol.* 1973;5:395–407.
2. Grech ED, Dodd NJ, Jackson MJ, Morrison WL, Faragher EB, Ramsdale DR. Evidence for free radical generation after primary percutaneous transluminal coronary angioplasty recanalization in acute myocardial infarction. *Am J Cardiol.* 1996;77:122–127.
3. Bolli R, Jeroudi MO, Patel BS, et al. Direct evidence that oxygen-derived free radicals contribute to postischemic myocardial dysfunction in the intact dog. *Proc Natl Acad Sci USA.* 1989;86:4695–4699.
4. Haramaki N, Stewart DB, Aggarwal S, Ikeda H, Reznick AZ, Packer L. Networking antioxidants in the isolated rat heart are selectively depleted by ischemia-reperfusion. *Free Radic Biol Med.* 1998;25:329–339.
5. Woo YJ, Zhang JC, Vijayarathay C, et al. Recombinant adenovirus-mediated cardiac gene transfer of superoxide dismutase and catalase attenuates postischemic contractile dysfunction. *Circulation.* 1998;98:11255–11260.
6. Chen Z, Siu B, Ho YS, et al. Overexpression of MnSOD protects against myocardial ischemia/reperfusion injury in transgenic mice. *J Mol Cell Cardiol.* 1998;20:2281–2289.
7. Yoshida T, Watanabe M, Engelman DT, et al. Transgenic mice overexpressing glutathione peroxidase are resistant to myocardial ischemia reperfusion injury. *J Mol Cell Cardiol.* 1996;28:1759–1767.
8. Lesnfsky EJ, Moghaddas S, Tandler B, Kerner J, Hoppel CL. Mitochondrial dysfunction in cardiac disease: ischemia-reperfusion, aging, and heart failure. *J Mol Cell Cardiol.* 2001;33:1065–1089.
9. Maulik N, Engelman RM, Rousou JA, Flack JE 3rd, Deaton D, Das DK. Ischemic preconditioning reduces apoptosis by upregulating anti-death gene *Bcl-2*. *Circulation.* 1999;100:11369–11375.
10. Hattori R, Hernandez TE, Zhu L, et al. An essential role of the antioxidant gene *Bcl-2* in myocardial adaptation to ischemia: an insight with antisense *Bcl-2* therapy. *Antioxid Redox Signal.* 2001;3:403–413

Metabolic actions of free radicals: walking the tightrope

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Abstract

Free radicals and the consequences of their actions have become a primary focus of biomedical research. The damage evoked by both reactive oxygen species and reactive nitrogen species contributes to a number of clinical phenotypes. Free radicals have been shown to play a major causative role in a number of disorders ranging from neurodegenerative diseases such as amyotrophic lateral sclerosis to cardiovascular diseases such as atherosclerosis. An understanding of the processes that underlie their formation and removal will contribute to an appreciation of the mechanisms of their regulation. However, it is equally important to view a more fundamental role for these so-called “agents of death.” Free radicals not only serve to trigger the apoptotic processes but are also involved in the activation of transcription factors as well as second messenger signaling pathways. This review attempts to summarize some of the observations that demonstrate the regulation of free radical metabolism via a sum of pathways that involve the formation, removal, and utilization of these radicals as second messengers. The focus will be on how the mitochondria, a major source of cellular free radicals, contributes to this overall process. ■ *Heart Metab.* 2003;19:4–10.

Keywords: Mitochondria, cellular signaling, oxidative stress

Introduction

Free radicals and the damage they effect have come to take a central role in a very large number of diseases and biochemical processes. The damaging effects of both oxygen- and nitrogen-derived free radicals with relation to aging and disease propagation have become a very active area of biomedical research [1, 2]. Free radical damage has been implicated in a range of diseases including atherosclerosis, diabetes mellitus, neurodegenerative disorders, hypertension, rheumatoid arthritis [3], and amyotrophic lateral sclerosis [4]. In the majority of these cases, the most destructive species of free radicals are thought to be hydroxyl and peroxynitrite radicals. The former arises as a result of the combination of

superoxide and hydrogen peroxide and the latter is formed from the reaction of superoxide with nitric oxide [5]. The primary source of superoxide radical formation is believed to be the mitochondrial electron transport chain. Approximately 1% to 2% of the electrons are lost to oxygen and result in the formation of superoxide [6, 7]. Superoxide formation also causes an accumulation of damage to DNA resulting in a shortening in the telomeres of nuclear DNA [7, 8]. This is hypothesized to be one of the major consequences of cumulative free radical damage during the aging process. This review will focus primarily on the mitochondria as the site of free radical formation, and the organelle where the majority of the “balancing act” associated with the regulation of radicals must occur.

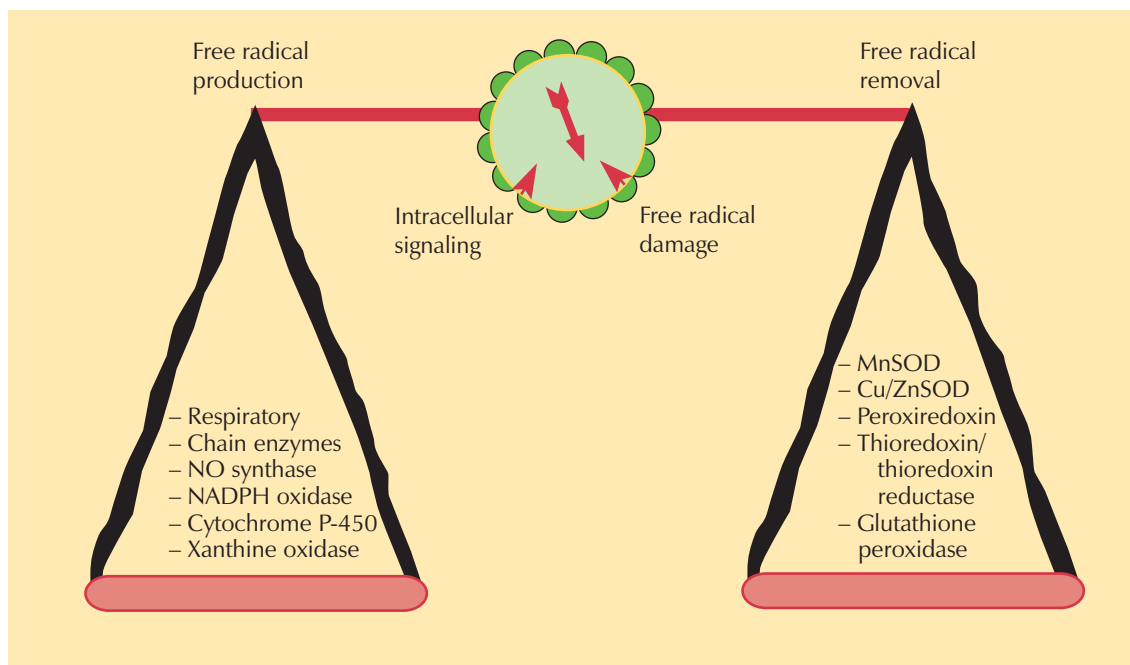


Figure 1. Schematic illustration of the interaction between production, removal, and utilization of cellular free radicals. Some representative systems that are known to produce free radicals are listed on one side of the “balance.” If there is an increase in the production of free radicals, combined with an inability to mitigate the resulting destructive species, then the scale will favor tipping towards cellular damage. However, under normal cellular conditions, the free radicals produced within the cell are utilized for a variety of regulating functions. In a “balanced” scenario, there is minimal cell damage as a majority of the free radicals are used for the purpose of signaling. NO, Nitric oxide; NADPH, reduced nicotinamide adenine dinucleotide phosphate; MnSOD, manganese superoxide dismutase; Cu/ZnSOD, copper-zinc superoxide dismutase.

It is estimated that well over 60 million North Americans suffer from cardiovascular diseases. Cardiovascular tissues, because they contain higher numbers of mitochondria and increased levels of respiratory chain components per milligram of mitochondrial protein, are subject to extensive damage resulting from elevated levels of superoxide. Diseases that affect mitochondrial function also impact severely on cardiomyocytes as well as on the surrounding vascular tissues [9, 10]. These include the development of atherosclerosis, hypertension [9], and arterial thrombosis [11]. Under normal conditions the vascular endothelium plays a pivotal role in inhibiting intravascular thrombus formation. Vascular endothelial cells play a crucial role in this pathway by synthesizing various substances such as thrombomodulin, tissue factor-pathway inhibitor, prostacyclin, and tissue plasminogen activator. Disruption of the pathways

involved in the release of these substances can affect the antithrombotic properties of the endothelium.

Much of this damage within the cardiovascular system results as a consequence of the activation of a number of apoptotic pathways [12]. Free radicals contribute to the apoptotic process in one of two ways: direct damage of proteins, or the activation of transcription factors culminating in a change in gene expression patterns [13]. Direct damage occurs as a result of oxidative damage to proteins and lipids. Oxidative damage of crucial redox-sensitive proteins can lead to an increase in the production of free radicals and an elevation in the level of damage incurred [14]. Triggering of key transcription factors such as hypoxia-inducible factor (HIF)-1 α [15] or nuclear factor kappaB (NF κ B) can result in the activation of genes related to the progression of the apoptotic pathway [13]. In addition to these

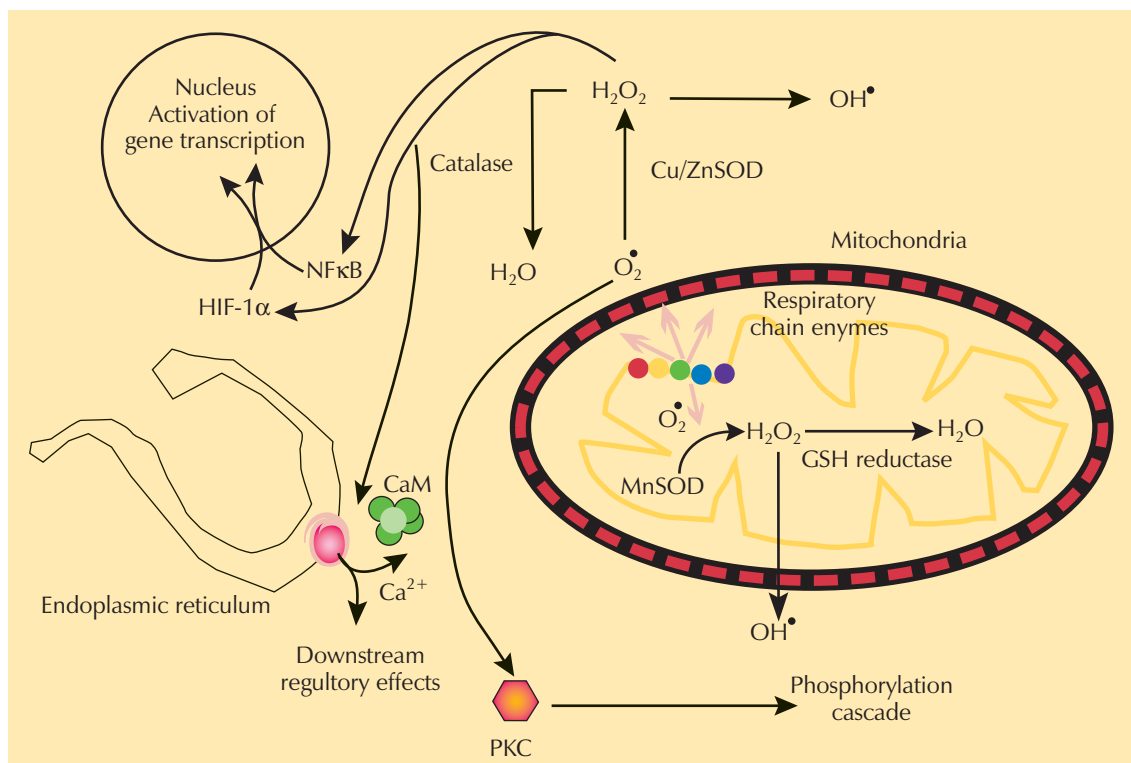


Figure 2. Putative second messenger roles for mitochondrially derived superoxides. The production of superoxide from the mitochondrial electron chain can go towards signaling a number of events. Superoxide radicals can be utilized to trigger a number of well-established signaling pathways. Activation of protein kinase C (PKC) directly, activation of Ca^{2+} release, and activation of transcription factors are just samples of the intracellular signaling roles attributed to superoxide. Cu/ZnSOD, Copper-zinc superoxide dismutase; MnSOD, manganese superoxide dismutase; GSH, glutathione.

roles, recent observations have demonstrated that free radicals can behave like conventional second messengers that can activate Ca^{2+} flux from IP_3 -sensitive and/or ryanodine-sensitive stores directly [16]. This review will focus on the balance between the production of free radicals and the pathways for their removal (Figure 1). More importantly, the basal level of free radical production that occurs during normal cellular metabolism constitutes an important mechanism of cellular communication (Figure 2). Significant changes beyond these basal levels can result in severe cellular damage leading to cell death. Networks of cellular systems help to maintain this delicate balance. Clearly the cell must “walk a tightrope” between an excess of free radicals, which may cause damage, and a sufficiency of free radicals in order to maintain the ability to evoke

gene regulatory function necessary for the induction of protective mechanisms.

Formation of reactive oxygen and nitrogen species

A number of reviews have addressed the contribution of increased oxidative stress towards the progression of cardiovascular disease [17]. In order to better understand this problem, an appreciation of the sources of free radical production within the cardiovascular system is required. Mitochondria are a major source of cellular free radicals and their contribution to oxidative injury in the cardiovascular system becomes important for two reasons. Firstly, cardiomyocytes are enriched in mitochondria because of their metabolic demands. Second-

ly, ischemic conditions can occur during myocardial infarction. Subsequent reperfusion can result in a dramatic elevation of free radical production [18] and lead to the activation of a host of other regulatory pathways.

Both reactive oxygen and reactive nitrogen species are responsible for damage to cardiovascular tissues. Endothelium-derived relaxing factor, or nitric oxide, is an important signaling molecule produced in a variety of tissues by nitric oxide synthase enzymes of which there are several isoforms [19]. Several groups have reported a mitochondrial form of the enzyme [20, 21].

The superoxide radical has also been shown to be particularly reactive. Aside from mitochondrial sources, a number of other enzyme systems are known to produce reactive oxygen species. These include xanthine oxidase, cytochrome P-450 mono-oxygenases, lipoxygenase, nitric oxide synthase, and reduced nicotinamide adenine dinucleotide phosphate oxidase [9]. Superoxides can react with H_2O_2 or nitric oxide to form either hydroxyl radicals or peroxynitrite radicals. The hydroxyl radical is extremely reactive, with an estimated lifetime of 10^{-9} seconds, and is widely known to cause lipid peroxidation and DNA damage [22]. Within the mitochondria, superoxides are thought to originate primarily from nicotinamide adenine dinucleotide-coenzyme Q-oxidoreductase complex (complex I) and ubiquinol-cytochrome c reductase (complex III) [23–25]. It should be borne in mind that there is a basal level of superoxide production, and management of these free radicals dictates the fate of the cells. This constant level of free radical insult is what contributes to the age-dependent damage to cellular elements.

Removal of free radicals

Recent attempts to identify enzymes involved in the regulation of the mitochondrial antioxidant defense system using a proteomics approach resulted in the identification of manganese superoxide dismutase (SOD), peroxiredoxin III, and mitochondrial thioredoxin

as proteins regulated by oxidative stress [26]. There exist a number of SOD [27] including copper-zinc SOD, to dissipate superoxides within the cytosol, and manganese SOD that regulate superoxide homeostasis within the mitochondria. It has been demonstrated that removal of manganese SOD (*sod2*) from rat heart mitochondria results in a significantly greater level of basal superoxide production [20]. The neonatal lethality of the *sod2*-mouse resulting from the inactivation of iron-sulfur centers within the electron transport chain and citric acid cycle enzymes underscores the importance of manganese SOD [28].

The product of superoxide dismutation is hydrogen peroxide, which can react via the Fenton reaction to form extremely toxic hydroxyl radicals. An extensive network of enzymes exists to facilitate the removal of H_2O_2 . Catalase, thioredoxin reductase, and glutathione peroxidase are primarily responsible for the removal of peroxide. However, there are also specific isoforms of these enzyme systems within the mitochondria for this purpose. Inactivating *Gpx1*, an isoform expressed in heart and muscle [29], increased mitochondrial H_2O_2 production and resulted in greater levels of lipid oxidation and decreased mitochondrial energy output.

Thioredoxin peroxidase (peroxiredoxin) also catalyzes the removal of H_2O_2 generated from cellular metabolism or during the cellular signaling processes. This enzyme relies on thioredoxin as a source for reducing equivalents. There are at least 12 varieties of peroxiredoxin in mammals and these can be subdivided into three distinct subclasses [30]. Peroxiredoxin III is localized to the mitochondria.

The thioredoxin, thioredoxin reductase, and peroxiredoxin system is one that is also involved in maintaining the free radical detoxification in several compartments inside and outside cells. Inhibition of thioredoxin-2, a mitochondrial-specific member of the thioredoxin superfamily, results in a marked increase in intracellular reactive oxygen species [31]. Conversely, overexpression of peroxiredoxin decreases levels of H_2O_2 which result from tumor necrosis factor- α activation

in NIH 3T3 fibroblasts. Moreover, the activation of NF κ B by exogenously added H₂O₂ was attenuated following overexpression of peroxiredoxin II [32].

Free radicals and cellular signaling

There are a number of diseases that are characterized by elevated levels of basal superoxide production. Conventionally, the increased level of free radical production was attributed to cellular dysfunction. However, recent evidence suggests that this shift in the equilibrium level of free radicals may serve to activate other second messenger pathways. The importance of understanding the signaling role of free radicals is underscored in the speculation by Toyokuni et al [33] that the consistently high levels of free radicals produced by cancerous cells have a role in promoting ongoing proliferation. The universality of these signaling molecules is demonstrated in the observation that they are capable of interacting with and regulating the function of membrane receptors, enzymes, or transcription factors.

The reactivity of oxygen and its intermediates towards the activation of a peripheral benzodiazepine receptor (PBR) suggests that it may function as a "superoxide receptor" [34]. These observations were made primarily on the basis of structural homologies between the mitochondrial PBR and the bacterial TspO protein, which is responsible for regulating oxygen sensitivity. It has also been demonstrated that transfection of Jurkat cells with human PBR cDNA exhibited an increased resistance to high levels of H₂O₂ [35].

Superoxides have also been linked with the activation of manganese SOD via a direct activation of protein kinase C [36]. This was demonstrated by measuring the activity of protein kinase C in the presence of a xanthine/xanthine oxidase-based system to generate superoxides [37]. The activation was postulated to be the result of a free radical-based thiol oxidation and the release of Zn²⁺ from a cysteine-rich zinc finger domain. This is supported by the observation that the addition of

dithioereitol, a reducing agent, prevented the observed increased in protein kinase C activity in the presence of the superoxide-generating system. This highlights recent evidence that the redox regulation of cellular proteins occurs primarily through sulfhydryl (RSH) groups. In most cases these groups are oxidized to form disulfide bonds (RSSR), sulfenic acid (RSOH), sulfinic acid (RSO₂H) or sulfonic acid (RSO₃H) [38].

Much of the gene transcription that results from exposure of cells to hypoxic conditions, especially in cardiovascular tissues, is likely due to activation by pathways involving free radical-mediated mechanisms. The regulatory effect of both reactive oxygen species and reactive nitrogen species on transcription factors and ultimately on gene expression is an indication as to the importance of their second messenger roles [5]. In general, the effects of gene expression can be divided into a number of different categories including ion transport, apoptosis, transcription, hormone action or neuromodulation. These effects have been well summarized in a number of reviews including that of Allen and Tresini [13]. Reactive oxygen species have been demonstrated to regulate both HIF-1 α [39] and NF κ B [40]. The interaction of oxygen and/or oxygen intermediates is postulated to affect HIF-1 α stability via modulation of the von Hippel-Lindau protein [41]. The regulation of HIF-1 α has been shown to be modulated as a function of the changes in reactive oxygen species such as H₂O₂ [39, 41].

Superoxides may directly regulate a number of very important cardiovascular control points (*Figure 2*). Protein kinase C activity and the release of Ca²⁺ from internal stores are important control points for signal transduction for any type of vascular muscle tissue. Superoxides increase both the release of Ca²⁺ and the activity of protein kinase C. In the case of protein kinase C [37], it was reported that the oxidation of cysteine and the release of Zn²⁺ was a prerequisite for the activation of the enzyme. In the case of intracellular Ca²⁺ release [42], the mechanism of action of superoxides was observed to be in a calmodulin-dependent fashion. Other studies have

also suggested that reactive nitrogen species, such as nitric oxide, can activate intracellular Ca^{2+} release by *S*-nitrosylating a single cysteine residue [43].

The dosage level is the distinguishing factor as whether these radicals are utilized for signaling events or evoke cellular damage.

To date, there are no clear-cut values on the “normal” dosages of free radicals within a cell. Therefore it is difficult to estimate the percentage of the total amount of cellular free radicals required for normal cellular communication. For this reason, constant administration of antioxidant drugs may actually be detrimental to the cell and interfere with its ability to actively recover from situations of oxidative stress.

Clearly, the exact mechanisms for the activation of second messenger pathways by reactive oxygen and nitrogen species will be difficult to elucidate because of the transient nature of these entities. Further detailed research towards the elucidation of the mechanisms of oxygen and nitrogen free radicals in intra and intercellular signaling events will help to more clearly define their role in cellular communication. ■

Acknowledgments

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REFERENCES

- Melov S, Ravenscroft J, Malik S, et al. Extension of life-span with superoxide dismutase/catalase mimetics. *Science*. 2000;289:1567–1569.
- Lefer DJ, Granger N. Oxidative stress and cardiac disease. *Am J Med*. 2000;109:315–323.
- Droge W. Free radicals in the physiological control of cell function. *Physiol Rev*. 2002;82:45–47.
- McEachern G, Kassoovska-Bratinova S, Raha S, et al. Manganese superoxide dismutase levels are elevated in a proportion of amyotrophic lateral sclerosis patient cell lines. *Biochem Biophys Res Commun*. 2000;273:359–363.
- Tsan M. Superoxide dismutase and pulmonary oxygen toxicity. *Proc Soc Exp Biol Med*. 1997;214:107–113.
- Raha S, Robinson BH. Mitochondria, oxygen free radicals and apoptosis. *Am J Med Genet*. 2001;106:62–70.
- Raha S, Robinson BH. Mitochondria, oxygen free radicals, disease and ageing. *Trends Biochem Sci*. 2000;25:502–508.
- Barja G. Mitochondrial free radical production and aging in mammals and birds. *Ann N Y Acad Sci*. 1998;854:224–238.
- Anversa P, Leri A, Beltrami CA, Guerra S, Kajstura J. Myocyte death and growth in the failing heart. *Lab Invest*. 1998;78:767–786.
- Fukai T, Folz RJ, Landmesser U, Harrison DG. Extracellular superoxide dismutase and cardiovascular disease. *Cardiovasc Res*. 2002;55:239–249.
- Ambrosio G, Tritto I, Golino P. Reactive oxygen metabolites and arterial thrombosis. *Cardiovasc Res*. 1997;34:445–452.
- Demaree SR, Lawler JM, Linehan J, Delp MD. Ageing alters aortic antioxidant enzyme activities in Fischer-344 rats. *Acta Physiol Scand*. 1999;166:203–208.
- Allen RG, Tresini M. Oxidative stress and gene regulation. *Free Radic Biol Med*. 2000;28:463–499.
- Jha N, Jurma O, Lalli G, et al. Glutathione depletion in PC12 results in selective inhibition of mitochondrial complex I activity. Implications for Parkinson's disease. *J Biol Chem*. 2000;275:26096–26101.
- Haddad JJE, Olver RE, Land SC. Antioxidant/pro-oxidant equilibrium regulates HIF-1 α and NF- κ B redox sensitivity: evidence for inhibition by glutathione oxidant in alveolar epithelial cells. *J Biol Chem*. 2002;275:21130–21139.
- Waypa GB, Schumacker PT. O_2 sensing in hypoxic pulmonary vasoconstriction: the mitochondrial door re-opens. *Respir Physiol Neurobiol*. 2002;132:81–91.
- Mak S, Newton GE. The oxidative stress hypothesis of congestive heart failure. *Chest*. 2001;120:2035–2046.
- Li C, Jackson RM. Reactive species mechanisms of cellular hypoxia-reoxygenation injury. *Am J Physiol Cell Physiol*. 2002;282:C227–C241.
- Brookes PS, Levenon A, Shiva S, Sarti P, Darley-Usmar VM. Mitochondria: regulators of signal transduction by reactive oxygen and nitrogen species. *Free Radic Biol Med*. 2002;33:755–764.
- Giulvi C, Poderoso JJ, Boveris A. Production of nitric oxide by mitochondria. *J Biol Chem*. 1998;273:11038–11043.
- Ghafourifar P, Ritcher C. Nitric oxide synthase activity in mitochondria. *FEBS Lett*. 1997;418:291–296.
- Pryor WA. Oxy-radicals and related species: their formation, lifetimes and reactions. *Annu Rev Biochem*. 1986;48:657–667.
- Raha S, Myint AT, Johnstone L, Robinson BH. Control of oxygen free radical formation from mitochondrial complex I: roles for protein kinase A and pyruvate dehydrogenase kinase. *Free Radic Biol Med*. 2002;32:421–430.
- Raha S, McEachern G, Myint AT, Robinson BH.

- Superoxides from mitochondrial complex III: the role of manganese superoxide dismutase. *Free Radic Biol Med.* 2000;29:170–180.
25. Pitkanen S, Robinson BH. Mitochondrial complex I deficiency leads to increased production of superoxide radicals and induction of superoxide dismutase. *J Clin Invest.* 1996;98:345–351.
 26. Rabiloult T, Heller M, Rigobello MP, Bindoli A, Abersold R, Lunardi J. The mitochondrial antioxidant defence system and its response to oxidative stress. *Proteomics.* 2001;1:1105–1110.
 27. Fredovich I. Superoxide radical and superoxide dismutases. *Annu Rev Biochem.* 1995;64:97–112.
 28. Melov S, Coskum P, Patel M, et al. Mitochondrial disease in superoxide dismutase 2 mutant mice. *Proc Natl Acad Sci USA.* 1999;96:846–851.
 29. Esposito LA, Kokoszka JE, Waymire KG, Cottrell B, MacGregor GR, Wallace DC. Mitochondrial oxidative stress in mice lacking the glutathione peroxidase-1 gene. *Free Radic Biol Med.* 2000;28:754–766.
 30. Chae HZ, Kim HJ, Kang SW, Rhee SG. Characterization of three isoforms of mammalian peroxiredoxin that reduce peroxides in the presence of thioredoxin. *Diabetes Res Clin Pract.* 1999;45:101–112.
 31. Tanaka T, Hosoi F, Tamaguchi-Iwai Y, et al. Thioredoxin-2 (TRX-2) is an essential gene regulating mitochondria-dependent apoptosis. *Embo J.* 2002;21:1695–1703.
 32. Kang SW, Chae HZ, Seo MS, Kim K, Baines IC, Rhee SG. Mammalian peroxiredoxin isoforms can reduce hydrogen peroxide generated in response to growth factors and tumor necrosis factor- α . *J Biol Chem.* 1998;273:6297–6303.
 33. Toyokuni S, Okamoto K, Yodoi J, Hiai H. Persistent oxidative stress in cancer. *Febs Lett.* 1995;358:1–3.
 34. Yeliseev A, Krueger K, Kaplan S. A mammalian mitochondrial drug receptor functions as a bacterial “oxygen” sensor. *Proc Natl Acad Sci USA.* 1997;94:5101–5106.
 35. Carayon P, Portier M, Dussosoy D, et al. Involvement of peripheral benzodiazepine receptors in the protection of hematopoietic cells against oxygen radical damage. *Blood.* 1996;87:3170–3178.
 36. Bianchi A, Becuwe P, Franck P, Dauca M. Induction of MnSOD gene by arachidonic acid is mediated by reactive oxygen species and p38 MAPK signaling pathway in human HepG2 hepatoma cells. *Free Radic Biol Med.* 2002;32:1132–1142.
 37. Knapp LT, Klann E. Superoxide-induced stimulation of protein kinase C via thiol modification and modulation of zinc content. *J Biol Chem.* 2000;275:24136–24145.
 38. Kamata H, Hirata H. Redox regulation of cellular signalling. *Cell Signal.* 1999;11:1–14.
 39. Chandel N, McClintok D, Feliciano C, et al. Reactive oxygen species generated at mitochondrial complex III stabilize HIF-1 α during hypoxia: a mechanism of O₂ sensing. *J Biol Chem.* 2000;275:25130–25138.
 40. Maehara K, Hasegawa T, Isobe K. An NF- κ B p65 subunit is indispensable for activating manganese superoxide dismutase gene transcription mediated by tumor necrosis factor- α . *J Cell Biochem.* 2000;77:474–486.
 41. Maxwell P, Wiesener M, Chang GW, et al. The tumour suppressor protein in VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature.* 1999;399:271–275.
 42. Kawakami M, Okabe E. Superoxide anion radical-triggered Ca²⁺ release from cardiac sarcoplasmic reticulum through ryanodine receptor Ca²⁺ channel. *Mol Pharmacol.* 1998;53:497–503.
 43. Eu JP, Sun J, Xu L, Stamler JS, Meissner G. The skeletal calcium release channel: coupled O₂ sensor and NO signaling functions. *Cell.* 2000;102:499–509.

Clinical effects of free radical scavengers

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Abstract

Increased production of free radicals has been implicated in key processes of atherosclerosis, including oxidative modification of low-density lipoprotein and endothelial dysfunction, thereby promoting a vascular inflammatory response. Recent negative results of large clinical trials using “antioxidant” supplementation have raised questions about the effect of vitamin E and the source of free radical production in vivo. Free radicals, such as superoxide, rapidly react with nitric oxide, leading to endothelial dysfunction, a well-known cardiovascular risk factor and feature of atherosclerosis. Recent studies have shown that endothelial dysfunction can serve as a prognostic marker of future adverse events. Since angiotensin-converting enzyme inhibitors and statins are known to improve endothelial function and to reduce vascular oxidative stress, these mechanisms may produce beneficial effects on morbidity and prognosis in high-risk patients. We need sensitive and specific methods to assess the oxidative burden in vivo and to identify patients at risk.

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Keywords: Atherosclerosis, free radicals, antioxidants, vitamin C, vitamin E, endothelium, prognosis, oxidative stress.

Introduction

In the 20 years since Brown and Goldstein’s observation that oxidative modification of low-density lipoprotein (LDL) leads to foam cell formation, there has been intense interest in the relationship between oxygen-derived free radical formation and atherosclerosis. In vitro and animal model studies provided a growing body of evidence that reactive oxygen species not only promote lipid peroxidation but also stimulate smooth muscle cell growth and initiate expression of proinflammatory responses, all of which contribute to the development and progression of atherosclerosis [1]. The most persuasive data came from animal models of atherosclerosis in

which studies with antioxidants, such as vitamin C, vitamin E, probucol, and coenzyme Q, showed a significant decrease in the degree of LDL oxidation and the extent of atherosclerotic lesions [2]. However, clinical studies investigating the effect of antioxidant supplementation on cardiovascular risk have produced mixed results.

Antioxidants and cardiovascular risk

Epidemiological studies have suggested that increased intake of dietary antioxidants lowers the risk of atherosclerosis. High-dose vitamin E intake has been associated with a significant reduction in cardiovascular diseases (*Table 1*)

Table 1. Observational and randomized trials of antioxidant vitamins.

Study name	Participants	Follow-up	Agents used	Outcome	Relative risk reduction
<i>Prognostic observational studies with vitamin E</i>					
Nurses' Health Study [3]	87,245 Female nurses	8 Years	Vitamin E supplement vs no supplement	437 Nonfatal MI 115 deaths from CHD	31% (3% to 51%)
Health Professionals' Follow-up Study [4]	39,910 Mal health professionals	4 Years	Upper and lower quintiles of intake	360 CABG or PTCA, 201 nonfatal MI, 106 deaths from CHD	40% (19% to 56%)
Iowa Women's Health Study [5]	34,486 Post-menopausal women	7 Years	Upper vs lower quintiles of vitamin E intake	242 Deaths from CHD	Dietary intake 65% supplement ($P = NS$)
<i>Randomized, placebo-controlled, double-blind studies with vitamin E</i>					
ATBC [6]	29,133 Male smokers in Finland	6.1 Years	Vitamin E 50 mg	Total mortality	-2%
Chinese study [7]	29,584 Subjects in Linxian province	5.2 Years	Vitamin E 30 mg + β -carotene 15 mg + selenium 50 μ g	Total mortality	-9%
CHAOS [8]	2002 Patients with CHD in the UK	510 Days	Vitamin E 400 or 800 IU	Total mortality	-29% (significant)
HOPE [9]	9541 Subjects at high risk	4.5 Years	Vitamin E 400 IU and/or ramipril	MI, death from CVD, stroke	No effect of vitamin E
GISSI [10]	11,324 Patients with CHD in Italy	3.5 Years	Vitamin E 300 mg	Total mortality + nonfatal MI + cerebrovascular events	-4.7% (NS)
Heart Protection Study [11]	20,536 Subjects at high risk	5 Years	Vitamin E 600 mg + vitamin C 250 mg + β -carotene 20 mg	Mortality, deaths from CVD and MI	No effect of vitamin supplementation

MI, Myocardial infarction; CHD, coronary heart disease; CABG, coronary artery bypass graft; PTCA, percutaneous transluminal coronary angioplasty; CVD, cardiovascular disease.

[3–5]. Similarly, an inverse relation between vitamin C intake and major coronary events was found when subjects reporting an intake of 50 mg or more of vitamin C per day were shown to have a lower death rate from all cardiovascular diseases [12]. However, a major limitation of such nonrandomized studies is the possibility that confounding factors may account for the decreased risk, because the effects of other aspects of diet or lifestyle on disease rates cannot be ruled out.

Several randomized, double-blind, placebo-controlled trials have been conducted in recent

years (Table 1). The Finnish Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) study failed to show any effect on coronary heart disease in male smokers [6]. The results of the Cambridge Heart Antioxidant Study (CHAOS) were encouraging: cardiovascular death and nonfatal myocardial infarction declined by 47% when patients with established coronary artery disease were treated with 400 to 800 IU vitamin E per day [8]. Three large, randomized, double-blind intervention trials using antioxidant vitamin supplementation have been reported and all three were negative [9–11].

The Heart Outcomes Prevention Evaluation (HOPE) trial in patients at high risk for cardiovascular events used 400 IU vitamin E daily together with ramipril in a 2×2 factorial design. Vitamin E had no effect, but ramipril conferred significant protection [9]. In patients after myocardial infarction, the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI) trial compared synthetic vitamin E (300 mg daily) and omega-3 polyunsaturated fatty acids (1 g daily) in a 2×2 factorial design [10]. Over a 3.5-year follow-up, vitamin E had no effect on the composite end point of death, nonfatal myocardial infarction, and stroke. The Heart Protection Study in 20,536 patients with atherosclerotic disease found no significant benefits on major coronary events using antioxidant vitamin supplementation [11].

The negative results of these trials raise important questions about the role of reactive oxygen species in atherosclerotic vascular disease.

Why has vitamin E supplementation failed?

The above studies seem to cast doubt on the oxidative modification theory of atherosclerosis, but there are several reasons to believe that this is not justified. (1) Although vitamin E effectively scavenges lipid peroxy radicals, it has limited activity against other oxidants, such as superoxide, peroxynitrite, and hypochlorous acid, which have been implicated in atherosclerosis. The rate constant for the reaction of vitamin E with O_2^- is five orders of magnitude slower than the rate of reaction of O_2^- with endogenous antioxidant enzymes and molecules such as superoxide dismutase and nitric oxide [13]. (2) Oral intake of vitamin E only modestly increases its plasma and tissue levels. Given the slow rate constants for vitamin E's reaction with O_2^- and other radicals, these modest increases in its concentration are unlikely to affect biological processes. (3) Many of the oxidative reactions that contribute to atherosclerosis occur in the cytoplasm, nucleus, and interstitial space. Vitamin E is concentrated in lipid layers and in the

LDL particle and is therefore unlikely to affect these events. (4) Vitamin E may have adverse, pro-oxidant effects. The tocopheroxyl radical generated when vitamin E reacts with a radical can promote lipid peroxidation by attacking polyunsaturated fatty acids [14].

Given these considerations, it is quite possible that the use of vitamin E or other antioxidant vitamins will not be the best approach to limit vascular oxidative stress.

What are the sources of pathogenic oxygen radicals in atherosclerosis?

Preventing free radical production may represent a much more efficient way to inhibit the detrimental effects of vascular oxidant stress than trying to scavenge free radicals using vitamins. This requires a better understanding of the enzyme systems involved in their production. There are several potential enzymatic sources of reactive oxygen species including xanthine oxidase, (reduced) nicotinamide adenine dinucleotide phosphate [NAD(P)H] oxidases, nitric oxide synthase, the mitochondrial electron transport chain, arachidonic acid pathway enzymes lipoxygenase and cyclo-oxygenase, cytochrome P-450, peroxidase, and other hemoproteins [15]. In addition, infiltrating phagocytic cells, which contain the high-capacity O_2^- -generating flavoenzyme NADPH oxidase, may be another important source of reactive oxygen species in atherosclerotic blood vessels. The nonphagocytic NAD(P)H oxidase seems to be a major source of reactive oxygen species in endothelial cells, vascular smooth muscle cells, and adventitial fibroblasts. Its activity is regulated by cytokines (tumor necrosis factor- α) and hormones (angiotensin II), and has been shown to be upregulated in experimental models of atherosclerosis, hyperlipidemia, diabetes, hypertension, and balloon denudation [16]. In addition, segments of human vessels obtained from patients undergoing routine coronary artery bypass surgery have shown increased NAD(P)H oxidase-dependent vascular O_2^- production [17].

Increased free radical production not only

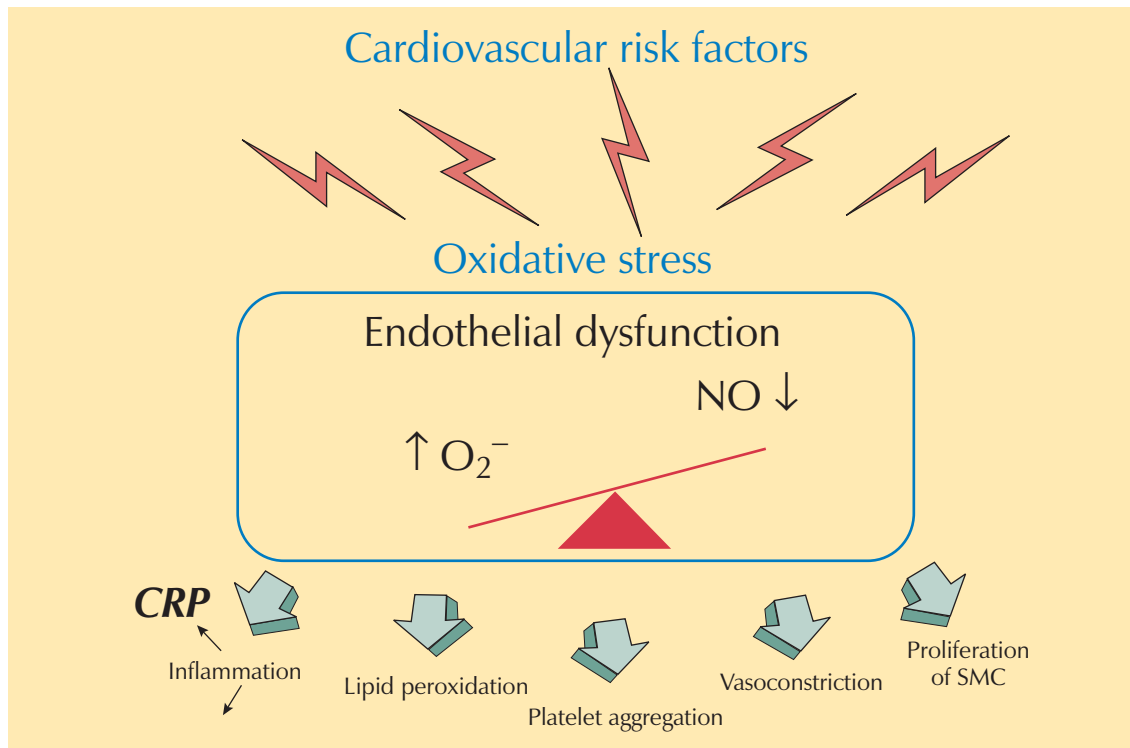


Figure 1. Mechanisms for oxidative stress-induced endothelial dysfunction leading to acceleration of atherosclerotic processes. O_2^- , superoxide; NO, nitric oxide.

leads to LDL peroxidation but also to endothelial dysfunction by inactivating nitric oxide, a well-known feature of atherosclerosis.

Oxidative stress induces endothelial dysfunction

The endothelium plays an integral role in maintaining vascular tone and function, in part by synthesis and release of vasoactive substances such as nitric oxide. Nitric oxide not only produces vasodilatation but also has potent antiatherogenic properties, including inhibiting adhesion molecule expression, preventing smooth muscle cell proliferation, reducing lipid peroxidation, and inhibiting platelet aggregation. Over the past decade, numerous experimental and clinical studies have demonstrated that oxidant stress is a major cause of endothelial dysfunction [18]. In particular, nitric oxide reacts rapidly with O_2^- , resulting in the formation of the perox-

ynitrite anion and loss of nitric oxide bioactivity (Figure 1). Accordingly, treatment with strong reducing agents such as vitamin C has consistently demonstrated beneficial effects on endothelial function in the coronary and peripheral circulation of patients [19]. Of note, because of the low rate constant of the reaction between vitamin C and superoxide, vitamin C must be given intra-arterially in sufficiently high concentrations (plasma concentration ≈ 10 mmol/L) to compete effectively with nitric oxide for superoxide [20]. Oral vitamin C has been shown to be rather unsuccessful. These findings are consistent with the notion that excessive production of superoxide contributes to endothelial dysfunction and decreased nitric oxide bioavailability.

Endothelial dysfunction, oxidative stress, and cardiovascular risk

Several groups have studied the prognostic

value of endothelial dysfunction by obtaining long-term follow-up of patients with and without significant coronary artery disease [21–23]. These studies demonstrated that patients with endothelial dysfunction are at increased risk for cardiovascular events, including death from cardiovascular causes, myocardial infarction, ischemic stroke, coronary angioplasty, and coronary bypass graft. Although the precise mechanisms underlying the association between endothelial dysfunction and cardiovascular risk are unknown, vascular oxidative stress may not only contribute to endothelial dysfunction but also to coronary artery disease activity. This suggestion is supported by a recent 5-year follow-up study, finding that the beneficial effects of intra-arterial administration of vitamin C on endothelial function are much more pronounced in patients with subsequent cardiovascular events compared with patients without adverse events (Figure 2) [23]. The degree of improvement by vitamin C may indicate the amount of vascular oxidative stress in these patients. This strongly supports the concept that increased oxidative stress contributes to the progression of atherosclerotic disease and may therefore be an important determinant of clinical events.

“Antioxidant” effects of angiotensin-converting enzyme (ACE) inhibitors and statins

Angiotensin II potently stimulates vascular free radical production from sources such as NAD(P)H oxidase, leading to endothelial dysfunction and promoting a vascular inflammatory response. Angiotensin II type 1 (AT₁) receptor antagonists or angiotensin-converting enzyme (ACE) inhibitors have been shown to normalize vascular oxidative stress and to reduce the progression of atherosclerosis [24, 25]. ACE inhibitors are known to improve endothelial dysfunction and reduce the rate of death from cardiovascular causes. Thus, ACE inhibitors and AT₁ receptor antagonists are believed to be potent antiatherosclerotic drugs likely due to their antioxidative properties.

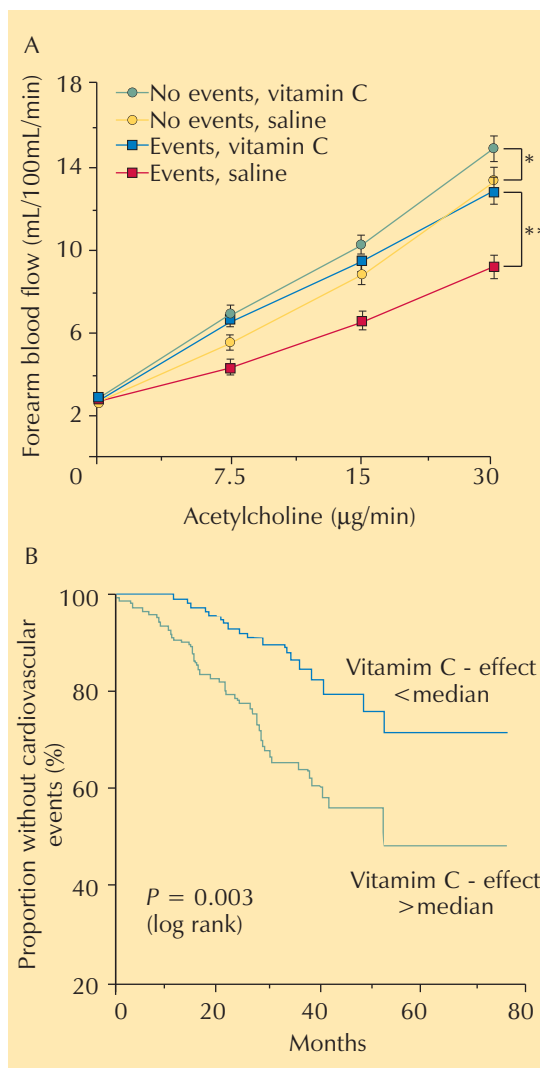


Figure 2. (A) Endothelial function assessed by acetylcholine-induced blood flow in patients with (squares) and without (circles) cardiovascular events during saline and vitamin C infusion. Vitamin C improved endothelial function in both groups. However, the effect of vitamin C was significantly larger in patients with events than in those without events. * $P < 0.05$ vs saline; ** $P < 0.001$ vs saline. (B) Kaplan-Meier analysis demonstrating cumulative proportion of patients without cardiovascular events during follow-up. Effect of vitamin C on endothelial function is divided into values below and above the median. (Adapted from [23].)

The beneficial effects of HMG-CoA reductase inhibitors appear to extend beyond their effects on serum cholesterol levels. Indeed, recent experimental and clinical evidence

indicates that some of the “pleiotropic” effects of statins involve improving endothelial function, enhancing the stability of atherosclerotic plaques, and decreasing oxidative stress and vascular inflammation [26]. Indeed, statins dramatically decrease superoxide production and increase bioavailability of nitric oxide by stimulating and upregulating endothelial nitric oxide synthase.

Search for markers of oxidative stress

Despite convincing evidence that free radicals play a central role in vascular disease, there are few methods of direct measurement of oxidative stress in patients. Assessment of plasma concentrations of antioxidants such as vitamin C, vitamin E, carotenoids, ubiquinol-10, and glutathione can be used [27]. Estimation of in vivo LDL oxidation has been proposed by measuring malondialdehyde, conjugated dienes in circulating LDL, or plasma titers of autoantibodies to oxidized LDL [28]. F2-isoprostanes have recently begun to emerge as a new class of free radical catalyzed products of arachidonic acid metabolism supposed to be far more specific than the conventional methods of malondialdehyde [29]. However, none of these markers has been accepted as a satisfactory candidate to identify patients at high risk due to oxidative stress. Biomarkers are urgently needed to identify such high-risk populations and to assess whether therapy effectively lowers the oxidative burden.

Conclusion

The recent disappointing trials of antioxidant therapy raise doubts about the ability of vitamins to augment antioxidant defence mechanisms in vivo. Despite ample experimental evidence that free radicals play a pivotal role in atherosclerotic disease, the methods currently available to assess the degree of oxidative stress and the efficacy of antioxidant therapy in vivo are quite limited. Quantitatively accurate indices of the oxidative burden of

patients at risk would substantially support clinical research in this area. ■

REFERENCES

1. Steinberg D, Lewis A. Conner Memorial Lecture. Oxidative modification of LDL and atherogenesis. *Circulation*. 1997;95:1062–1071.
2. Díaz MN, Frei B, Vita JA, Keaney JF. Antioxidants and atherosclerotic heart disease. *N Engl J Med*. 1997;337:408–416.
3. Stampfer MJ, Hennekens CH, Manson JE, et al. Vitamin E consumption and the risk of coronary disease in women. *N Engl J Med*. 1993;328:1444–1449.
4. Rimm EB, Stampfer MJ, Ascherio A, Giovannucci E, et al. Vitamin E consumption and the risk of coronary heart disease in men. *N Engl J Med*. 1993;328:1450–1456.
5. Kushi L, Folsom A, Princas R, et al. Dietary antioxidant vitamins and death from coronary heart disease in postmenopausal women. *N Engl J Med*. 1996;334:1156–1162.
6. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study: the effect of vitamin E and beta-carotene on the incidence of lung cancer and other cancers in male smokers. *N Engl J Med*. 1994;330:1029–1035.
7. Blot WJ, Li JY, Talor PR, et al. Nutrition intervention trials in Linxian, China: supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general population. *J Natl Cancer Inst*. 1993;85:1483–1492.
8. Stephens S, Parsons A, Schofield P, et al. Randomized controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS). *Lancet*. 1996;347:781–786.
9. Yusuf S, Dagenais G, Pongue J, et al, for the Heart Outcomes Prevention Evaluation Study Investigators. Vitamin E supplementation and cardiovascular events in high-risk patients. *N Engl J Med*. 2000;342:154–160.
10. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. *Lancet*. 1999;354:447–455.
11. Heart Protection Study Collaborative Group. MRC/BHF Heart Protection Study of antioxidant vitamin supplementation in 20536 high-risk individuals: a randomised placebo-controlled trial. *Lancet*. 2002;360:23–33.
12. Enstrom JE, Kanim LE, Klein MA. Vitamin C intake and mortality among a sample of the United States population. *Epidemiology*. 1992;3:194–202.
13. Gotoh H, Niki E. Rates of interactions of superoxide with vitamin E, vitamin C, and related compounds

- as measured by chemiluminescence. *Biochim Biophys Acta*. 1992;1115:201–207.
14. Upston JM, Terentis AC, Stocker R. Tocopherol-mediated peroxidation of lipoproteins: implications for vitamin E as a potential antiatherogenic supplement. *Faseb J*. 1999;13:977–994.
 15. Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. *Circ Res*. 2000;87:840–844.
 16. Griendling KK, Sorescu D, Ushio-Fukai M. NAD(P)H oxidase: role in cardiovascular biology and disease. *Circ Res*. 2000;86:494–501.
 17. Guzik TJ, West NE, Black E, et al. Vascular superoxide production by NAD(P)H oxidase: association with endothelial dysfunction and clinical risk factors. *Circ Res*. 2000;86:E85–E90.
 18. Keaney JF, Vita JA. Atherosclerosis, oxidative stress, and antioxidant protection in endothelium-derived relaxing factor actions. *Prog Cardiovasc Dis*. 1995;38:129–154.
 19. Carr AC, Zhu BZ, Frei B. Potential antiatherogenic mechanisms of ascorbate (vitamin C) and alpha-tocopherol (vitamin E). *Circ Res*. 2000;87:349–354.
 20. Jackson TS, Xu A, Vita JA, Keaney JF. Ascorbate prevents the interaction of superoxide and nitric oxide only at very high physiological concentrations. *Circ Res*. 1998;83:916–922.
 21. Suwaidi JA, Hamasaki S, Higano ST, et al. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. *Circulation*. 2000;101:948–954.
 22. Schachinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation*. 2000;101:1899–1906.
 23. Heitzer T, Schlinzig T, Krohn K, et al. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation*. 2001;104:2673–2678.
 24. Munzel T, Keaney JF. Are ACE inhibitors a “magic bullet” against oxidative stress? *Circulation*. 2001;104:1571–1574.
 25. Yusuf S, Sleight P, Pogue J, et al, for the Heart Outcomes Prevention Evaluation Study Investigators. Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. *N Engl J Med*. 2000;342:145–153.
 26. Takemoto M, Liao JK. Pleiotropic effects of 3-hydroxy-3-methylglutaryl coenzyme reductase inhibitors. *Arterioscler Thromb Vasc Biol*. 2001;21:1712–1719.
 27. Polidori MC, Stahl W, Eichler O, et al. Profiles of antioxidants in human plasma. *Free Radic Biol Med*. 2001;30:456–462.
 28. Ahotupa M, Vasankari T. Baseline diene conjugation in LDL lipids: an indicator of circulating oxidized LDL. *Free Radic Biol Med*. 1999;27:1141–1150.
 29. Patrono C, FitzGerald GA. Isoprostanes: potential markers of oxidant stress in atherothrombotic disease. *Arterioscler Thromb Vasc Biol*. 1997;17:2309–2315.

Metabolic imaging of myocardial stunning

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Abstract

Myocardial stunning refers to a reversible state of regional contractile dysfunction that can occur after restoration of coronary blood flow following a brief episode of ischemia despite the absence of necrosis. Stunned myocardium can also result from repeated ischemic episodes caused by increases in oxygen demand in the setting of chronic coronary artery disease. During acute myocardial ischemia, there is a sharp decline in free fatty acid oxidation that is followed by a markedly increased rate of glucose utilization. However, soon after reperfusion, glucose utilization in stunned myocardium is reduced compared with that in normal regions. This relative reduction in glucose uptake in stunned regions is usually restored to control levels within a week after reperfusion, depending on the severity and duration of the initial flow deficit. These abnormalities in glucose metabolism can be assessed noninvasively with the glucose analog ^{18}F -deoxyglucose and SPECT or PET imaging. These metabolic alterations present in stunning appear different from those typically associated with hibernation. Thus, this different metabolic adaptation may prove useful for the noninvasive characterization of infarcted and viable (stunned and/or hibernating) myocardium in patients with severe left ventricular dysfunction who are being considered for potential myocardial revascularization. ■ *Heart Metab.* 2003;19:18–22.

Keywords: Myocardial stunning, metabolic imaging, glucose utilization, free fatty acid oxidation, ^{18}F -deoxyglucose, PET, myocardial hibernation.

Introduction

Myocardial stunning refers to a reversible state of regional contractile dysfunction that can occur after restoration of coronary blood flow following a brief episode of ischemia despite the absence of necrosis [1]. It is considered a form of reperfusion injury, whereby reintroduction of oxygen after a period of ischemia induces a transient calcium overload that

damages the contractile apparatus. The postischemic contractile abnormality is fully reversible provided that recurrent ischemia (followed by stunning) does not occur and sufficient time is allowed for the myocardium to recover. Stunned myocardium has been described in animals [2] and subsequently documented in humans [1], where it is considered to play a role in the prolonged contractile dysfunction seen in patients undergoing reperfusion therapy for acute myocardial infarction, following attacks of unstable angina, and in some patients with exercise-induced ischemia. Although commonly

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regarded as an acute phenomenon, stunned myocardium may also occur in patients with chronic coronary stenoses who experience recurrent episodes of ischemia (symptomatic or asymptomatic) in the same territory (so-called repetitive stunning) [3]. The latter mechanism is probably the most common form of stunning in patients with chronic left ventricular dysfunction due to coronary artery disease and will be the focus of this review.

Metabolic abnormalities during ischemia-reperfusion

Metabolic alterations during myocardial ischemia

A reduction in oxygen supply or an inadequate blood flow response to increased demand is associated with an almost instantaneous decline or loss of contractile function. The inadequate oxygen supply or supply-demand imbalance causes profound metabolic alterations. There is a sharp decline in free fatty acid oxidation, which is followed by an increased flux of glucose through the glycolytic pathway [4]. The increased glycolytic flux during ischemia appears to involve a specific stimulation of membrane transport of glucose through the rapid translocation of glucose transporters (GLUT4 and GLUT1 isoforms) and an increased activity of key glycolytic enzymes [5, 6]. Such increases in glucose uptake during acute ischemia have been demonstrated in experimental animals and in patients with chronic coronary artery disease [4, 7].

Metabolic changes post reperfusion

Several metabolic abnormalities have been described in stunned myocardium, including alterations in glucose as well as in fatty acid kinetics. In experimental models, myocardial stunning induced by a single or multiple brief episodes of low-flow ischemia has been consistently associated with a reduction in myocardial glucose utilization (approximately 30% compared with controls), at least early after reperfu-

sion [8–12]. Although in some studies glucose utilization was shown to increase after 24 hours of reperfusion, such changes have not been universally observed. For example, Buxton et al [13] showed regional increases in fluorodeoxyglucose uptake in stunned myocardium 24 hours after reperfusion. In contrast, we have

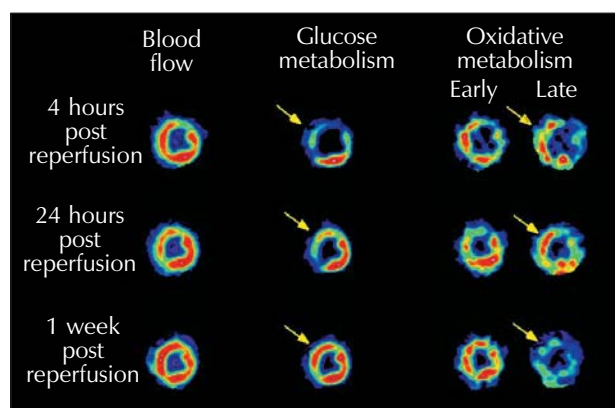


Figure 1. PET images of a dog heart in short-axis views obtained at corresponding midventricular levels obtained post reperfusion after four, 5-minute LAD coronary occlusions, each followed by 5 minutes of reperfusion. The images are oriented with the anterior wall at the top, the inferior wall at the bottom, the interventricular septum to the left, and the lateral wall to the right. Each image is scaled to its own maximum. Images of blood flow (left column) were obtained with ^{13}N -ammonia and images of glucose metabolism (middle column) with ^{18}F -deoxyglucose. Images of oxidative metabolism (MVO_2) (right column) were obtained with ^{11}C -acetate; the early phase denotes delivery of the tracer to the myocardium while the late phase represents regional washout of the tracer through the tricarboxylic acid cycle (oxidation). (Top panel): Depicts corresponding midventricular short-axis sections of regional blood flow, glucose and MVO_2 4 hours post reperfusion. The flow images (left) demonstrate near-normal perfusion in the stunned regions (ie, anterior and anteroseptum). However, stunned regions demonstrated reduced glucose utilization (arrow) and slow clearance of ^{11}C -acetate (impaired oxidation) relative to normal myocardium (lateral wall). (Middle panel): One day post reperfusion. Myocardial perfusion in stunned myocardium is near-normal, glucose uptake (arrow) remains depressed and the MVO_2 is still lower (arrow) than in normal myocardium. (Bottom panel): One week after reperfusion. Blood flow, glucose uptake, and MVO_2 are largely homogenous. Wall motion and metabolism demonstrated a parallel recovery with time. (Reproduced from Di Carli et al [12] with permission from the Society of Nuclear Medicine.)

shown a prolonged reduction of glucose utilization in stunned myocardium subjected to multiple cycles of ischemia and reperfusion (Figure 1). These apparently contradictory results appear to be more related to differences in experimental design than to physiologic discrepancies. Indeed, when studies are performed under fasting conditions, which reduces glucose utilization by normal myocardium, stunned regions show a relative increase in glucose uptake. However, when such changes are evaluated during standardized substrate availability (as assessed by the hyperinsulinemic-euglycemic clamp) to reduce the normal physiologic inhomogeneity in glucose uptake in normal myocardium during fasting conditions [9, 13], relative glucose uptake appears reduced in stunned myocardium. The acute reduction in glucose utilization appears to improve gradually to control levels between 48 hours and 1 week after reperfusion. The relative reduction in glucose uptake in postischemic stunning appears to relate to the severity and duration of the preceding flow deficit. Thus, stunned myocardium can demonstrate normal or a relative reduction in glucose uptake.

Human studies also suggest that similar metabolic alterations can be seen in some patients with ischemic left ventricular dysfunction. Perrone-Filardi et al [14] observed decreased glucose utilization in dysfunctional myocardial regions with normal resting blood flow. Interestingly, they also reported that 63% of those regions had reversible perfusion defects on stress thallium imaging, suggesting that stunning (caused by transient but repetitive ischemic episodes) was the underlying mechanism. We have shown similar findings in 15 patients with coronary artery disease and severe left ventricular dysfunction, in whom we evaluated the clinical, functional, and arteriographic correlates of myocardial regions showing decreased glucose utilization and normal blood flow (so-called reversed mismatch) on PET imaging [15]. All these regions showed severe wall motion abnormalities despite relatively normal resting blood flow. Consistent with the results of Perrone-Filardi et al, these dysfunctional regions showed a consistent reduction in glucose

uptake (~30% lower than normal) and oxidative metabolism (~15% lower than normal) (Figure 2). Coronary angiography demonstrated highly significant stenoses in the coronary arteries supplying these segments. Thus, this perfusion-contraction “uncoupling” with decreased metabolism agrees with our experimental observations post reperfusion and suggests that it probably reflects the metabolic correlate of “repetitive” stunning.

Potential mechanisms of metabolic alterations

Oxidation of all major substrates, including glucose, is depressed post reperfusion, and-

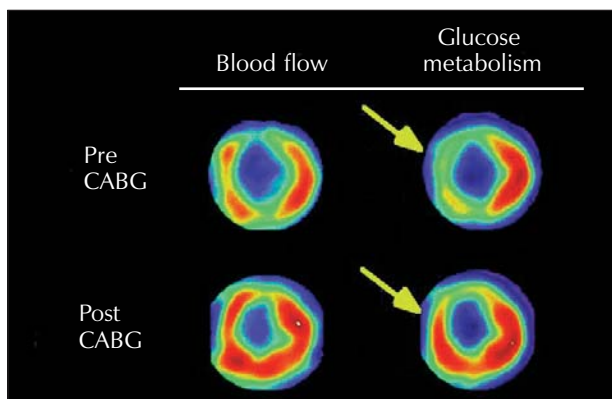


Figure 2. PET images of a human heart in short-axis views obtained in corresponding midventricular levels before and after coronary artery bypass surgery (CABG). The images have the same orientation as those of Figure 1. Images of blood flow (left column) were obtained with ^{13}N -ammonia and images of glucose metabolism (right column) with ^{18}F -deoxyglucose. (Top panel): A patient with three-vessel coronary artery disease and a severe wall motion abnormality in the anterior and septal walls. The resting flow images (left) demonstrate a small perfusion defect in the anterior wall and near-normal perfusion in the interventricular septum. A $^{99\text{m}}\text{Tc}$ sestamibi perfusion scan (not shown) demonstrated exercise-induced ischemia in these regions. Glucose metabolism in the anterior and septal walls is markedly reduced compared with normal myocardium (lateral wall). (Bottom panel): Same patient 4 weeks after CABG. Blood flow and glucose metabolism are largely homogenous. A 2-D echocardiogram post CABG demonstrated improvement in systolic wall motion in the anterior and septal regions.

nonglucose substrates are the preferred substrate for oxidative metabolism [16, 17]. Indeed, previous studies have demonstrated that reperfused myocardium has a strong preference for and aerobic use of fatty acids during reflow [16, 17]. Further, carbohydrate utilization for oxidative metabolism during reflow is significantly reduced [8, 18]. One possible mechanism for the reduced glucose uptake in reperfused tissue may be that shifting levels of metabolites following ischemia and reperfusion may have decreased the activity of key regulatory enzymes of the glycolytic pathway [19]. Another possibility may be that multiple cycling of ischemia and reperfusion or their metabolic byproducts could have decreased glucose uptake by decreasing the number of insulin receptors or their sensitivity to insulin [20], or by altering the process in which insulin signals GLUT4 translocation [21, 22], or both.

Clinical implications

The experimental and clinical data presented above suggest that the metabolic alterations present in stunning are different from those typically associated with hibernation. Hibernating myocardium appears to show increased glucose uptake in areas with reduced blood flow at rest, so-called perfusion-metabolism mismatch on PET imaging. In contrast, stunned myocardium caused by multiple cycles of ischemia-reperfusion (as it may occur in patients with chronic coronary artery disease) may show normal or reduced glucose uptake in areas with preserved blood flow at rest, so-called reverse mismatch on PET imaging.

This different metabolic adaptation may prove useful for the noninvasive characterization of infarcted and viable (stunned and/or hibernating) myocardium in patients with severe left ventricular dysfunction who are being considered for potential myocardial revascularization. ■

REFERENCES

1. Bolli R. Myocardial "stunning" in man. *Circulation*. 1992;86:1671–1691.
2. Heyndrickx GR, Millard RW, McRitchie RJ, Maroko PR, Vatner SF. Regional myocardial functional and electrophysiological alterations after brief coronary artery occlusion in conscious dogs. *J Clin Invest*. 1975;56:978–985.
3. Vanoverschelde JL, Wijns W, Depre C, et al. Mechanisms of chronic regional postischemic dysfunction in humans. New insights from the study of noninfarcted collateral-dependent myocardium. *Circulation*. 1993;87:1513–1523.
4. Opie LH. Effects of regional ischemia on metabolism of glucose and fatty acids. Relative rates of aerobic and anaerobic energy production during myocardial infarction and comparison with effects of anoxia. *Circ Res*. 1976;38:152–174.
5. Bell GI, Kayano T, Buse JB, et al. Molecular biology of mammalian glucose transporters. *Diabetes Care*. 1990;13:198–208.
6. Wheeler TJ. Translocation of glucose transporters in response to anoxia in heart. *J Biol Chem*. 1988;263:19447–19454.
7. Camici P, Araujo LI, Spinks T, et al. Increased uptake of ¹⁸F-fluorodeoxyglucose in postischemic myocardium of patients with exercise-induced angina. *Circulation*. 1986;74:81–88.
8. Liedtke AJ, Renstrom B, Hacker TA, Nellis SH. Effects of moderate repetitive ischemia on myocardial substrate utilization. *Am J Physiol*. 1995;269:H246–H253.
9. Schwaiger M, Schelbert HR, Ellison D, et al. Sustained regional abnormalities in cardiac metabolism after transient ischemia in the chronic dog model. *J Am Coll Cardiol*. 1985;6:336–347.
10. Buxton DB, Schelbert HR. Measurement of regional glucose metabolic rates in reperfused myocardium. *Am J Physiol*. 1991;261:H2058–H2068.
11. McFalls EO, Ward H, Fashingbauer P, Gimmestad G, Palmer B. Myocardial blood flow and FDG retention in acutely stunned porcine myocardium. *J Nucl Med*. 1995;36:637–643.
12. Di Carli MF, Prcevski P, Singh TP, et al. Myocardial blood flow, function, and metabolism in repetitive stunning. *J Nucl Med*. 2000;41:1227–1234.
13. Buxton DB, Nienaber CA, Luxen A, et al. Noninvasive quantitation of regional myocardial oxygen consumption in vivo with [¹¹C]acetate and dynamic positron emission tomography. *Circulation*. 1989;79:134–142.
14. Perrone-Filardi P, Bacharach SL, Dilsizian V, et al. Clinical significance of reduced regional myocardial glucose uptake in regions with normal blood flow in patients with chronic coronary artery disease. *J Am Coll Cardiol*. 1994;23:608–616.
15. Di Carli M, Choi Y, Schelbert H, Phelps M, Madahi J. Clinical significance of reduced glucose uptake in myocardial regions with preserved blood

- flow in patients with coronary artery disease. *J Am Coll Cardiol.* 1996;27:163A.
16. Myears DW, Sobel BE, Bergmann SR. Substrate use in ischemic and reperfused canine myocardium: quantitative considerations. *Am J Physiol.* 1987;253:H107–H114.
 17. Liedtke AJ, DeMaison L, Eggleston AM, Cohen LM, Nellis SH. Changes in substrate metabolism and effects of excess fatty acids in reperfused myocardium. *Circ Res.* 1988;62:535–542.
 18. Renstrom B, Nellis SH, Liedtke AJ. Metabolic oxidation of glucose during early myocardial reperfusion. *Circ Res.* 1989;65:1094–1101.
 19. Kobayashi K, Neely JR. Effects of ischemia and reperfusion on pyruvate dehydrogenase activity in isolated rat hearts. *J Mol Cell Cardiol.* 1983;15:359–367.
 20. Dodds K, Lamb P, Pentecost B, Nattrass M. Erythrocyte insulin receptors following myocardial infarction in non-diabetic subjects. *Ann Clin Biochem.* 1986;23:657–660.
 21. Harada K, Maekawa T, Abu Shama KM, Yamashima T, Yoshida K. Translocation and down-regulation of protein kinase C-alpha, -beta, and -gamma isoforms during ischemia-reperfusion in rat brain. *J Neurochem.* 1999;72:2556–2564.
 22. Shepherd PR, Kahn BB. Glucose transporters and insulin action — implications for insulin resistance and diabetes mellitus. *N Engl J Med.* 1999;341:248–257.

Modifying oxidative stress by nutritional intervention

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Abstract

Oxidative stress is the consequence of oxidative metabolism and of some spontaneous oxidative reactions in the organism. Its magnitude is reduced by endogenous antioxidant enzymatic processes and specific molecules, as well as by exogenous antioxidants, mostly of plant origin. In chronic diseases inflammation causes oxidative stress, thus contributing to cell and tissue injury. In addition, specific oxidative processes appear as the main pathogenic mechanism in some conditions like atherosclerosis, Parkinson's disease, or ischemia-reperfusion tissue damage. The magnitude of an oxidative stress condition can be estimated from measurement of ongoing oxidative processes or the magnitude and quality of antioxidant mechanisms. A more accurate estimation comes from the measurement of oxidative stress markers like 8-OH-deoxyguanosine in DNA, covalent protein modifications, lipid oxidation products, and oxidative stress sensitive processes like endothelial function. Food components other than antioxidant vitamins, mainly plant phenolic antioxidants with flavonoids among them, have been recognized in recent years as key components of a healthy diet. Moreover, the beginning of a systematic collection of data is allowing a better characterization of the dietary antioxidant supply, yet their bioavailability and overall effectiveness require more investigation. Part of the information will come from dietary intervention studies through antioxidant capacity, antioxidant levels and oxidative stress marker measurements, in normal and disease conditions. ■ *Heart Metab.* 2003;19:23–29.

Keywords: Antioxidants; oxidative stress; 8-OHdG; endothelial function; diet; wine; mediterranean diet.

Oxidative stress

Oxidative stress, the consequence of a pro-oxidant imbalance between pro-oxidants and antioxidants in the human body, has been increasingly implicated during the last decade in the pathogenesis of chronic diseases and aging. Since antioxidants reduce oxidative stress, they could control the damage caused by reactive oxygen or nitrogen species and decrease the risk and consequences of chronic diseases. Diet composition influences both

oxidative damage and antioxidant mechanisms, thus explaining, at least in part, the relationship between diet and chronic diseases such as atherosclerosis and cancer [1–3].

Mediterranean diet

The Mediterranean population has a low mortality rate, a fact attributed in part to the dietary habits of the region. Indeed, the Mediterranean diet has been proposed as a prototype for a healthy diet. The Mediter-

anean diet is high in monounsaturated fatty acids, fiber, and antioxidants, balanced in omega-6/omega-3 polyunsaturated fatty acids, and low in saturated fat. People from the Mediterranean regions of southern Europe eat more fish, white meat, olive oil, legumes, vegetables, and fruit; less red meat and animal fat; and consume a moderate quantity of red wine with meals [4–7]. Conversely, people from the USA and some other continental and northern European populations eat more red meat, animal fat, dairy products, and sugar; and fewer legumes, vegetables and fruit, and, in many populations, sea food. This diet, known as the occidental diet, is high in saturated fats and omega-6 polyunsaturated fatty acids, in refined or simple carbohydrates, and low in antioxidants and fiber [8–12].

Epidemiological studies have shown that the occidental diet is associated with a high incidence of cardiovascular disease and other chronic diseases, in contrast with diets rich in fruit and vegetables [13–15]. Wine, a rich source of antioxidants, has been associated with a low risk of cardiovascular disease [16, 17].

Biomarkers of oxidative stress

Lipid peroxidation and oxidative DNA damage constitute good biomarkers of oxidative damage [18]. Furthermore, oxidative DNA damage is mechanistically involved in cancer and aging, and lipid peroxidation plays a key role in cardiovascular disease. The oxidative hypothesis of atherogenesis postulates that oxidized LDL is the main agent of damage, and the endothelial cell the main target [19–21]. Recently, Natella et al [22] reported that supplementing a meal with grape seed proanthocyanidins, which are present in red wine, markedly reduces postprandial lipid peroxides. Oxidative DNA damage can be measured as 8-OH-deoxyguanosine (8-OHdG) in leukocytes and also shows good correlation with atherosclerosis status [23, 24]. In rabbits, dietary lipid lowering reduces oxidative DNA damage [25].

Endothelial dysfunction

The loss of endothelial function can be considered as evidence of oxidative stress. Nitric oxide, essential for normal vasodilatation, has antiatherogenic properties and its concentration decreases in the presence of the radical superoxide. Endothelial dysfunction or reduced endothelial function measured as flow-dependent vascular reactivity is present in early atherosclerosis families, hypercholesterolemia, hypertension, diabetes, hyperhomocysteinemia, and in smokers. Antioxidant administration restores endothelial function, at least in part through prevention of nitric oxide interaction with superoxide [25–28].

Intervention studies

The use of 8-OHdG as a marker in dietary antioxidant intervention studies has recently been questioned [29]. However, we have accumulated experimental evidence that peripheral leukocyte 8-OHdG content is a reliable marker of oxidative damage [30, 31]. We have performed intervention studies using controlled diets, a Mediterranean and an occidental diet, with and without wine, to evaluate the changes on antioxidants and oxidative stress in volunteers. Among other parameters, we measured 8-OHdG in leukocyte DNA as a marker of systemic oxidative stress [32]; thiobarbituric acid-reacting substances (TBARS) and 7 β -hydroxycholesterol as markers of lipid peroxidation [32]; plasma antioxidant levels [33], total antioxidant reactivity (TAR), and total radical antioxidant potential (TRAP) [34] as markers of antioxidant status; and endothelial function [35] as a marker of atherogenic risk. The results show close correlation between plasma antioxidants and oxidative damage and favor the hypothesis that diet effectively regulates oxidative stress in humans.

Total plasma antioxidant capacity

In an intervention study we measured the total plasma antioxidant capacity as TAR; the

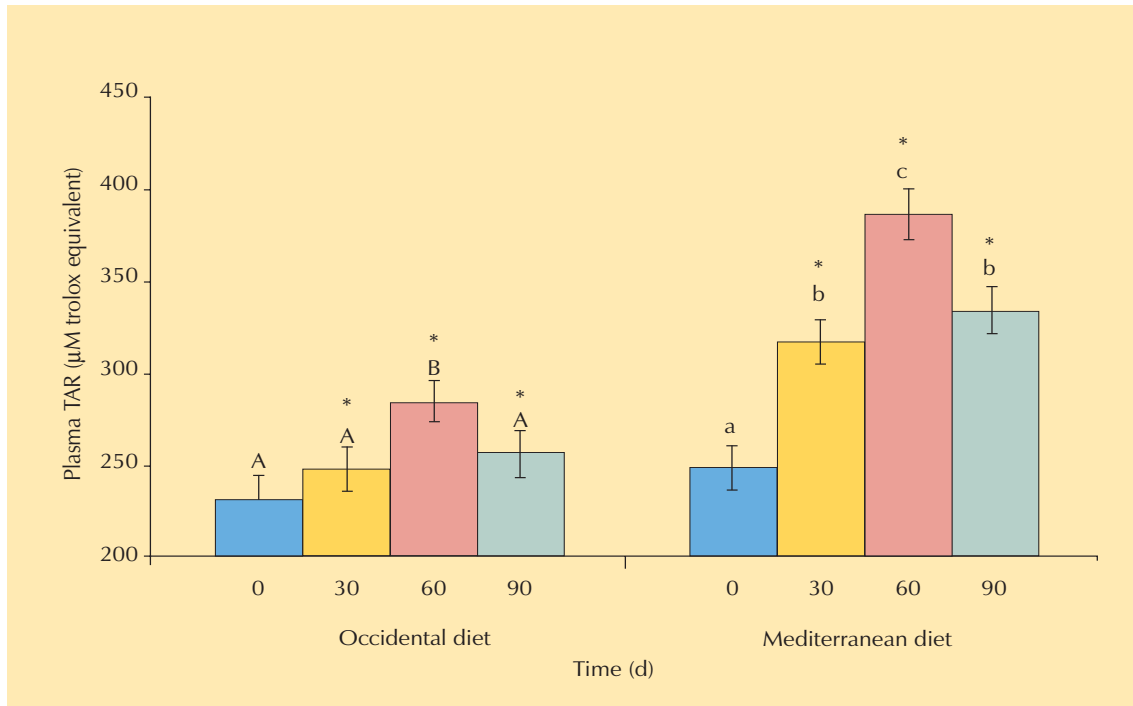


Figure 1. Effects of an occidental diet, Mediterranean diet, and wine consumption on plasma TAR. Values within the same diet, denoted by different capital or lower case letters, are significantly different ($P < 0.003$). *Values for the same time interval in different diets are significantly different ($P < 0.05$). Mean value \pm SD. (Reproduced from Leighton et al [30].)

results are shown in Figure 1. In this study we compared a Mediterranean diet with an occidental diet, and the effect of wine supplementation, administered as shown in Figure 2. TAR detects hydrosoluble antioxidants, with urate and ascorbate as the major known contributors. Volunteers were given either a

Mediterranean diet or an occidental diet for 3 months. During the second month they additionally received 240 mL/day red wine of the Cabernet Sauvignon variety.

With the Mediterranean diet the TAR values increased above basal levels: 28% at day 30 and a further 56% increase after the addition

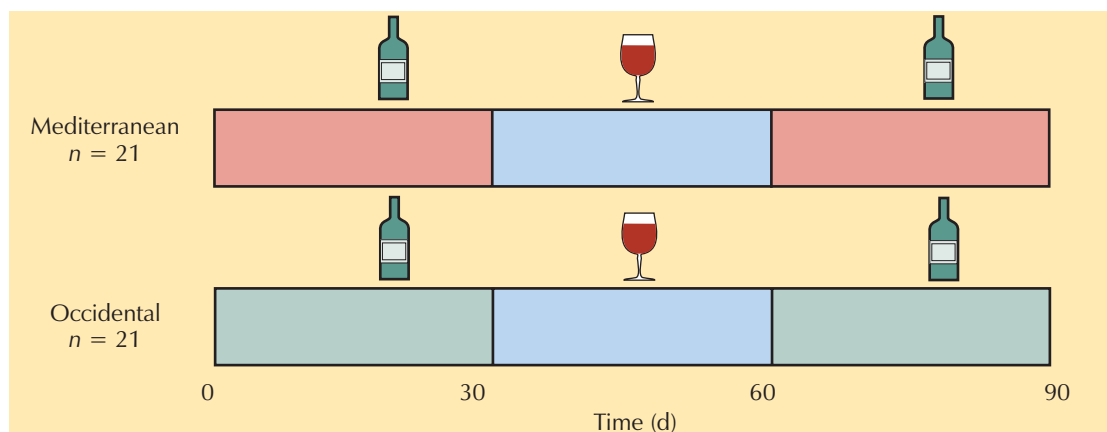


Figure 2. Intervention study design. Diets were supplied for 90 days; from days 30 to 60 red wine was supplied isocalorically. Clinical and biochemical evaluations were carried out at days 0, 30, 60, and 90.

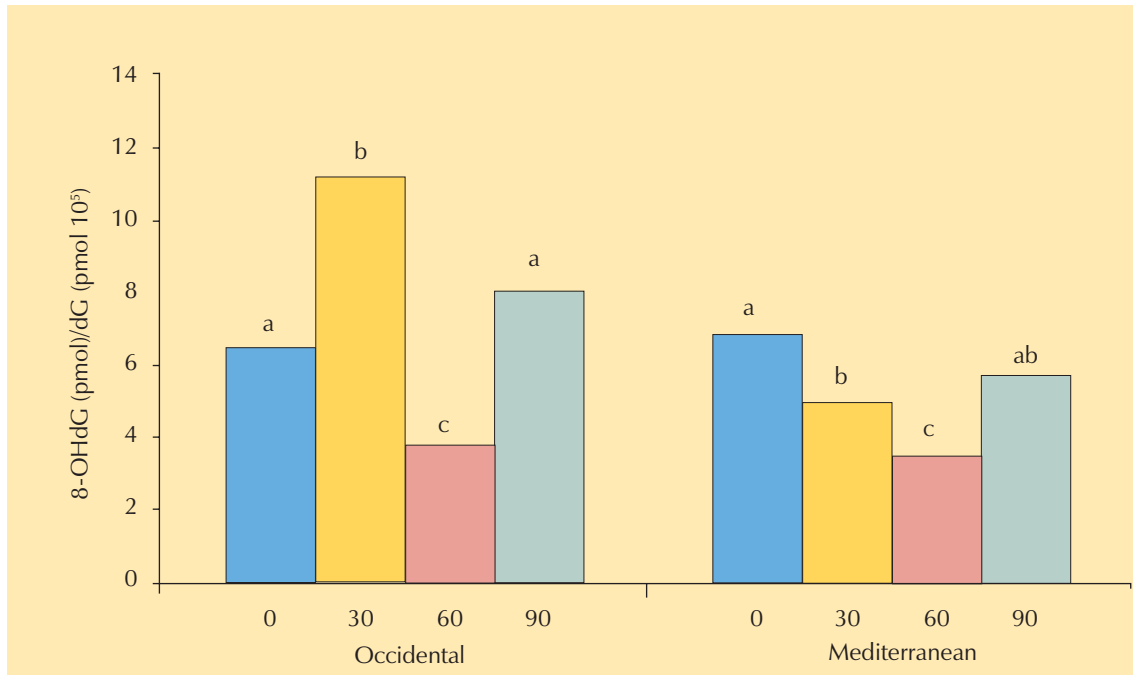


Figure 3. Effects of an occidental diet, Mediterranean diet, and wine consumption on 8-OHdG content in blood leukocytes. Results are presented as picomoles of 8-OHdG per 10⁵ picomoles of deoxyguanosine (dG). Mean value \pm SD. Different lower case letters within the same dietary group denote statistically different values ($P < 0.05$). (Reproduced from Leighton et al [30].)

of wine. The changes after commencing the Mediterranean diet and again after the addition of wine were statistically significant. By contrast, with the occidental diet the TAR values were unmodified, but a significant 23% increase above day 0 or basal level was observed with the addition of wine. Thus in the occidental diet only wine increased plasma TAR values above those corresponding to the volunteers' usual diet.

When Mediterranean and occidental diet values were compared for the same time intervals, the former were all significantly higher than the latter: 29%, 37% and 31% at days 30, 60, and 90, respectively. Clearly the Mediterranean diet per se induces a higher antioxidant capacity, just as wine does when added to a Mediterranean or occidental diet.

Oxidative DNA damage

To evaluate the oxidative damage in DNA, the content of 8-OHdG was measured in DNA

from peripheral blood leukocytes. The levels of 8-OHdG in both the Mediterranean and occidental groups, at different times of the study, are shown in Figure 3. The level of 8-OHdG detected in the Mediterranean group was lower than that in the occidental group at day 30 ($P < 0.05$), suggesting that the Mediterranean diet is able to decrease the level of DNA oxidative damage. Conversely, an occidental diet low in fruit and vegetables and rich in fat induces oxidative DNA damage.

During the period when wine was added, a substantially reduced level of oxidative DNA damage was observed equally in both diets. In fact, in the occidental group, which showed a diet-induced increase in the level of 8-OHdG at day 30, wine consumption led to a sharp decrease in the level of 8-OHdG to a value even lower than the basal level. These results show that the high oxidative DNA damage induced by an occidental diet can apparently be prevented with moderate wine consumption.

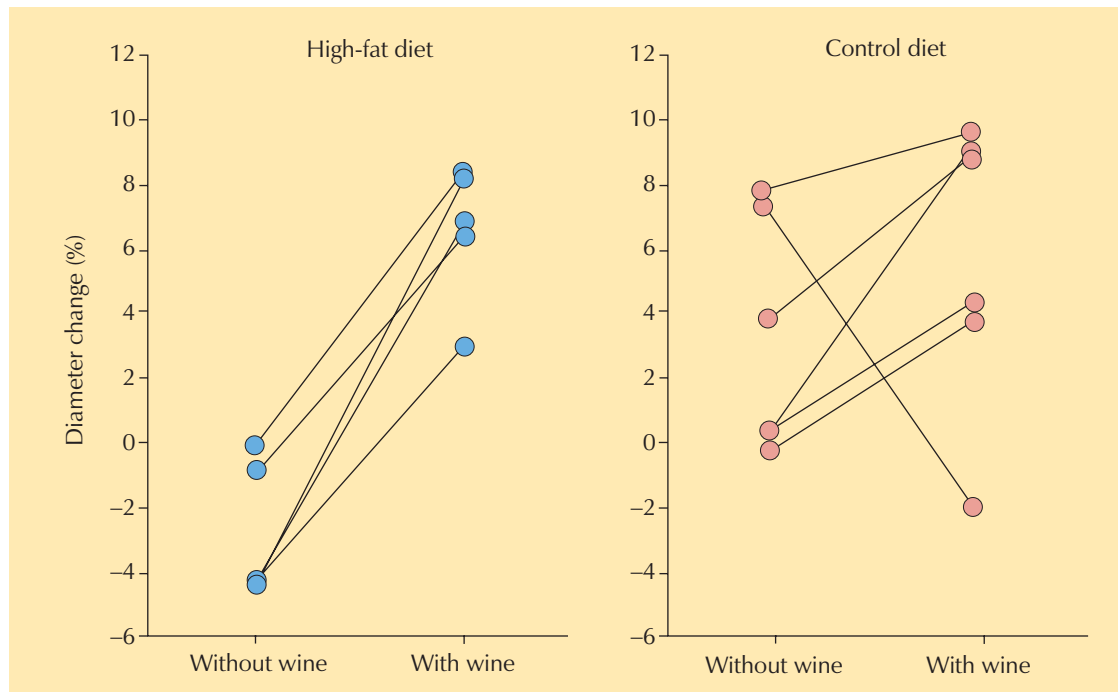


Figure 4: Effects of an occidental diet, Mediterranean diet, and wine supplementation on endothelial function. (Reproduced from Cuevas et al [26].)

In the Mediterranean group, no statistically significant differences were seen between basal values and those after diet at day 90. Nevertheless, values after 30 days of diet were lower. When wine was added to the Mediterranean diet, the level of 8-OHdG was significantly lowered (day 60). These results suggest that, in contrast with an occidental diet, a Mediterranean diet leads to a decrease in oxidative DNA damage and that moderate wine supplementation confers additional protection.

Endothelial function

Endothelial function was assessed noninvasively as flow-mediated vasodilatation of the brachial artery. The measurements, expressed as percentages of arterial diameter change compared with the basal recording, were made 1 minute after releasing the blood flow arrest imposed by forearm arterial occlusion. Individual measurements are shown in Figure 4, and mean values and statistical analysis in Table I. [26].

Endothelial function was significantly reduced in the occidental diet compared with the Mediterranean diet ($P = 0.014$). After the period with wine this difference disappeared. In fact, in the occidental group a complete loss of vascular reactivity was observed, which reappeared with the addition of wine to the diet. Remarkably, after the addition of wine, both groups showed the same, normal endothelial function values.

The limited number of analyses and the

Table I. Effects of an occidental diet, Mediterranean diet, and wine supplementation on endothelial function. (Reproduced from Cuevas et al [26].)

	Brachial artery flow-mediated diameter change (%)	
	Without wine	With wine
Occidental diet	-2.9 ± 2.1^a	6.6 ± 2.2^b
Mediterranean diet	3.1 ± 3.9^c	5.8 ± 4.6^d

Results are mean \pm SD.
^a vs ^c, $P = 0.014$; ^b vs ^d, $P = \text{NS}$; ^a vs ^b, $P = 0.001$; ^c vs ^d, $P = \text{NS}$.

variability of the detection procedure do not allow a definitive conclusion on the effect of wine on endothelial function in volunteers following a Mediterranean diet. Nevertheless, the results suggest that both a Mediterranean diet and wine preserve or increase endothelial function, while they clearly show that an occidental diet suppresses flow-mediated vascular reactivity, in a process fully reversed by wine supplementation.

Correlations between oxidative damage and plasma antioxidants

As stated above, oxidative damage is the consequence of an imbalance between pro-oxidant reactions, mediated by reactive oxygen and nitrogen species, and antioxidants or antioxidant activity present in the body. In several intervention studies, we evaluated oxidative damage using biomarkers such as 8-OHdG in human DNA leukocytes, plasma TBARS, and 7 β -hydroxycholesterol and correlated the results with various plasma antioxidants [31]. The results shown in Table II illustrate that the three biomarkers for oxidative

damage behave in a similar fashion: 8-OHdG content in leukocyte DNA correlates significantly with plasma TBARS and plasma 7 β -hydroxycholesterol (0.215, $P < 0.002$, and 0.152, $P < 0.012$, respectively). Also shown in Table II is the negative correlation between 8-OHdG in leukocytes and each of the plasma antioxidants measured. The Pearson correlation coefficients between the level of 8-OHdG in leukocytes and plasma polyphenol antioxidants was -0.448 ($P < 0.001$), the highest value; followed by β -carotene, -0.425 ($P < 0.001$); lycopene, -0.362 ($P < 0.001$); ubiquinol, -0.336 ($P < 0.001$); vitamin C, -0.236 ($P < 0.001$); and vitamin E, -0.158 ($P < 0.003$).

The plasma polyphenol antioxidants correspond to the sum of catechin, protocatechuic acid, and gallic acid, and appear to be at least as effective as the other antioxidants. Interestingly, urine total polyphenol content is also a good indicator of antioxidant capacity. Vegetables, fruit, and red wine supplied most of the polyphenols measured in plasma. In urine, polyphenols originate both from endogenous and exogenous sources. The total plasma antioxidant capacity values measured as TAR and TRAP also have a significant negative correlation with leukocyte 8-OHdG, a result that validates their use as indicators of antioxidant capacity. These results also support the validity of the biomarkers employed to assess oxidative damage in humans; yet in our hands, 8-OHdG was the most reliable. ■

Table II. Correlation of 8-OHdG with oxidative damage, plasma antioxidants, and antioxidant capacity. Pooled data from several intervention studies. (Reproduced from Perez et al [31].)

	R	P	n
TBARS	0.215	0.002	211
7 β -Hydroxycholesterol	0.152	0.012	269
Vitamin C	-0.236	<0.001	363
Vitamin E	-0.158	0.003	363
β -Carotene	-0.425	<0.001	363
Lycopene	-0.362	<0.001	363
Ubiquinol	-0.336	<0.001	363
Catechin + protocatechuic acid + gallic acid	-0.448	<0.001	266
Total polyphenol urine	-0.183	0.001	348
TAR	-0.113	0.032	363
TRAP	-0.240	<0.001	363

REFERENCES

1. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc Natl Acad Sci USA*. 1993;90:7915-7922.
2. Steinberg D. Antioxidant vitamins and coronary heart disease. *N Engl J Med*. 1993;328:1487-1489.
3. Halliwell B. Antioxidants and human disease: a general introduction. *Nutr Rev*. 1997;55:S44-S49.
4. Kromhout D, Keys A, Arsvanian C, et al. Food consumption patterns in the 1960s in seven countries. *Am J Clin Nutr*. 1989;49:889-894.
5. Ferro-Luzzi A, Branca F. Mediterranean diet, Italian-style: prototype of a healthy diet. *Am J Clin Nutr*. 1995;61:1338S-1345S.

6. Renaud S, de Lorgeril M, Delaye J, et al. Cretan Mediterranean diet for prevention of coronary heart disease. *Am J Clin Nutr.* 1995;6:1360S–1367S.
7. Simopoulos A. The Mediterranean diets: what is so special about the diet of Greece? The scientific evidence. *J Nutr.* 2001;131:3065S–3073S.
8. Berrino F. Western diet and Alzheimer's disease. *Epidemiol Prev.* 2002;26:107–115.
9. Buzina R, Suboticanec K, Saric M. Diet patterns and health problems: diet in southern Europe. *Ann Nutr Metab.* 1991;35(suppl 1):32–40.
10. de Wilde M, Farkas E, Gerrits M, et al. The effect of n-3 polyunsaturated fatty acid-rich diets on cognitive and cerebrovascular parameters in chronic cerebral hypoperfusion. *Brain Res.* 2002;947:166.
11. Hallgren CG, Hallmans G, Jansson JH, et al. Markers of high fish intake are associated with decreased risk of a first myocardial infarction. *Br J Nutr.* 2001;86:397–404.
12. Satia-Abouta J, Patterson RE, Neuhouser ML, Elder J. Dietary acculturation: applications to nutrition research and dietetics. *J Am Diet Assoc.* 2002;102:1105–1118.
13. Rimm EB, Ascherio A, Giovannucci E, Spiegelman D, Stampfer MJ, Willet WC. Vegetable, fruit, and cereal fiber intake and risk of coronary heart disease among men. *JAMA.* 1996;275:447–451.
14. Steinmetz KA, Potter JD. Vegetables, fruit, and cancer prevention: a review. *J Am Diet Assoc.* 1996;96:1027–1039.
15. Willett WC. Nutrition and cancer. *Salud Publ Mex.* 1997;39:298–309.
16. Renaud S, de Lorgeril M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet.* 1992;339:1523–1526.
17. Renaud S, Ruf JC. The French paradox: vegetables or wine. *Circulation.* 1994;90:3118–3119.
18. Halliwell B. Can oxidative DNA damage be used as a biomarker of cancer risk in humans? Problems, resolutions and preliminary results from nutritional supplementation studies. *Free Radic Res.* 1998;29:469–486.
19. Ehara S, Ueda M, Naruko T, et al. Elevated levels of oxidized low density lipoprotein show a positive relationship with the severity of acute coronary syndromes. *Circulation.* 2001;103:1955–1960.
20. Mata P, Alonso R, López-Farré JM, et al. Effect of dietary fat saturation on LDL oxidation and monocyte adhesion to human endothelial cells in vitro. *Arterioscler Thromb Vasc Biol.* 1996;16:1347–1355.
21. Steinberg D. Low density lipoprotein oxidation and its pathobiological significance. *J Biol Chem.* 1997;272:20963–20966.
22. Natella F, Beelli F, Gentili V, et al. Grape seed proanthocyanidins prevent plasma postprandial oxidative stress in humans. *J Agric Food Chem.* 2002;50:7720–7725.
23. Bennett M. Reactive oxygen species and death. Oxidative DNA damage in atherosclerosis. *Circ Res.* 2001;88:648–650.
24. Martinet W, Knaapen MW, De Meyer GR, Herman AG, Kockx MM. Oxidative DNA damage and repair in experimental atherosclerosis are reversed by dietary lipid lowering. *Circ Res.* 2001;88:733–739.
25. Aikawa M, Sugiyama S, Hill C, et al. Lipid lowering reduces oxidative stress and endothelial cell activation in rabbit atheroma. *Circulation.* 2002;106:1390–1396.
26. Cuevas AM, Guasch V, Castillo O, et al. A high-fat diet induces and red wine counteracts endothelial dysfunction in human volunteers. *Lipids.* 2000;35:143–148.
27. Heitzer T, Schlinzig T, Krohn K, et al. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation.* 2001;104:2673–2678.
28. Landmesser U, Harrison D. Oxidant stress as a marker for cardiovascular events. Ox marks the spot. *Circulation.* 2001;104:2638–2640.
29. Moller P, Loft S. Oxidative DNA damage in human white blood cells in dietary antioxidant intervention studies. *Am J Clin Nutr.* 2002;76:303–310.
30. Leighton F, Cuevas A, Guasch V, et al. Plasma polyphenols and antioxidants, oxidative DNA damage, and endothelial function, in a diet and wine intervention study in humans. *Drugs Exp Clin Res.* 1999;25:133–141.
31. Perez DD, Strobel P, Foncea R, et al. Wine, diet, antioxidant defenses, and oxidative damage. *Ann N Y Acad Sci.* 2002; 957: 136–145.
32. Salonen JT. Markers of oxidative damage and antioxidant protection: assessment of LDL oxidation. *Free Radic Res.* 2000;33:S41–S46.
33. Motchnick PA, Frei B, Ames B. Measurement of antioxidants in human blood plasma. *Methods Enzymol.* 1994;234:269–279.
34. Lissi EA, Salim-Hanna M, Pascual C, Del Castillo MD. Evaluation of total antioxidant potential (TRAP) and total antioxidant reactivity from lumino-enhanced chemiluminescence measurements. *Free Radic Biol Med.* 1995;18:153–158.
35. Adams MR, Kinlay S, Blake CJ, et al. Atherogenic lipids and endothelial dysfunction: mechanisms in the genesis of ischemic syndromes. *Annu Rev Med.* 2000;51:149–167.

Manipulation of cardiac metabolism and oxidative stress

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Abstract

Oxidative stress, during postischemic reperfusion for example, increases the generation of reactive oxygen species and alters the defense mechanisms against free radicals. Reactive oxygen species appear to be the mediators of myocardial stunning that accompanies reperfusion and are involved in heart failure progression. Clinical and experimental data suggest that trimetazidine, a metabolic agent with anti-ischemic properties, is able to reduce the accumulation of free radicals in patients with ischemic heart disease and heart failure. It has proven cytoprotective effects when administered before coronary angiography due to an indirect antioxidative effect.

■ *Heart Metab.* 2003;19:30–31

Keywords: Oxidative stress, reactive oxygen species, reperfusion tissue injury, ischemic heart disease, trimetazidine, cytoprotection, antioxidative effect

Cardiomyocytes suppress contraction and oxygen consumption during hypoxia. Oxidative stress increases the generation of reactive oxygen species by altering mitochondrial reactions. During postischemic reperfusion, for example, reactive oxygen species are formed at an accelerated rate and the defense mechanisms against oxygen free radicals are also altered.

Exposure of myocardial cellular components to exogenous reactive oxygen species could lead to cellular dysfunction and necrosis.

There is strong evidence that reactive oxygen species are mediators of the reversible ventricular dysfunction (stunning) that often accompanies reperfusion [1]. The widespread introduction of fibrinolysis and PTCA in the treatment of myocardial infarction has changed the outlook of modern cardiology, but it also raises new problems. One is the occurrence of extensive tissue injury caused by reperfusion, with the generation of oxygen free radicals.

Trimetazidine (Vastarel 20 mg) is a well-established anti-ischemic drug belonging to a new class of metabolic agents known as 3-ketoacyl-CoA-thiolase inhibitors. It has been used for the treatment of conditions related to the generation of reactive oxygen species. The ability of trimetazidine to protect low-density lipoproteins from oxidation, and cultured cells from H₂O₂-induced DNA damage, has been investigated and the results indicate that this agent can modulate the action of oxidants in different systems [2]. In one study performed in patients with ischemic heart disease who underwent coronary angiography, it was found that this intervention provokes oxidative stress and membrane-destructive processes. Trimetazidine given 10 days before the procedure was shown to produce a cytoprotective effect [3].

Evidence that trimetazidine has antioxidant effects is suggested by reduced accumulation of free radicals in experimental conditions of high oxidative stress. Clinical trial data con-

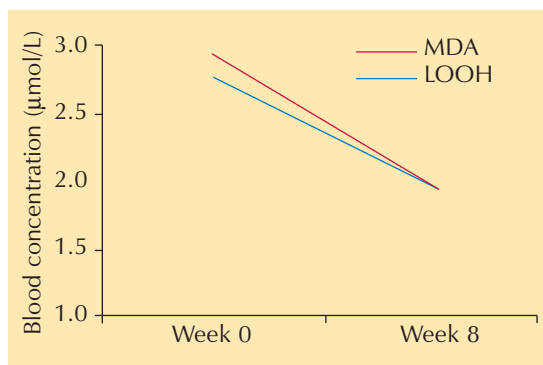


Figure 1. Reduction in lipid peroxidation products after 8 weeks' treatment with trimetazidine.

cluded that 1 month's therapy with trimetazidine significantly decreased the content of free radical oxidation products in patients with ischemic heart disease. These data suggest that trimetazidine's antioxidant effect is indirectly mediated via activation of antioxidant enzymes, which diminishes the tissue damage caused by ischemia [4].

Interestingly, chronic release of reactive oxygen species has recently been limited to heart failure progression [5]. The release of reactive oxygen species appears to derive from the nonphagocytic (reduced) nicotinamide adenine dinucleotide phosphate oxidase and mitochondria. Fibrosis, collagen deposition and metalloprotease activation involved in the progression of heart failure are dependent on the release of reactive oxygen species. A study conducted by Belardinelli et al [6] aimed to assess the antioxidant effects of trimetazidine in patients with documented coronary artery disease and left ventricular systolic dysfunction. Lipid peroxidation product malonyldialdehyde (MDA) and lipid hydroperoxides (LOOH) were measured, and endothelium-dependent and -independent vasodilatation of

radical artery was determined on study entry and after 4 weeks' treatment with trimetazidine or placebo. The findings of the study suggest that trimetazidine reduces both plasma MDA and LOOH levels (Figure 1) along with endothelial dysfunction and improves functional capacity in patients with chronic heart failure. These benefits are likely to be linked to the antioxidant properties of trimetazidine.

The therapeutic potential of free radical-directed drugs in heart disease has not been fully investigated. Due to its specific metabolic mode of action free of any hemodynamic impact and its excellent tolerance, trimetazidine appears to be an interesting therapeutic option to protect tissues from ischemia-induced oxidative stress. ■

REFERENCES

1. Ferrari R, Agnoletti L, Camini L, et al. Oxidative stress during myocardial ischaemia and heart failure. *Eur Heart J*. 1998;suppl 19B:B2-B11.
2. Tselepis A, Doulias P, Lourida E, et al. Trimetazidine protects low-density lipoproteins from oxidation and cultured cells exposed to H₂O₂ from DNA damage. *Free Radic Biol Med*. 2001;30:1357-1364.
3. Logacheva IV, Leschinskii LA, Romanova ZD, et al. Use of antioxidants and trimetazidine in preparation of patients with ischaemic heart disease for coronary angiography. *Klin Med (Mosk)*. 2001;79:30-33.
4. Tikhaze AK, Lankin VZ, Zharova EA, Kolycheva SV. Trimetazidine as indirect antioxidant. *Bull Exp Biol Med*. 2000;130:951-953.
5. Sorescu D, Griendling KK. Reactive oxygen species, mitochondria and NAD(P)H oxidases in the development and progression of heart failure. *Congest Heart Fail*. 2002;8:132-140.
6. Belardinelli R, Solenghi M, Volpe L. Effects of trimetazidine on endothelial dysfunction on chronic heart failure: an antioxidant effect? *Circulation*. 2001;104(17 suppl):II-337.

Effects of xanthine oxidase inhibition with allopurinol in chronic heart failure

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Abstract

Patients with chronic heart failure (CHF) are characterized by impaired peripheral blood flow and reduced vasodilator capacity. These features are closely related to prominent clinical symptoms such as reduced exercise capacity and early muscle fatigue. Indeed, the symptoms relate more closely to peripheral than to central hemodynamic abnormalities. An important role of the endothelium has been recognized in the regulation of vascular tone and tissue perfusion, and, accordingly, endothelial function and vasodilator reactivity to exercise have been shown to be significantly impaired in patients with CHF. One major factor responsible for the impaired regulation of vascular tone in CHF is the reduced bioavailability of nitric oxide resulting from accelerated degradation of nitric oxide by free oxygen radicals. An important source of increased free oxygen radical load in CHF has been recognized in the enzyme xanthine oxidase. Abnormalities of the xanthine oxidase metabolic pathway may be viewed in the context of derangements within a complex metabolic web. This review will focus on the role of xanthine oxidase as an oxygen radical-generating enzyme and the final step of purine degradation resulting in the formation of uric acid. First results indicating a potential benefit of therapeutically targeting xanthine oxidase in CHF (with allopurinol) will be discussed. ■ *Heart Metab.* 2003;19:32–39.

Keywords: Chronic heart failure, xanthine oxidase, free oxygen radicals, vasodilator capacity, allopurinol, insulin resistance, metabolism

Significance of the xanthine oxidase metabolic pathway in chronic heart failure (CHF)

In humans, uric acid forms the metabolic endpoint of purine degradation. The last metabolic steps in this process (from hypoxanthine to

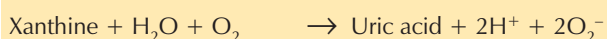
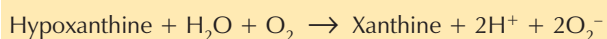
xanthine and from xanthine to uric acid) are promoted by the enzyme xanthine oxidoreductase (EC1.1.3.22). This enzyme is a flavo-protein that contains both iron and molybdenum and uses NAD⁺ as electron acceptor. It exists in two interconvertible forms, xanthine dehydrogenase and xanthine oxidase. In its oxidase form, the enzyme transfers the reducing equivalent generated by oxidation of substrates to molecular oxygen with the resultant production of superoxide anion and hydrogen peroxide (Figure 1). Hydrogen peroxide can

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Case report

Xanthine oxidase inhibition with allopurinol in CHF



Hydrogen peroxide can be converted to free hydroxyl radicals:

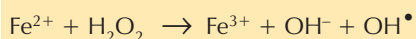


Figure 1. Free oxygen radical production in the xanthine oxidase-mediated reaction from hypoxanthine to xanthine and from xanthine to uric acid.

be converted to free hydroxyl radicals.

The generation of free oxygen radicals by xanthine oxidase may have an important pathophysiological role in tissues and in vascular regulation in the setting of chronic heart failure (CHF). It is of interest that in 1968 the cytosolic xanthine oxidase was the first documented putative biological generator of oxygen-derived free radicals [1]. Since then it has been established that xanthine oxidase is a major source of free oxygen radical production in the human body [2–4]. This metabolic pathway is of particular significance in conditions of tissue hypoxia and ischemia-reperfusion [5], as increased degradation of adenosine triphosphate via adenosine leads to an increased substrate load for xanthine oxidase [6]. Accordingly, elevation of serum uric acid has been observed in hypoxic states, such as obstructive pulmonary disease [7], neonatal hypoxia [8, 9], cyanotic heart disease [10, 11], and acute heart failure [12]. Uric acid levels have been shown to increase also in the coronary sinus following consecutive balloon inflations during angioplasty [13, 14] and during coronary bypass operations [15]. Simultaneously, in ischemia-hypoxia, xanthine dehydrogenase is increasingly converted to xanthine oxidase, which further adds to accelerated radical production [2, 16]. In CHF, elevated uric acid levels might therefore be expected,

since patients with CHF have impaired uptake of oxygen at rest and during exercise.

In CHF, hyperuricemia is a consistent finding reflecting impaired oxidative metabolism [17, 18]. High serum uric acid levels indicate the degree of xanthine oxidase activation in CHF [19] and occur independently of the effects of diuretics and renal dysfunction [17]. Indirect measurement of endothelium-bound xanthine oxidase has shown increased xanthine oxidase enzyme activity in CHF compared with healthy control subjects [20]. We can therefore conclude that in patients with CHF there is an increased free radical oxygen load [21–23].

In CHF, endothelial dysfunction and reduced vasodilator capacity are consistent findings that relate closely to prominent clinical symptoms such as reduced exercise capacity and early muscle fatigue [24, 25]. Decreased perfusion of skeletal muscle in CHF is neither primarily related to central hemodynamic abnormalities [26, 27] nor to arterial hypotension [28] but, more importantly, to endothelial dysfunction [29] and inflammation [30]. Increased oxidative stress is seen as one major factor responsible for the impaired regulation of vascular tone due to its effect of diminishing vasoactive nitric oxide [31, 32]. Xanthine oxidase-generated free oxygen radicals interact with endothelium-derived nitric oxide to form peroxynitrite (in itself a highly active oxygen radical), starting a cascade of detrimental oxygen radical effects (Figure 2). Endothelial dysfunction has been shown to be related to increased scavenging, ie, degradation, of nitric oxide by free oxygen radicals rather than impaired generation of nitric oxide [3]. It should be noted that in humans the tissue with the highest activity of xanthine oxidase (beside the epithelium of the mammary gland) is the capillary endothelium and the endothelium of the small arteries [33, 34].

There is increasing evidence to suggest that the xanthine oxidase metabolic pathway is not merely the final step in purine degradation, with the formation of uric acid as a metabolically inert waste product. In humans the organs with the highest xanthine oxidase

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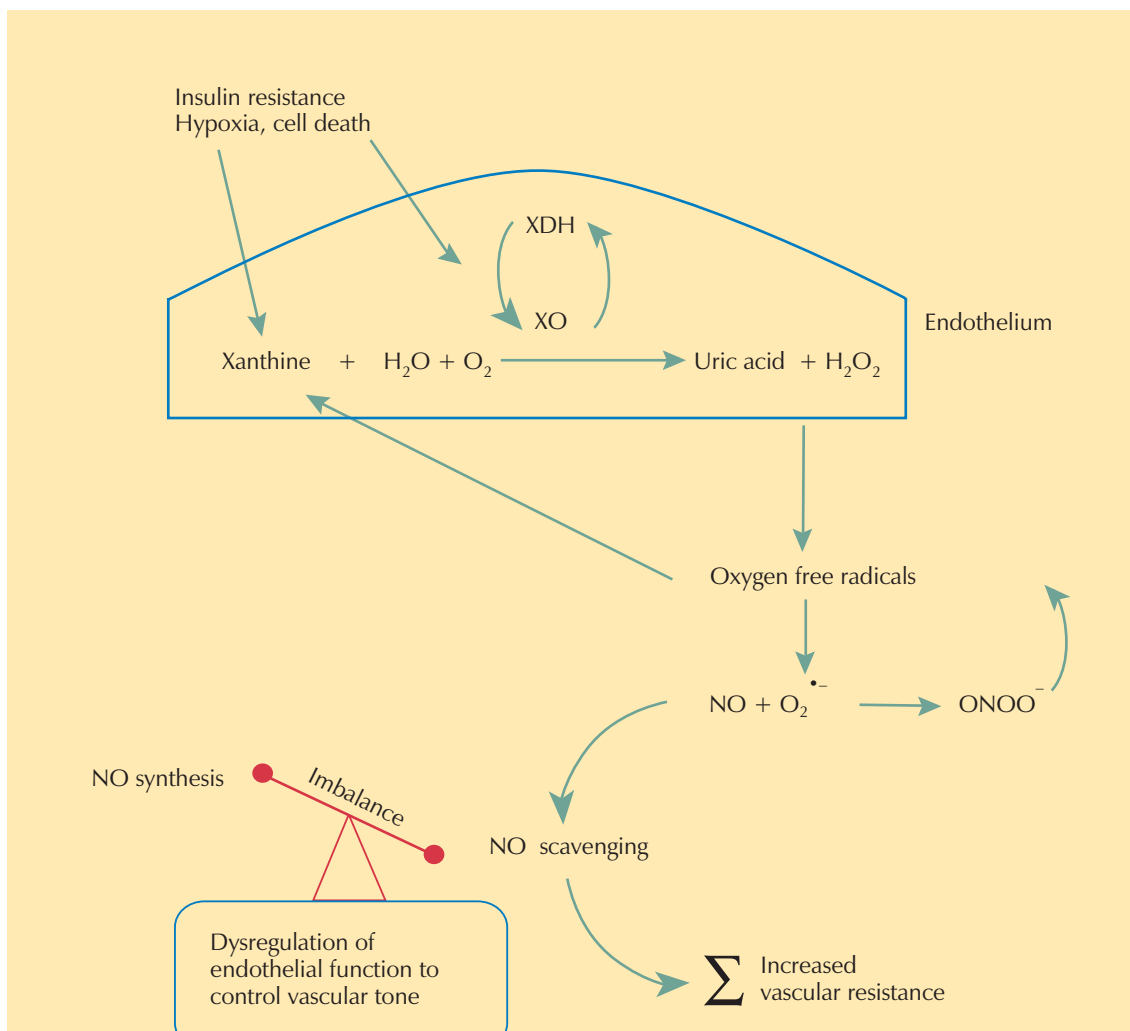


Figure 2. Effects of xanthine oxidase-derived free oxygen radicals on endothelial function. The capillary endothelium is one of the tissues with the highest activity of xanthine oxidase (XO). In CHF, several conditions contribute to increased XO-derived free oxygen radical production. Hypoxia and insulin resistance result in increased substrate supply for the enzyme XO. The balance of the XO/xanthine dehydrogenase (XDH) system is shifted towards increased XO activity. Superoxide anions react with endothelium-derived nitric oxide (NO) to form peroxynitrite (ONOO⁻) thus initiating a cascade of detrimental oxygen radical effects. Due to NO scavenging, NO-mediated regulation of vascular tone is impaired.

activity are the intestine and the liver, with low or undetectable levels in the brain, kidney, lung, and muscle [35]. The localization of xanthine oxidase primarily in the endothelial cells of the capillaries suggests that it is involved in specific functions of the vascular system [33]. Given the capacity to generate free oxygen radicals, this enzyme might have a role in bactericidal defence mechanisms [36, 37], especially at the barrier between

intestinal lumen and body tissues. This physiologic mechanism may provide an acute adaptive response to environmental factors. One could hypothesize, however, that long-term stimulation of xanthine oxidase may result in chronic activation of this mechanism leading to maladaptive processes and eventually damaging effects. The latter provides the pathophysiologic link between uric acid generation and a large variety of detrimental processes,

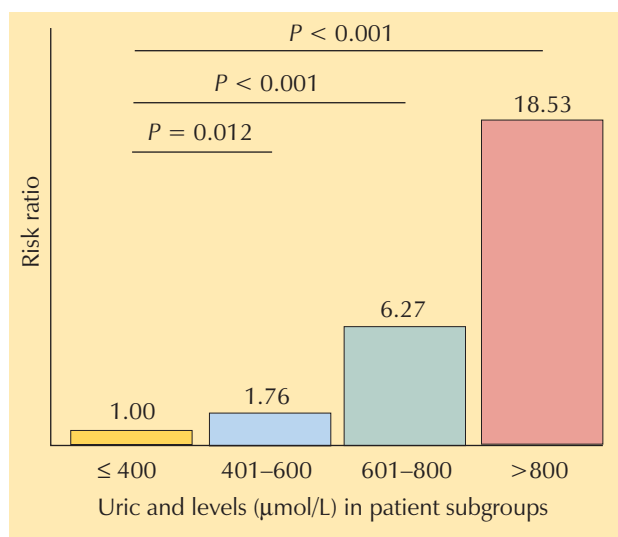


Figure 3. Risk ratios in CHF subgroups with elevated uric acid levels. A graded relationship is shown between serum uric acid levels and survival in 294 patients with CHF; risk ratios are compared with uric acid ≤ 400 $\mu\text{mol/L}$ (unpublished data, proceedings of the Working Group on Myocardial Function of the ESC, Isola, 2003).

including increased cytokine production, cell apoptosis, and endothelial dysfunction, all of which occur in CHF patients [38–40]. Indeed, in a prospective series of studies of patients with CHF, it has been shown that hyperuricemia is a marker of impaired oxidative metabolism and hyperinsulinemia [17], inflammatory cytokine activation [41], and impaired vascular function [42].

The option to therapeutically target raised xanthine oxidase activity in CHF might therefore be an intriguing concept in order to counteract maladaptive chronic upregulation of the xanthine oxidase metabolic pathway. In fact, it has been shown that in CHF patients with hyperuricemia, treatment with the xanthine oxidase inhibitor allopurinol improved endothelial function and peripheral blood flow [43, 44], while markers of free oxygen radical generation were reduced [43]. In a placebo-controlled, randomized, double-blind, crossover study we demonstrated that allopurinol (100 mg once daily), after just 1 week of treatment, reduced uric acid levels by 39% while vasodilator capacity improved in

the arm and leg vascular bed by 24% and 23%, respectively [43]. It was found that the treatment-induced reduction of uric acid significantly correlated with the improvement of vasodilator capacity. This might raise the possibility that, independently of the oxygen radicals, uric acid itself may have an adverse effect on the regulation of peripheral vascular tone. This is supported by the finding that high uric acid levels in CHF predict impaired peripheral blood flow and reduced vasodilator capacity [45], independently of renal dysfunction, diuretic dose, and CHF etiology. It has been shown that allopurinol treatment can improve forearm blood flow and endothelial dysfunction also in other conditions such as in type 2 diabetes mellitus and mild hypertension [46]. In the context of reperfusion injury, it is understood that xanthine oxidase-derived free oxygen radicals are a major contributor to impaired blood flow and tissue damage, and that allopurinol may exert protective effects against these reperfusion injuries [47].

Beside its role in affecting peripheral vascular tone, inhibition of xanthine oxidase appears to exert direct myocardial effects in CHF. In animal models, allopurinol reduces myocardial oxygen consumption [48] and improves systolic function [49, 50], resulting in increased myocardial energetic efficiency. Recently, this has been confirmed in human CHF [51]. Although the underlying mechanism is not yet fully uncovered, some authors have suggested a specific effect of allopurinol in sensitizing cardiac myofilaments to Ca^{2+} [52].

Hyperuricemia as a novel prognostic marker

In addition to its involvement in the regulation of vascular tone, there is increasing evidence to suggest that the xanthine oxidase metabolic pathway has prognostic significance. Recently, it has been shown that in CHF patients high uric acid levels are a predictor of impaired survival, independently of and better than other well-established parameters such as clinical status, exercise capacity, parameters of

kidney function, and diuretic therapy [53]. Data have indicated a stepwise increase of mortality risk in parallel with rising uric acid levels (Figure 3).

This is in line with the findings of a recent retrospective study that examined the effect of allopurinol in CHF on mortality and hospitalization [54]. It was observed that in these patients long-term high-dose allopurinol (≥ 300 mg/day) may be associated with better all-cause mortality (adjusted relative risk 0.59, 95% CI 0.37–0.95, $P < 0.05$) than low-dose allopurinol (< 300 mg/day), assuming a dose-related effect of allopurinol. Treatment with allopurinol is, however, not free of problems. It can induce gout attacks, kidney dysfunction, or skin reactions, and further work is required to confirm the potential benefit of this treatment.

The xanthine oxidase metabolic pathway as part of a complex metabolic web

Abnormal regulation of the xanthine oxidase pathway may be seen in the context of more complex metabolic derangements. Epidemiologic studies have documented an increase in serum uric acid as a characteristic finding in patients with obesity and hypertension [55–57], hypertriglyceridemia [58], and coronary artery disease [59, 60]. The metabolic syndrome is a constant etiologic factor in these diseases and, indeed, recent studies have identified a significant relation between hyperuricemia and insulin resistance — the widely accepted causative factor of the metabolic syndrome [56, 61, 62]. The relationship between hyperuricemia and insulin resistance persisted when differences in age, sex, body mass index, and waist-to-hip ratio were taken into account, and were also seen in normal healthy individuals [63, 64]. Based on these data, an expanded definition of the insulin resistance syndrome was suggested that added hyperuricemia to the cluster of metabolic abnormalities comprising the syndrome [65].

The pathophysiologic connection of hyperuricemia and the insulin resistance syndrome

has been hypothesized to result from the accumulation and diversion of glycolytic intermediates towards the pentose phosphate pathway [66].

Its increased metabolic turnover results in accumulation of phosphoribosylpyrophosphate [67], in itself a key precursor of the purine de novo synthesis. Increased substrate supply is followed by upregulated purine metabolism and hence purine production. One branch point for diversion of glycolytic intermediates towards the pentose phosphate pathway is controlled by the enzyme glyceraldehyde 3-phosphate dehydrogenase (GA3PDH). This enzyme catalyzes the only oxidative step in glycolysis and its activity is regulated by insulin [68, 69]. Thus the link between insulin resistance and uric acid production could be mediated by impairment of GA3PDH. Although further work is needed to fully uncover the etiological relationship between hyperuricemia and insulin resistance, the significance of high uric acid levels as an additional risk factor within the metabolic syndrome has repeatedly been confirmed [70–73].

Summary

The traditional view of CHF as a mere pumping disorder has shifted to a more complex approach including hormonal, immune, and metabolic aspects, and secondary changes. Increasing data suggest that the xanthine oxidase metabolic pathway is a significant contributor to the pathophysiology of CHF, with both symptomatic and prognostic implications.

Preliminary results suggest a beneficial effect of lowering uric acid by xanthine oxidase inhibition. Whether one could also use uricosuric treatments (to increase excretion of uric acid) or newer, more selective xanthine oxidase inhibitors potentially with fewer side effects in chronic or acute heart failure needs to be established in further studies. ■

REFERENCES

- McCord JM, Fridovich I. The reduction of cytochrome c by milk xanthine oxidase. *J Biol Chem.* 1968;243:5753–5760.
- Terada LS, Guidot DM, Leff JA, et al. Hypoxia injures endothelial cells by increasing endogenous xanthine oxidase activity. *Proc Natl Acad Sci USA.* 1992;89:3362–3366.
- Harrison DG. Cellular and molecular mechanisms of endothelial cell dysfunction. *J Clin Invest.* 1997;100:2153–2157.
- Cai H, Harrison DG. Endothelial dysfunction in cardiovascular disease. *Circ Res.* 1997;100:2153–2157.
- Zweier JL, Kuppusamy P, Lutty GA. Measurement of endothelial cell free radical generation: evidence for a central mechanism of free radical injury in postischemic tissues. *Proc Natl Acad Sci USA.* 1988;85:4046–4050.
- McCord JM, Roy RS. The pathophysiology of superoxide: roles in inflammation and ischemia. *Can J Physiol Pharmacol.* 1982;60:1346–1352.
- Braghiroli A, Sacco C, Erbetta M, Ruga V, Donner CF. Overnight urinary uric acid:creatinine ratio for detection of sleep hypoxemia. Validation study in chronic obstructive pulmonary disease and obstructive sleep apnea before and after treatment with nasal continuous positive airway pressure. *Am Rev Respir Dis.* 1993;148:173–178.
- Toguzov RT, Demin VF, Produkin VY, Tikhonov YV, Pimenov AM. Influence of perinatal hypoxia on purine contents in erythrocytes of newborn infants. *Biomed Biochim Acta.* 1989;48:S279–S281.
- Porter KB, O'Brien WF, Benoit R. Comparison of cord purine metabolites to maternal and neonatal variables of hypoxia. *Obstet Gynecol.* 1992;79:394–397.
- Hayabuchi Y, Matsuoka S, Akita H, Kuroda Y. Hyperuricaemia in cyanotic congenital heart disease. *Eur J Pediatr.* 1993;152:873–876.
- Marro PJ, Baumgart S, Delivoria-Papadopoulos M, et al. Purine metabolism and inhibition of xanthine oxidase in severely hypoxic neonates going onto extracorporeal membrane oxygenation. *Pediatr Res.* 1997;41:513–520.
- Woolliscroft JO, Colfer H, Fox IH. Hyperuricemia in acute illness: a poor prognostic sign. *Am J Med.* 1982;72:58–62.
- De Jong JW, van der Meer P, Huizer T, Serruys PW, Bos E, Roelandt JR. Does xanthine oxidase cause damage during myocardial ischemia? *Bratisl Lek Listy.* 1991;92:41–47.
- Huizer T, de Jong JW, Nelson JA, et al. Urate production by human heart. *J Mol Cell Cardiol.* 1989;21:691–695.
- Lazzarino G, Raatikainen P, Nuutinen M, et al. Myocardial release of malonyldialdehyde and purine compounds during coronary bypass surgery. *Circulation.* 1994;90:291–297.
- Ashraf M, Samra ZQ. Subcellular distribution of xanthine oxidase during cardiac ischemia and reperfusion: an immunocytochemical study. *J Submicrosc Cytol Pathol.* 1993;25:193–201.
- Leyva F, Anker S, Swan JW, et al. Serum uric acid as an index of impaired oxidative metabolism in chronic heart failure. *Eur Heart J.* 1997;18:858–865.
- Leyva F, Chua TP, Anker SD, Coats AJ. Uric acid in chronic heart failure: a measure of the anaerobic threshold. *Metabolism.* 1998;47:1156–1159.
- Bakhtiarov ZA. Changes in xanthine oxidase activity in patients with circulatory failure [in Russian]. *Ter Arkh.* 1989;61:68–69.
- Landmesser U, Spiekermann S, Dikalov S, et al. Vascular oxidative stress and endothelial dysfunction in patients with chronic heart failure: role of xanthine-oxidase and extracellular superoxide dismutase. *Circulation.* 2002;106:3073–3078.
- Belch JJ, Bridges AB, Scott N, Chopra M. Oxygen free radicals and congestive heart failure. *Br Heart J.* 1991;65:245–248.
- Ghatak A, Brar MJ, Agarwal A, et al. Oxy free radical system in heart failure and therapeutic role of oral vitamin E. *Int J Cardiol.* 1996;57:119–127.
- Keith M, Geranmayegan A, Sole MJ, et al. Increased oxidative stress in patients with congestive heart failure. *J Am Coll Cardiol.* 1998;31:1352–1356.
- Zelis R, Flaim SF. Alterations in vasomotor tone in congestive heart failure. *Prog Cardiovasc Dis.* 1982;24:437–459.
- Anker SD, Swan JW, Volterrani M, et al. The influence of muscle mass, strength, fatigability and blood flow on exercise capacity in cachectic and non-cachectic patients with chronic heart failure. *Eur Heart J.* 1997;18:259–269.
- Franciosa JA, Park M, Levine TB. Lack of correlation between exercise capacity and indexes of resting left ventricular performance in heart failure. *Am J Cardiol.* 1981;47:33–39.
- Myers J, Fröhlicher VF. Hemodynamic determinants of exercise capacity in chronic heart failure. *Ann Intern Med.* 1991;115:377–386.
- Sullivan MJ, Knight JD, Higginbotham MB, Cobb FR. Relation between central and peripheral hemodynamics during exercise in patients with chronic heart failure. Muscle blood flow is reduced with maintenance of arterial perfusion pressure. *Circulation.* 1989;80:769–781.
- Drexler H, Hayoz D, Munzel T, et al. Endothelial function in chronic congestive heart failure. *Am J Cardiol.* 1992;69:1596–1601.
- Anker SD, Volterrani M, Egerer KR, et al. Tumour necrosis factor alpha as a predictor of impaired peak leg blood flow in patients with chronic heart failure. *Q J Med.* 1998;91:199–203.
- Keaney JF Jr, Vita JA. Atherosclerosis, oxidative stress, and antioxidant protection in endothelium-derived relaxing factor action. *Prog Cardiovasc Dis.* 1995;38:129–154.
- Indik JH, Goldman S, Gaballa MA. Oxidative stress contributes to vascular endothelial dysfunction in

- heart failure. *Am J Physiol Heart Circ Physiol*. 2001;281:H1767–H1770.
33. Jarasch ED, Grund C, Bruder G, Heid HW, Keenan TW, Franke WW. Localization of xanthine oxidase in mammary-gland epithelium and capillary endothelium. *Cell*. 1981;25:67–82.
 34. Jarasch ED, Bruder G, Heid HW. Significance of xanthine oxidase in capillary endothelial cells. *Acta Physiol Scand Suppl*. 1986;548:39–46.
 35. Sarnesto A, Linder N, Raivio KO. Organ distribution and molecular forms of human xanthine dehydrogenase/xanthine oxidase protein. *Lab Invest*. 1996;74:48–56.
 36. Tubaro E, Lotti B, Cavallo G, Croce C, Borelli G. Liver xanthine oxidase increase in mice in three pathological models. A possible defence mechanism. *Biochem Pharmacol*. 1980;29:1939–1943.
 37. McCord JM. The evolution of free radicals and oxidative stress. *Am J Med*. 2000;108:652–659.
 38. Bolger AP, Anker SD. Tumour necrosis factor in chronic heart failure: a peripheral view on pathogenesis, clinical manifestations and therapeutic implications. *Drugs*. 2000;60:1245–1257.
 39. Adams V, Jiang H, Yu J, et al. Apoptosis in skeletal myocytes of patients with chronic heart failure is associated with exercise intolerance. *J Am Coll Cardiol*. 1999;33:959–965.
 40. Hornig B, Maier V, Drexler H. Physical training improves endothelial function in patients with chronic heart failure. *Circulation*. 1996;93:210–214.
 41. Leyva F, Anker SD, Godsland I, et al. Uric acid in chronic heart failure: a marker of chronic inflammation. *Eur Heart J*. 1998;19:1814–1822.
 42. Anker SD, Leyva F, Poole-Wilson PA, Kox WJ, Stevenson JC, Coats AJ. Relation between serum uric acid and lower limb blood flow in patients with chronic heart failure. *Heart*. 1997;78:39–43.
 43. Doehner W, Schoene N, Rauchhaus M, et al. The effects of xanthine oxidase inhibition with allopurinol on endothelial function and peripheral blood flow in hyperuricemic patients with chronic heart failure — results from two placebo controlled studies. *Circulation*. 2002;105:2619–2624.
 44. Farquharson CA, Butler R, Hill A, Belch JJ, Struthers AD. Allopurinol improves endothelial dysfunction in chronic heart failure. *Circulation*. 2002;106:221–226.
 45. Doehner W, Rauchhaus M, Florea VG, et al. Uric acid in cachectic and non-cachectic CHF patients — relation to leg vascular resistance. *Am Heart J*. 2001;141:792–799.
 46. Butler R, Morris AD, Belch JJ, et al. Allopurinol normalizes endothelial dysfunction in type 2 diabetics with mild hypertension. *Hypertension*. 2000;35:746–751.
 47. Coghlan JG, Flitter WD, Clutton SM, et al. Allopurinol pretreatment improves postoperative recovery and reduces lipid peroxidation in patients undergoing coronary artery bypass grafting. *J Thorac Cardiovasc Surg*. 1994;107:248–256.
 48. Ekelund UE, Harrison RW, Shokek O, et al. Intravenous allopurinol decreases myocardial oxygen consumption and increases mechanical efficiency in dogs with pacing-induced heart failure. *Circ Res*. 1999;85:437–445.
 49. Ukai T, Cheng CP, Tachibana H, et al. Allopurinol enhances the contractile response to dobutamine and exercise in dogs with pacing-induced heart failure. *Circulation*. 2001;103:750–755.
 50. Saavedra WF, Paolucci N, St John ME, et al. Imbalance between xanthine oxidase and nitric oxide synthase signaling pathways underlies mechanoeenergetic uncoupling in the failing heart. *Circ Res*. 2002;90:297–304.
 51. Cappola TP, Kass DA, Nelson GS, et al. Allopurinol improves myocardial efficiency in patients with idiopathic dilated cardiomyopathy. *Circulation*. 2001;104:2407–2411.
 52. Perez NG, Gao WD, Marban E. Novel myofilament Ca²⁺-sensitizing property of xanthine oxidase inhibitors. *Circ Res*. 1998;83:423–430.
 53. Anker SD, Doehner W, Rauchhaus M, et al. Uric acid and survival in chronic heart failure: validation and application in metabolic, functional and hemodynamic staging. *Circulation* 2003 (in press).
 54. Struthers AD, Donnan PT, Lindsay P, McNaughton D, Broomhall J, MacDonald TM. Effect of allopurinol on mortality and hospitalisations in chronic heart failure: a retrospective cohort study. *Heart*. 2002;87:229–234.
 55. Klein R, Klein BE, Cornoni JC, Maready J, Cassel JC, Tyroler HA. Serum uric acid. Its relationship to coronary heart disease risk factors and cardiovascular disease, Evans County, Georgia. *Arch Intern Med*. 1973;132:401–410.
 56. Brand FN, McGee DL, Kannel WB, Stokes J 3rd, Castelli WP. Hyperuricemia as a risk factor of coronary heart disease: The Framingham Study. *Am J Epidemiol*. 1985;121:11–18.
 57. Bonora E, Targher G, Zenere MB, et al. Relationship of uric acid concentration to cardiovascular risk factors in young men. Role of obesity and central fat distribution. The Verona Young Men Atherosclerosis Risk Factors Study. *Int J Obes Relat Metab Disord*. 1996;20:975–980.
 58. Fox IH, John D, DeBruyne S, Dwosh I, Marliss EB. Hyperuricemia and hypertriglyceridemia: metabolic basis for the association. *Metabolism*. 1985;34:741–746.
 59. Myers AR, Epstein FH, Dodge HJ, Mikkelsen WM. The relationship of serum uric acid to risk factors in coronary heart disease. *Am J Med*. 1968;45:520–528.
 60. Yano K, Rhoads G, Kagan A. Epidemiology of serum uric acid among 8000 Japanese-American men in Hawaii. *J Chronic Dis*. 1977;30:171–184.
 61. Persky VW, Dyer AR, Idris-Soven E, et al. Uric acid: a risk factor for coronary heart disease? *Circulation*. 1979;59:969–977.
 62. Lee J, Sparrow D, Vokonas PS, Landsberg L, Weiss ST. Uric acid and coronary heart disease risk: evi-

Case report

Effects of xanthine oxidase inhibition in chronic heart failure

- dence for a role of uric acid in the obesity-insulin resistance syndrome. The Normative Aging Study. *Am J Epidemiol.* 1995;142:288–294.
63. Modan M, Halkin H, Karasik A, Lusky A. Elevated serum uric acid — a facet of hyperinsulinaemia. *Diabetologia.* 1987;30:713–718.
64. Facchini F, Chen YD, Hollenbeck CB, Reaven GM. Relationship between resistance to insulin-mediated glucose uptake, urinary uric acid clearance, and plasma uric acid concentration. *JAMA.* 1991;266:3008–3011.
65. Reaven GM. Role of insulin resistance in human disease (syndrome X):an expanded definition. *Annu Rev Med.* 1993;44:121–131.
66. Leyva F, Wingrove CS, Godsland IF, Stevenson JC. The glycolytic pathway to coronary heart disease: a hypothesis. *Metabolism.* 1998;47:657–662.
67. Kunjara S, Sochor M, Ali SA, Greenbaum AL, McLean P. Hepatic phosphoribosyl pyrophosphate concentration. Regulation by the oxidative pentose phosphate pathway and cellular energy status. *Biochem J.* 1987;244:101–108.
68. Alexander M, Curtis G, Avruch J, Goodman HM. Insulin regulation of protein biosynthesis in differentiated 3T3 adipocytes. Regulation of glyceraldehyde-3-phosphate dehydrogenase. *J Biol Chem.* 1985;260:11978–11985.
69. Alexander MC, Lomanto M, Nasrin N, Ramaika C. Insulin stimulates glyceraldehyde-3-phosphate dehydrogenase gene expression through cis-acting DNA sequences. *Proc Natl Acad Sci USA.* 1988;85:5092–5096.
70. Liese AD, Hense HW, Lowel H, Doring A, Tietze M, Keil U. Association of serum uric acid with all-cause and cardiovascular disease mortality and incident myocardial infarction in the MONICA Augsburg cohort. *Epidemiology.* 1999;10:391–397.
71. Levy WC, Nye RG. Uric acid is an independent predictor of mortality in heart failure — analysis from PRAISE. *Circulation.* 1999;100(suppl):I411–I412.
72. Fang J, Alderman MH. Serum uric acid and cardiovascular mortality the NHANES I epidemiologic follow-up study, 1971–1992. National Health and Nutrition Examination Survey. *JAMA.* 2000;283:2404–2410.
73. Bickel C, Rupprecht HJ, Blankenberg S, et al. Serum uric acid as an independent predictor of mortality in patients with angiographically proven coronary artery disease. *Am J Cardiol.* 2002;89:12–17.

Biochemistry of free radicals

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Abstract

Partial reduction of molecular oxygen can generate reactive oxygen species (ROS), including hydrogen peroxide or singlet oxygen, and the free radicals superoxide and hydroxyl. ROS are constantly formed in the human body and removed by endogenous antioxidants which constitute a primary means of detoxifying ROS and preventing ROS-induced cellular damage. Most of the ROS formed within cells are highly reactive and they are able to oxidise most of the biomolecules within the cell, leading to tissue injury and cell death. Cells have developed an impressive array of antioxidant defences to prevent free radical formation or limit their damaging effects. In particular, these include antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) which scavenge ROS. When these mechanisms of defence fail or are overwhelmed by an excessive production of ROS, oxidative stress occurs, leading to marked biochemical, metabolic, and functional abnormalities. If it is now well recognized that oxidative stress and ROS overproduction may play a role in many disease states, the discovery of the physiological role of nitric oxide $^{\circ}\text{NO}$, a nitrogen-derived radical, in 1987, has led to new and very exciting developments in free radical research. The role of ROS in cellular signal transduction pathways has exploded in recent years suggesting that oxidants are major determinants in a variety of biological functions. ■ *Heart Metab.* 2003;19:40–44.

Keywords: free radicals, oxidative stress, cellular injury

Introduction

In the mid 1950s, a small number of scientists first postulated the role of free radicals in the pathophysiological mechanisms of various types of human disease [3]. However, the importance of free radicals in biological systems was not recognized until the early 1970s. Indeed these reactive chemical species are short-lived species and their detection requires the use of sophisticated techniques. Prior to 1975, owing to the technical difficulty of measuring free radical amounts and of quantitating oxidative dam-

age, it was very difficult to prove that free radicals could contribute to cell pathology. In the early 80s, tools such as EPR spectroscopy or fluorescent probes allowed to study free radical biochemistry and to get useful informations about the nature and consequences of free radical-induced protein and lipid oxidation in vitro as well as in vivo.

Reactive oxygen, free radicals and pathology

Today, it is widely accepted that molecular

oxygen, although essential for maintaining cell viability in living organisms, may also act as one of the primary factors involved in the pathogenesis of several forms of tissue injury, for instance that occurring when ischemic tissues are reperfused ('reperfusion injury'). Nowadays, overproduction of free radicals is considered as a common feature of a large broad of diseases and oxidative stress is generally thought to make a significant contribution to ischemic diseases, drug-induced cardiomyopathy, heart failure, hypertension, inflammatory diseases, cancer, adult respiratory distress syndrome, organ transplantation, neurologic diseases, smoking-related diseases, and AIDS, as well as many others [9]. Moreover oxidative damage is considered to be a major factor in the progressive decline of tissue functions associated with aging [10].

However, in 1987, the discovery of nitric oxide (a nitrogen-derived free radical) and its identification as the endothelium-derived relaxing factor led to the concept that, beyond their toxic effects, free radicals may also play a useful role as second messengers in cellular signal transduction. As such, they are involved in the maintenance of cellular homeostasis and in the communication of the cell with its external milieu [7].

In 1973, the pioneering work of David Hearse and colleagues [4] clearly demonstrated that the reintroduction of oxygen after a period of severe hypoxia in an isolated, buffer-perfused heart preparation, is associated with the occurrence of 'oxygen paradox'. Oxygen paradox is characterized by the development of a sudden alteration in the integrity of cardiac cells and a definitive loss of myocardial contractile function, associated with severe and irreversible ultrastructural damage. The demonstration that molecular oxygen is involved in the development of such reoxygenation injury (or reperfusion injury) was provided by the observation that if reperfusion of the anoxic heart with an oxygenated buffer enhanced cellular injury, anoxic reperfusion did not produce any extent of tissue injury [5]. In clinical conditions of myocardial ischemia,

early reperfusion is imperative for salvage of the remaining viable myocardial cells within the jeopardized area. However, post-ischemic reperfusion is often associated with some specific alterations brought about by oxidative stress upon reflow, such as life-threatening ventricular arrhythmias and persistent contractile dysfunction (myocardial stunning).

Biochemistry of free radicals

The way by which oxygen may exert such deleterious effects is through its reactive forms (reactive oxygen species: ROS, or reactive oxygen metabolites: ROM), including oxygen-derived free radicals as well as nonradical reactive species. Chemically, a free radical is any chemical species (atom, ion, or molecule) containing a single, unpaired electron in its outer orbitals. This single electron confers to the radical a very high reactivity. Oxygen-derived free radicals, including superoxide radical ($^{\circ}\text{O}_2^-$) and hydroxyl radical ($^{\circ}\text{OH}$), or nonradical ROS (hydrogen peroxide H_2O_2 , singlet oxygen $^1\text{O}_2$) are partially reduced forms of molecular oxygen (Figure 1). They are highly unstable and extremely reactive.

All cellular components can be attacked by free radicals. Thus, oxygen free radicals react with membrane lipids, especially those containing unsaturated double bonds, leading to the formation of lipid peroxides or hydroperoxides, and aldehydes. Membrane proteins, especially those containing sulfhydryl groups, are also important targets for oxyradicals, leading to marked alterations in cellular ionic homeostasis. Finally, several enzymes are inactivated by ROS, including catalase, glyceraldehyde-3-phosphate dehydrogenase, glutathione peroxidase, adenylate cyclase, myofibrillar ATPase, and creatine kinase [9].

The one-electron reduction product of molecular oxygen is the superoxide radical (superoxide anion $^{\circ}\text{O}_2^-$), the half-life of which is 10^{-9} - 10^{-11} sec (10^{-15} sec in presence of superoxide dismutase) (Table I). Superoxide radical is a requirement for the production of hydrox-

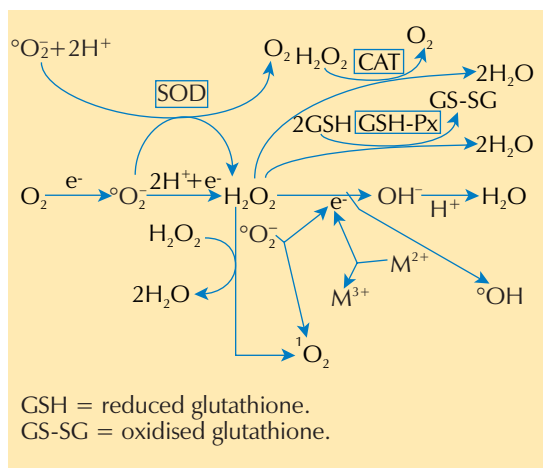


Figure 1. Univalent reduction of oxygen leading to the formation of ROS.

The reduction of superoxide anion ($^{\circ}\text{O}_2^-$) can occur spontaneously or can be catalysed by superoxide dismutase (SOD) or diverse organic SOD-like compounds [1, 11].

The resulting hydrogen peroxide (H_2O_2) can be: i) divalently reduced by catalase (CAT) or glutathione peroxidase (GSH-Px), ii) divalently reduced by diverse peroxidases producing singlet oxygen ($^1\text{O}_2$), iii) monovalently reduced to produce hydroxyl radical ($^{\circ}\text{OH}$) through the Fenton reaction catalysed by transition metals (M) such as iron or copper, or iv) monovalently reduced by reaction with $^{\circ}\text{O}_2^-$ to produce $^1\text{O}_2$.

yl radical $^{\circ}\text{OH}$. It can be dismutated into hydrogen peroxide H_2O_2 , a nonradical oxygen species (half-life : 10^{-3} sec ; 10^{-8} sec in presence of catalase), either spontaneously, or, at a much higher rate, under the catalyzing effect of superoxide dismutases SOD (Figure 1). In mammalian cells, there are two different SODs. These enzymes are metallo-proteins and their metal center is essential for their catalytic activity. Manganese-centered SOD (Mn-SOD) is located in mitochondria, and copper-zinc-centered SOD (Cu/Zn-SOD) in the cytosol.

Hydrogen peroxide is a strong oxidant which is able to interact slowly with most organic substrates. In presence of transition metals such as iron or copper (Table I), hydrogen peroxide can oxidize superoxide radical at rapid rates (Fenton reaction) to produce hydroxyl

radical $^{\circ}\text{OH}$, the most highly reactive oxidant, which, unlike superoxide radical or hydrogen peroxide, is reactive with most biological substrates. Due to its high reactivity, hydroxyl radical immediately reacts with surrounding target molecules at the site where it is generated. The Fenton reaction is the major source of hydroxyl radicals under physiological conditions. Alternatively, $^{\circ}\text{OH}$ can be generated through the Haber-Weiss reaction (Table I) when a superoxide radical and a H_2O_2 molecule spontaneously combine to form molecular oxygen and two hydroxyl radicals [2]. Moreover, $^{\circ}\text{OH}$ can be generated through a metal-independent pathway involving the interaction of superoxide radical and nitric oxide.

Singlet oxygen ($^1\text{O}_2$) is formed if one of the unpaired electrons of molecular oxygen absorbs energy to undergo spin inversion and

Table I. Formation of oxygen-derived free radicals and reactive species. (Production and dismutation of superoxide radical, Fenton and Haber-Weiss reactions).

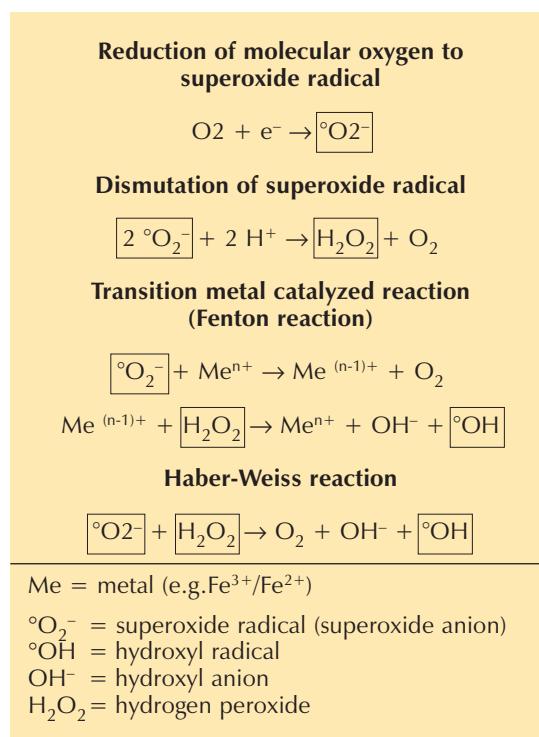


Table II. Formation of oxygen-derived free radicals and reactive species through reactions catalyzed by xanthine oxidase, myeloperoxidase, and NADPH oxidase.

<p>Xanthine oxidase (XO) reaction</p> $\text{Hypoxanthine} + \text{H}_2\text{O} + 2 \text{O}_2 \longrightarrow \text{Xanthine} + 2 \text{}^{\circ}\text{O}_2^- + 2 \text{H}^+$ $\text{Xanthine} + \text{H}_2\text{O} + 2 \text{O}_2 \longrightarrow \text{Uric acid} + 2 \text{}^{\circ}\text{O}_2^- + 2 \text{H}^+$ <p>Neutrophil myeloperoxidase (MPO) reaction</p> $\text{H}_2\text{O}_2 + \text{Cl}^- + \text{H}^+ \longrightarrow \text{HOCl} + \text{H}_2\text{O}$ <p>Neutrophil NADPH oxidase reaction</p> $\text{NADPH} + 2 \text{O}_2 \longrightarrow \text{NADP}^+ + 2 \text{}^{\circ}\text{O}_2^- + 2 \text{H}^+$
<p>Xanthine dehydrogenase/oxidase (capillary endothelial cells) Myeloperoxidase (neutrophil polymorphonuclear leucocytes) NADPH (reduced nicotinamide adenine dinucleotide phosphate) oxidase (neutrophil polymorphonuclear leucocytes, macrophages)</p>

orbital transition. Its half-life is approximately $2 \cdot 10^{-6}$ sec. Intracellular generation of $^1\text{O}_2$ may take place as a reaction product of hypochlorite and H_2O_2 , dismutation of $^{\circ}\text{O}_2^-$, or Haber-Weiss reaction. Although there is currently no satisfying way to provide direct evidence of $^1\text{O}_2$ formation in vivo, recent experimental studies have suggested that singlet oxygen might play an important role in myocardial ischemia-reperfusion injury [13].

Sources of oxygen radicals

In normal aerobic tissue, there is a continuous generation of oxygen radicals, which are detoxified by the endogenous antioxidants. Approximately 2% to 5% of the electron flow through the electron transport chain during normal mitochondrial respiration is subjected to partial univalent reduction of molecular oxygen, producing ROS including superoxide radical. In such conditions, Fenton reaction appears to be the major source of hydroxyl radical [2]. In various pathological conditions, for instance upon reperfusion of the ischemic myocardium, the activity of cellular antioxidant enzymes is considerably reduced, whereas the rate of oxyradical production is greatly

increased, due to reintroduction of molecular oxygen [11]. Such an imbalance between production of ROS and antioxidant defence can result in oxidative stress. Under these conditions, the most important sources of oxyradical generation are mitochondrial electron transport system (univalent reduction of molecular oxygen, NADH dehydrogenase complex) (Figure 1), endothelial cells (xanthine oxidase reaction), inflammatory cells (myeloperoxidase, NADPH oxidase), catecholamine oxidation, and metabolism of arachidonic acid (Table II) [11].

Conclusion

During the last decade, the terms 'free radicals', 'oxidative stress', and 'antioxidants' have become commonly used to discuss the cellular mechanisms of an increasing number of human diseases even though there are still many gaps in our understanding of the role of free radicals in the pathogenesis of such diseases. Available evidence from animal and human studies illustrate that antioxidant reserve might well be an important factor in promoting tissue protection against oxidative stress-mediated injury. Antioxidant enzyme

mimics (mostly SOD mimics) or catalytic drugs hold much promise for treating conditions in which the damaging oxidant molecule is continuously overproduced following an insult such as myocardial ischemia followed by reperfusion [1, 6, 12]. Recently, the research in the area of SOD mimics has been particularly fascinating because the discovery of these new catalysts has evolved in parallel to greater understanding of the biological role of ROS in general and superoxide radical in particular. It is clear that antioxidants are not a panacea for treating or preventing aging and diseases, but the importance of certain dietary antioxidants in preventing life-threatening diseases, such as heart disease and certain types of cancer, is still debated. Finally, if antioxidant therapy may be of some interest for limiting the damage caused by ROS, an important question now arises regarding their physiological role in biological signaling.

REFERENCES

1. Gershman R, Gilbert DL, Nye SW, Dwyer P, Fenn WO. Oxygen poisoning and X-irradiation: a mechanism in common. *Science*. 1954;119:623–626.
2. McCord JM. The evolution of free radicals and oxidative stress. *Am J Med*. 2000;108:652–659.
3. Papa S, Skulachev VP. Reactive oxygen species, mitochondria, apoptosis and aging. *Mol Cell Biochem*. 1997;174:305–319.
4. Hensley K, Floyd RA. Reactive oxygen species and protein oxidation in aging: a look back, a look ahead. *Arch Biochem Biophys*. 2002;397:377–383.
5. Hearse DJ, Humphrey SM, Chain EB. Abrupt reoxygenation of the anoxic potassium-arrested perfused rat heart: a study of myocardial enzyme release. *J Mol Cell Cardiol*. 1973;5:395–407.
6. Hearse DJ, Humphrey SM, Nayler WG, Slade A, Border D. Ultrastructural damage associated with reoxygenation of the anoxic myocardium. *J Mol Cell Cardiol*. 1975;7:315–324.
7. Barandier C, Tanguy S, Pucheu S, Boucher F, de Leiris J. Effect of antioxidant trace elements on the response of cardiac tissue to oxidative stress. *Ann N Y Acad Sci*. 1999;874:138–155.
8. Singal PK, Khaper N, Palace V, Kumar D. The role of oxidative stress in genesis of heart disease. *Cardiovasc Res*. 1998;40:426–432.
9. Lucchesi BR. Free radicals and tissue injury. *Dialogues Cardiovasc Med*. 1998;3:3–22.
10. Freeman BA, Crapo JD. Biology of disease. Free radicals and tissue injury. *Lab Invest*. 1982;47:412–426.
11. Toufektsian M-C, Boucher FR, Tanguy S, Morel S, de Leiris JG. Cardiac toxicity of singlet oxygen: implication in reperfusion injury. *Antioxid Redox Signal*. 2001;3:63–69.
12. Henke SL. Superoxide dismutase mimics as future therapeutics. *Exp Opin Ther Patents*. 1999;9:169–180.
13. Tanguy S, Boucher F, Besse S, de Leiris J. Cytoprotection against oxidative stress in rat isolated cardiomyocytes: effect of EUK8, a non-protein catalytic antioxidant. *Cardiovasc Drugs Ther*. 1998;12:355–357.

Featured research

Abstracts and commentaries

Inflammatory biomarkers, hormone replacement therapy, and incident coronary heart disease: prospective analysis from the Women's Health Initiative observational study

Pradhan A, Manson JE, Rossouw JE, et al. *JAMA*. 2002;288:980–987.

Postmenopausal hormone replacement therapy (HRT) has been shown to elevate C-reactive protein (CRP) levels. Several inflammatory biomarkers, including CRP, are associated with increased cardiovascular risk. However, whether the effect of HRT on CRP represents a clinical hazard is unknown. The study objectives were to assess the association between baseline levels of CRP and interleukin 6 (IL-6) and incident coronary heart disease (CHD), and to examine the relationship between baseline use of HRT, CRP, and IL-6 levels as they relate to subsequent vascular risk. This was a prospective, nested case-control study of postmenopausal women, forming part of the Women's Health Initiative, a large, nationwide, observational study. Among 75,343 women with no history of cardiovascular disease or cancer, 304 women who developed incident CHD were defined as cases and matched by age, smoking status, ethnicity, and follow-up time with 304 study participants who remained event-free during a median observation period of 2.9 years. The main outcome measure was the incidence of first myocardial infarction or death from CHD. Median baseline levels of CRP (0.33 vs 0.25 mg/dL; interquartile range [IQR] 0.14–0.71 vs 0.10–0.47; $P < 0.001$) and IL-6 (1.81 vs 1.47 pg/mL; IQR 1.30–2.75 vs 1.05–2.15; $P < 0.001$) were significantly higher among cases compared with controls. In matched analyses, the odds ratio for incident CHD in the highest vs lowest quartile was 2.3 for CRP (95% CI 1.4–3.7; P for trend = 0.002) and 3.3 for IL-6

(95% CI 2.0–5.5; P for trend < 0.001). After additional adjustment for lipid and nonlipid risk factors, both inflammatory markers were significantly associated with a twofold increase in odds for CHD events. As anticipated, current use of HRT was associated with significantly elevated median CRP levels. However, there was no association between HRT and IL-6. In analyses comparing individuals with comparable baseline levels of either CRP or IL-6, those taking or not taking HRT had similar CHD odds ratios. In analyses stratified by HRT, we observed a positively graded relationship between plasma CRP levels and the odds ratio for CHD among both users and nonusers of HRT across the full spectrum of baseline CRP. These prospective findings indicate that CRP and IL-6 independently predict vascular events among apparently healthy postmenopausal women and that HRT increases CRP. However, use or nonuse of HRT had less importance as a predictor of cardiovascular risk than did baseline levels of either CRP or IL-6.

Commentary

The failure in randomized trials of HRT to replicate the benefit seen in observational studies has been an enormous disappointment. One of the mechanisms postulated is the increase in markers of systemic inflammation following oral unopposed estrogen and oral combination therapy. In this study (the senior author is Paul Ridker, who has published extensively on inflammatory biomarkers) an observational subset of a much larger study (304 of 75,343 women) were evaluated to assess the potential link between elevated CRP and IL-6 and subsequent incidence of first myocardial infarction or death from CHD. The authors demonstrated that both CRP and

IL-6 predict cardiovascular risk in healthy postmenopausal women irrespective of whether or not they use HRT. HRT was associated with elevated CRP but not IL-6, possibly pointing to the absence of a generalized inflammatory effect. Importantly, the risk for a CAD event was the same for HRT users and nonusers, suggesting that HRT was of less importance than baseline levels of CRP or IL-6. The reduction of cardiovascular risk should therefore be focused on established therapies (lifestyle, aspirin, statins) rather than HRT. It is unlikely that HRT forms that do not impact on inflammatory markers (eg, transdermal preparations) will reduce CHD risk in the context of the information already available. In contrast, the anti-inflammatory properties of the statins suggest benefit beyond low-density lipoprotein cholesterol reduction.

(See link: Statins as potent anti-inflammatory drugs. *Circulation*. 2002;106:2041–2042).

Graham Jackson

Effects of hyperglycemia and fatty acid oxidation inhibition during aerobic conditions and demand-induced ischemia

Chavez PN, Stanley WC, McElfresh TA, Huang H, Sterk JP, Chandler MP. *Am J Physiol Heart Circ Physiol*. 2003.

Metabolic interventions improve performance during demand-induced ischemia by reducing myocardial lactate production and improving regional systolic function. We tested the hypotheses that: (1) stimulation of glycolysis would increase lactate production and improve ventricular wall motion; and (2) the addition of fatty acid oxidation inhibition would reduce lactate production and further improve contractile function. Measurements were made in anesthetized open-chest swine hearts. Three groups, hyperglycemia (HG), hyperglycemia + oxfenicine (HG + Oxf), and control (CTRL), were treated under aerobic conditions and during demand-induced

ischemia. During demand-induced ischemia, HG resulted in greater lactate production and tissue lactate content but had no significant effect on glucose oxidation. HG + Oxf significantly lowered lactate production and increased glucose oxidation compared with both CTRL and HG. Myocardial energy efficiency was greater in HG and HG + Oxf under aerobic conditions but did not change during demand-induced ischemia. Thus, enhanced glycolysis resulted in increased energy efficiency under aerobic conditions, but significantly enhanced lactate production, with no further improvement in function during demand-induced ischemia. Partial inhibition of free fatty acid oxidation in the presence of accelerated glycolysis increased energy efficiency under aerobic conditions and significantly reduced lactate production and enhanced glucose oxidation during demand-induced ischemia.

Commentary

Although glycolysis is not a major contributor to overall energy production in the normal heart, it is thought to be a much more important source of energy during myocardial ischemia. However, accumulation within the myocardium of glycolytic byproducts, namely lactate and protons, during ischemia is a potential adverse effect of high rates of glycolysis. Lactate and protons accumulate when glucose oxidation is inhibited while glycolytic rates are either maintained or accelerated. This problem is exacerbated if fatty acid oxidation rates are high, since this further decreases glucose oxidation rates. While the accumulation of glycolytic byproducts can be detrimental to the severely ischemic heart, it is controversial whether this is a problem in moderately ischemic hearts.

In the study by Chavez et al, demand-induced ischemia was produced in pig hearts exposed to high glucose levels. This increased lactate production and decreased cardiac efficiency during ischemia. However, inhibition of fatty acid oxidation resulted in an increase in glucose oxidation, a decrease in lactate

production, and an increase in cardiac efficiency in these ischemic hearts. This study supports previous studies that show that inhibition of fatty acid oxidation (such as with trimetazidine) can improve cardiac efficiency and benefit the ischemic heart.

Gary Lopaschuk

Association between hyperglycemia and the no-reflow phenomenon in patients with acute myocardial infarction

Iwakura K, Ito H, Ikushima M, et al. *J Am Coll Cardiol.* 2003;41:1–7.

We investigated the association between hyperglycemia and the no-reflow phenomenon in patients with acute myocardial infarction (AMI). Hyperglycemia is associated with increased risk of heart failure, cardiogenic shock, and death after AMI, but its underlying mechanism remains unknown. A total of 146 consecutive patients with a first AMI were studied by intracoronary myocardial contrast echocardiography after successful reperfusion within 24 hours of symptom onset. Two-dimensional echocardiography was recorded on day 1 and again 3 months later to determine the change in wall motion score (Δ WMS; sum of 16 segmental scores: dyskinesia = 4 to normokinesia = 0). The no-reflow phenomenon was found in 49 (33.6%) of 146 patients; their glucose levels on hospital admission were significantly higher than those of patients who did not exhibit this phenomenon (209 ± 79 vs 159 ± 56 mg/dL; $P < 0.0001$). There was no difference in glycosylated hemoglobin nor in the incidence of diabetes mellitus between the two subsets of patients. The no-reflow phenomenon was more often observed in the 75 patients with hyperglycemia (≥ 160 mg/dL) than in those without hyperglycemia (52.0% vs 14.1%; $P < 0.0001$). Patients with hyperglycemia had a higher peak creatine kinase level (2497 ± 1603 vs 1804 ± 1300 IU/L; $P = 0.005$) and a lower Δ WMS (3.7 ± 4.8 vs 5.7 ± 4.3 ; $P =$

0.01) than did those without hyperglycemia. The blood glucose level was an independent prognostic factor for no-reflow, along with age, gender, absence of preinfarction angina, complete occlusion of the culprit lesion, and anterior AMI. Hyperglycemia might be associated with impaired microvascular function after AMI, resulting in a larger infarct size and poorer functional recovery.

Commentary

It has long been observed that there is an association between admission glucose levels and morbidity and mortality following myocardial infarction. This holds true for patients with and for those without known diabetes mellitus. In a recent meta-analysis, Capes et al [1] reported that patients without diabetes who had glucose concentrations ≥ 6.1 to 8.0 mmol/L had a 3.9-fold higher risk of death than patients without diabetes who had lower glucose concentrations. Glucose concentrations > 8.0 to 10.0 mmol/L on admission were associated with an increased risk of congestive heart failure or cardiogenic shock in patients without diabetes. In patients with diabetes who had glucose concentrations ≥ 10.0 to 11.0 mmol/L the risk of death was moderately increased (relative risk 1.7). The relation between admission glucose levels vs morbidity and mortality remains unchanged despite modern infarction treatment with primary PTCA, thrombolysis, antithrombotics, β -blockers, and ACE inhibitors [2].

The mechanisms underlying the unfavorable relation between hyperglycemia and infarct prognosis are unclear, but there are a number of factors which may explain it. High catecholamine levels are found during infarction, which adversely affect glucose and fatty acid metabolism. High fatty acid levels may be toxic to the myocardium and may increase O_2 demand and reduce contractility. Hyperglycemia induces capillary plugging by leukocytes and diminishes endothelium-dependent vasodilatation, increases thrombus formation, reduces macrophage and lymphocyte function, results in less ischemic preconditioning,

and reduces collateral flow. Hyperglycemia may reduce circulating volume by osmotic diuresis. Finally, stress hyperglycemia may be a marker of more extensive myocardial damage in acute infarction. More damage leads to a greater rise in stress hormones, increasing myocardial oxygen consumption, and more congestive heart failure. However, the relationship between hyperglycemia and infarct size has been questioned as some studies have only found a weak relation or no relationship at all between infarct size and hyperglycemia.

In the present study the authors correlated the absence of reflow (by contrast echocardiography) with hyperglycemia. They found that no-reflow was more frequently observed in patients with high glucose levels on admission and that these patients had larger infarctions and poorer recovery of ventricular function during follow-up.

This study carries two important messages. First, the data nicely confirm, in patients, some of the mechanisms described above.

Second, despite modern treatment of infarction by thrombolysis or primary PTCA (which have clearly reduced infarct size and prognosis), hyperglycemia plays an important role in the ultimate damage caused by infarction. Further studies are needed to demonstrate that insulin intervention reduces infarct size and improves prognosis in patients undergoing thrombolysis or primary PTCA.

REFERENCES

1. Capes SE, Hunt D, Malmberg K, Gerstein HC. Stress hyperglycaemia and increased risk of death after myocardial infarction in patients with and without diabetes: a systematic overview. *Lancet*. 2000;355:773–778.
2. Wahab NN, Cowden EA, Pearce NJ, Gardner MJ, Merry H, Cox JL. Is blood glucose an independent predictor of mortality in acute myocardial infarction in the thrombolytic era? *J Am Coll Cardiol*. 2002;40:1748–1754.

Frans.C. Visser

Glossary

Gary D. Lopaschuk

Arachidonic acid pathway enzymes lipoxygenase

The lipoxygenase pathway is an important group of enzymes involved in the metabolism of arachidonic acid to leukotrienes. Leukotrienes have diverse biological actions in the body.

Calmodulin-dependent fashion

Calmodulin is an important molecule that binds calcium and stimulates the activity of calmodulin-dependent kinases. Calmodulin mediates many important reactions in the cell, including excitation-contraction coupling of muscle cells.

Carotenoids

Carotenoids are natural antioxidants that are abundant in fruit and vegetables. It has been proposed that the epidemiological association between high fruit and vegetable consumption and lower cancer rates may be related to an increased consumption of carotenoids.

Catalase

Catalase is an important enzyme that converts hydrogen peroxide to water and oxygen. This prevents hydrogen peroxide from forming hydroxyl radical, a highly reactive free radical.

Catechin

Catechin is a flavonoid, sometimes called flavanol. Catechin has antioxidant properties and can prevent free radical injury. It is present in grapes and has been suggested to contribute to the antioxidant properties of wine.

Conjugated dienes in circulating low-density lipoproteins

Conjugated dienes are a product of free radical reaction with lipids. The presence of conjugated dienes in circulating low-density lipoproteins is used as a marker of oxidant stress by free radicals.

Cyclo-oxygenase

The cyclo-oxygenase enzymes are an important group of enzymes involved in the metabolism of arachidonic acid to prostaglandins. Prostaglandins have diverse biological actions in the body.

Cytochrome P-450 mono-oxygenases

Cytochrome P-450 mono-oxygenases play an important role in steroid metabolism and in drug clearance in the liver.

Dismutase

Dismutase (usually called superoxide dismutase) is an enzyme that converts superoxide radicals to hydrogen peroxide.

Disulfide bonds (RSSR)

A disulfide bond is a bond in proteins between two sulfur molecules. Disulfide bonds are an important mechanism by which proteins maintain their three-dimensional configuration. If the disulfide bond is broken (for instance due to a reaction with a free radical), the function of the protein can be compromised.

Dithiothreitol

Dithiothreitol is a strong thiol reductant. It prevents sulfhydryl groups from being oxidized.

F2-isoprostanes

Free radical peroxidation of lipids can produce F2-isoprostanes. As a result, the production of F2-isoprostanes has been used as a measure of oxidant stress.

Free radicals

Free radicals are usually either oxygen or hydroxyl groups that have an unpaired electron. These free radicals are unstable and react with lipids, proteins, or DNA and RNA. This can result in tissue damage.

Gallic acid

Gallic acid is a tannin that is thought to exert antioxidant activity. It has been proposed that the presence of gallic acid in red wine has beneficial antioxidant effects.

Glutathione

Glutathione is an amino acid that acts as a substrate for glutathione peroxidase. Increasing glutathione levels are thought to increase the antioxidant actions of glutathione peroxidase.

Glutathione peroxidase

Glutathione peroxidase is an antioxidant enzyme that reacts with hydrogen peroxide and decreases the levels of this source of free radicals.

Glyceraldehyde 3-phosphate (GA3PDH)

Glyceraldehyde 3-phosphate (GA3PDH) is a key enzyme of glycolysis. During myocardial ischemia, GA3PDH can become rate-limiting for glycolysis. GA3PDH mRNA expression levels are often used as a control in many Northern blot experiments in order to ensure that equal amounts of mRNA have been loaded on the gel.

Gpx1

Gpx1 is an abbreviation for glutathione peroxidase.

Hemoproteins

Hemoproteins are proteins that contain a heme molecule, which is an iron-containing protoporphyrin. Cytochrome P-450 and hemoglobin are two examples of hemoproteins.

Human menopausal gonadotropin (hMG)-coenzyme A reductase inhibitors

Human menopausal gonadotropin (hMG)-coenzyme A reductase is important in the production of gonadal steroid hormones. There has been concern that the commonly used hMG reductase inhibitors used to lower cholesterol may also inhibit gonadotropin-coenzyme A reductase, therefore altering gonadal steroid hormone production.

Hydrogen peroxide

Hydrogen peroxide is an intermediate between two free radicals. Superoxide radicals can be acted upon by superoxide dismutase to produce hydrogen peroxide. However, if hydrogen peroxide is not subsequently removed from the cell (usually by catalase), a nonenzymatic reaction can occur in which highly reactive hydroxyl radicals are produced.

7 β -Hydroxycholesterol

7 β -Hydroxycholesterol is a cholesterol ester. Since free radicals can produce 7 β -hydroxycholesterol, the levels of plasma 7 β -hydroxycholesterol are sometimes used as a marker of free radical injury.

Hydroxyl

A hydroxyl free radical is a hydroxyl group with an unpaired electron. It is a highly reactive free radical that can cause serious damage to a cell by reacting with lipids, proteins, or DNA.

Hyperinsulinemic-euglycemic clamp

During a hyperinsulinemic-euglycemic clamp, high insulin levels are infused into a patient (or animal) and glucose is subsequently infused at a rate which maintains normal blood glucose levels. This procedure is used to determine the insulin sensitivity of the muscle. The more glucose infused under conditions of hyperinsulinemia the more insulin-sensitive is the muscle.

Hypoxia-inducible factor-1 α (HIF-1 α)

Hypoxia-inducible factor-1 α (HIF-1 α) is a transcriptional factor that is activated by stresses such as hypoxia. HIF-1 α then modifies the transcription of a number of genes, including glycolytic enzymes.

IP₃-sensitive

IP₃ is an important intracellular signaling molecule that is released from phosphatidylinositol present in the phospholipid cell membrane. IP₃-sensitive refers to any signaling pathway that involves IP₃.

Lipid hydroperoxides (LOOH)

Lipid hydroperoxides (LOOH) are produced when free radicals react with lipids. The levels of LOOH are often used as a measure of the amount of free radical production or injury.

Lipid peroxidation

Lipid peroxidation is the term used to describe the reaction of free radicals with lipids.

Lipid peroxyl radicals

Reaction of free radicals with lipids produces lipid peroxyl radicals. This is an unstable intermediate that then forms a more stable lipid hydroperoxide.

Lipoxygenase

The lipoxygenase pathway comprises an important group of enzymes involved in the metabolism of arachidonic acid to leukotrienes. Leukotrienes have diverse biological actions in the body.

Lycopene

Lycopenes are antioxidants that are abundant in tomatoes and tomato juice. Clinical studies are presently assessing whether lycopenes can be used to prevent free radical injury.

Malonyldialdehyde (MDA)

Malonyldialdehydes (MDA) are produced when free radicals react with lipids. The levels of MDA in the blood are often used as a measure of the amount of free radical production.

Mitochondrial electron transport chain

The mitochondrial electron transport chain is a number of mitochondrial proteins involved in energy production. Nicotinamide adenine dinucleotide (NADH) and reduced flavin adenine dinucleotide (FADH₂) are used as substrates for the electron transport chain. In the course of oxidizing NADH and FADH₂, protons are pumped out of the mitochondria. This sets up an electrochemical gradient in which adenosine diphosphate is phosphorylated to adenosine triphosphate (ATP) (by an ATP synthase) as protons flow back into the mitochondria.

Mitochondrial thioredoxin

Thioredoxin is an antioxidant enzyme that prevents the oxidation of thiol (sulfur) groups of proteins.

Nicotinamide adenine dinucleotide (NADH)-coenzyme Q-oxidoreductase complex (complex I)

Nicotinamide adenine dinucleotide (NADH)-coenzyme Q-oxidoreductase complex (complex I) is the first complex of the electron transport chain. It oxidizes NADH in the first reaction of the electron transport chain.

NIH 3T3 fibroblasts

NIH 3T3 fibroblasts are a commonly used fibroblast cell line that was produced by the National Institutes of Health.

Nitric oxide synthase

Nitric oxide synthase is the enzyme responsible for the production of nitric oxide (NO). NO is a very important molecule in many cellular events and was identified as the molecule released by endothelial cells that can cause vascular relaxation.

Nuclear factor kappaB (NFκB)

Nuclear factor kappaB (NFκB) is a nuclear transcription factor that is important in the control of cell growth.

8-OH-deoxyguanosine (8-OHdG)

8-OH-deoxyguanosine (8-OHdG) is produced when DNA is subjected to free radical attack. Leukocyte DNA 8-OHdG levels are sometimes used as a measure of free radical injury to DNA.

Peroxiredoxin III

Peroxiredoxin III is specifically localized to mitochondria and is believed to play an important role in the regulation of cellular redox status by serving as a primary line of defense against H₂O₂ produced during respiration.

Peroxynitrite (ONOO⁻)

Peroxynitrite (ONOO⁻) is formed by the combination of NO with O₂⁻. ONOO⁻ then rapidly decomposes to form highly reactive oxidant species.

Phosphoribosylpyrophosphate

Phosphoribosylpyrophosphate is an intermediate in the purine biosynthesis pathway. Purines are an important component of DNA and RNA.

Proanthocyanidins

Proanthocyanidins are components of grape seeds and are the main phenolic antioxidant of red wine. They are thought to have beneficial antioxidant effects.

Prostacyclin

Prostacyclin is a prostaglandin that has a number of important biological effects, one of which is a potent vasodilatory action in blood vessels.

Protein kinase C (PKC)

Protein kinase C (PKC) is an important kinase involved in cellular signaling. It is activated by lipids (diacylglycerols) released from the phospholipid membrane. There is a considerable research interest in the role of PKC modification in the ischemic heart.

Protocatechuic acid

Protocatechuic acid is a naturally occurring antioxidant. It is a phenolic compound found in various plants, including grapes.

Reduced nicotinamide adenine dinucleotide phosphate oxidase

Reduced nicotinamide adenine dinucleotide phosphate oxidase is an enzyme present in phagocytes such as neutrophils and macrophages. This enzyme is an important source of free radicals.

Ryanodine

Ryanodine is an inhibitor of the calcium channel that releases calcium from the sarcoplasmic reticulum. It was an important tool in first characterizing this channel, which is often called the ryanodine-sensitive calcium channel.

Superoxide

Superoxide is a free radical. It is an oxygen molecule that has an unpaired electron. This molecule can react with lipids, proteins, DNA, and RNA, causing tissue damage.

Thiobarbituric acid-reacting substances (TBARS)

Thiobarbituric acid-reacting substances (TBARS) is a simple assay that is used to measure malonyldialdehyde, a product of free radical lipid peroxidation.

Thioredoxin peroxidase

Thioredoxin peroxidase is a peroxiredoxin that uses thiols as reductants. Since they use hydrogen peroxide as a substrate, they are an antioxidant enzyme.

Thioredoxin reductase

Thioredoxin reductase is a major cellular protein disulfide reductase. This enzyme has antioxidant activity.

Thrombomodulin

Thrombomodulin is a key component of the anticoagulant protein C pathway and is a major contributor to vascular thromboresistance. It inhibits blood coagulation.

Tissue factor pathway inhibitor

Tissue factor pathway inhibitor is a critical inhibitor that modulates tissue factor-induced coagulation.

Tissue plasminogen activator

Tissue plasminogen activator is a thrombolytic agent that disrupts blood clots. It is widely used to break up thrombus in patients with acute myocardial infarction or strokes.

Tocopheroxyl radical

Tocopheroxyl radicals are formed from α -tocopherol (vitamin E). Vitamin E is an antioxidant, since it is an efficient scavenger of lipid peroxy radicals. Tocopheroxyl radicals are formed during this process, which can then be recycled back to α -tocopherol.

Tryptophan-rich sensory protein (TspO)

Tryptophan-rich sensory protein (TspO) is a tryptophan-rich protein that negatively affects the transcriptional expression of several genes.

Ubiquinol-10

Ubiquinol-10 is a reduced form of coenzyme Q.

Glossary

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Ubiquinol-cytochrome c reductase (complex III)

Ubiquinol-cytochrome c reductase is the third complex of the electron transport chain. It is involved in mitochondrial respiration, which is a major source of energy for cells.

von Hippel-Lindau protein

von Hippel-Lindau protein is a tumor-suppres-

sor protein. It targets proteins for ubiquitination and degradation. For instance, von Hippel-Lindau protein targets HIF-1 α for ubiquitination and proteasomal degradation.

Xanthine oxidase

Xanthine oxidase is an enzyme that oxidizes xanthine. This reaction is also a source of free radicals, as superoxide radicals are produced.