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COMMENTARIES

Commentary: oxidative stress reconsidered

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Abstract All definitions of the terms 'oxidative stress' and 'antioxidants' implicate that oxidants are just damaging. However, there is increasing evidence that reactive oxygen species (ROS) are not only toxic but that we need them for healthy life. This change in paradigm has been discussed at the third international symposium on 'Nutrition, oxygen biology and medicine—micronutrients, exercise, energy and aging disorders', of the Society for Free Radical Research France and the Oxygen Club of California on April 8–10, 2009 in Paris. The beneficial effect of a low to moderate concentration of oxidants produced during exercise was taken as most discussed example. In this case, ROS are required for normal force production in skeletal muscle, for the development of training-induced adaptation in endurance performance, as well as for the induction of endogenous defense systems. Taking antioxidants during training prevents adaptation. Although substantial progress on the understanding of the physiological functions of ROS was communicated at the meeting, it remained obvious that a lot of work is needed to fully understand the conditions and individual situations under which ROS are beneficial or detrimental.

Keywords Oxidative stress · Physiological functions · Adaptive response · Exercise

The term oxidative stress is frequently used but rarely defined. Helmut Sies described it as the 'imbalance between oxidants and antioxidants in favour of the oxidants, potentially leading to damage' [15]. Oxidative stress, thus, refers to a serious imbalance between the production of reactive oxygen species (ROS) and antioxidant defenses. An antioxidant was defined as 'any substance that delays, prevents or removes oxidative damage to a target molecule' [10]. All definitions implicate that oxidants are damaging only. ROS comprise oxygen radicals like the superoxide anion ($O_2^{\bullet -}$), alkoxy radicals ($RO^{\bullet}$), peroxy radicals ($ROO^{\bullet}$), and the hydroxyl radical ($^{\bullet}OH$) as well as non-radical forms like H_2O_2 or other hydroperoxides (ROOH). ROS, mainly because of their radical form, can damage all kind of molecules. The reaction with DNA to cause cancer or with lipoproteins, for long been considered the atherosclerosis-inducing event, has gained most attraction. However, there is increasing evidence that ROS are not only toxic, but we need them for a healthy life. The best examples are the production of $O_2^{\bullet -}$ by phagocytosing cells to kill invaded bacteria or the up-regulation of endogenous defense systems to eliminate xenobiotics or pro-carcinogens. To figure out further situations where ROS act beneficial was the aim of the third international symposium on 'Nutrition, oxygen biology and medicine—micronutrients, exercise, energy and aging disorders', of the Society for Free Radical Research France and the Oxygen Club of California on April 8–10, 2009 in Paris.

Numerous contributions at this meeting revealed that the simple concept of 'dangerous reactive oxygen species must be balanced by beneficial antioxidants' can be put to rest. Clearly, H_2O_2 of mitochondrial origin, since the times of its discovery [3, 11], considered to represent a disastrous construction failure of the respiratory chain, has been recognized, i.a., as a regulator of cell death pathways [2, 6]. Long ago, the superoxide anion, which first attracted the interest of gas-phase physico-chemists [9], radiologist, and toxicologists, has become known to be a physiological

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metabolite of the phagocytes' NOX2 [1] that is of outstanding importance in the defence against microbial infections. Further, the superoxide anion is continuously produced by a diversified family of enzymes (NOX1, NOX3-5 and the two dual oxidases DUOX 1 and 2). Their distinct tissue localization [5] and subcellular compartmentation [16] points to fine-tuned site-specific metabolic functions of their product $O_2^{\bullet -}$ or the H_2O_2 formed there from. The basket of compounds subsumed under the term ROS (see above) also contains the products of a realm of lipoxygenases which in part at least have been characterized as mediators of inflammatory responses or metabolic regulators. Not surprisingly therefore, ROS production, *alias* "oxidative stress", turns out not to be a threat to aerobic life, which has to be strictly avoided, but a phenomenon that constitutes the homeostasis of normal life. ROS, commonly $O_2^{\bullet -}$ and/or H_2O_2, can further be generated by cytochrome P450, various oxidases, lipoxygenases and dehydrogenases The complexity of ROS *producing* processes is mirrored in an equally complex set of 'reducing' enzymes comprising three superoxide dismutases, catalase, eight distinct glutathione peroxidases, from which five are selenoproteins, six peroxiredoxins with possibly more to be detected. This ever-changing scenario of competing processes prompted a round table discussion which centred on problems such as the interference of ROS with signaling cascades, ROS-mediated metabolic regulation, adaptive responses, and responsiveness or, in more general terms, on the question whether we have to rethink about the biological impact of oxidative stress. Participants were: George Brooks, Malcolm Jackson, Kelvin Davies, Arlette Gratas-Delamarche, LiLi Ji; the discussion was moderated by Maret Traber and Regina Brigelius-Flohé.

Facts and evidences presented at the meeting corroborated that usually low to moderate concentrations of oxidants, as, e.g., produced during exercise beneficially affect endurance development, aging, and the metabolic syndrome. From the talks of José Vina, M. Jackson, K. Davies, Scott Powers et al. it became clear that the ROS produced during exercise are by no means detrimental. Instead, they are required for normal force production in skeletal muscle, for the development of training-induced adaptation in endurance performance, as well as for the induction of endogenous defense systems [7, 8, 14]; reviewed by Powers and Jackson [12]. All this happens under conditions of moderate exercise, while exhaustive exercise can clearly be damaging. The adaptive response, however, does not only depend on the degree of stress but also on pre-existing conditions and the age of the exercising subject. The success of training, thus, depends on many factors among them, the individual capability of a person to compensate the challenge among them, and the response to oxidative stress from exercise.

How exercise may be linked to adaptive processes was elaborated by George Brooks: In his talk he recalled that during exercise muscle produces and consumes lactate as energy source. Lactate is taken up by the mitochondria via the mitochondrial lactate/pyruvate transporter, a monocarboxylate transporter (MCT1) found in mitochondria of muscles, sperm, brain, heart, liver, and in tumor cells. Lactate stimulates cell respiration and, in consequence, mitochondrial ROS production. The ROS thus produced mediate signals, set the stimulus, and trigger the adaptive response.

Potential molecular links between ROS production and adaptive response are legion, activation of the NFκB and the Keap1/Nrf2 system being the best investigated ones. The latter one provides a particularly revealing paradigm of historical misconceptions in the field. Originally, Nrf2 was claimed to activate antioxidant response element (ARE) since it is commonly activated by plant-derived 'antioxidants'. Meanwhile Nrf2 was shown to be activated by oxidation of a particular SH group in its cytosolic inhibitor Keap1. It is, therefore, an *oxidative* process that activates the Nrf2 system to induce protective enzymes, the so-called phase II enzymes. This change in paradigm shows how dangerous it is to classify a xenobiotic as antioxidant by means of in vitro tests demonstrating the protection of some organic compound against destruction by free radicals, usually $^{\bullet}OH$. In vivo, e.g., such 'antioxidant' plant polyphenols readily react with the most abundant radical, i.e., molecular oxygen, to initiate an oxidant chain reaction. The biological benefit of such a bioactive compound, if any, results from the induction of antioxidant and other protective enzymes which are regulated by the Keap1/Nrf2/ARE system. In this sense, the mild oxidative stress exerted by exercise or autooxidizable bioactive compounds may be considered as kind of vaccination that protects the organism against a detrimental oxidative challenge.

In the context of adaptation to exercise, additional molecular mechanisms were discussed at the meeting. Of particular relevance is the up-regulation of the expression of critical factors that regulate the mitochondriogenic pathway such as PGC-1α (co-activator of PPARγ), NRF1 (nuclear respiratory factor-1), and TFAM (mitochondrial transcription factor A). In parallel, enzymes such as MnSOD and catalase, heat shock proteins 60 and 70, HSF1, AP-1, NFκB, and p53 are up-regulated. The importance of PGC-1α could be demonstrated by PGC-1α null mice. Irrespective of age, they did not gain endurance by exercise and, instead, underwent senile sarcopenia. These observations link moderate exercise to longevity as did the observation that old muscles did not produce Hsps and could not further activate the already activated NFκB. Having these processes in mind, it can no longer surprise that antioxidants do not improve endurance exercise

performance and do not prevent fatigue. Instead antioxidant treatment hampers training-induced adaptation and depresses muscle force. A very recent, very striking example was just published; intake of antioxidants (1,000 mg vitamin C and 400 IU Vitamin E per day) prevented exercise-mediated increase in insulin sensitivity together with all measured ROS-sensitive transcriptional regulators there of (PPARγ, PGC1α and β, SOD1 and 2, GPx1) in exercised skeletal muscle. Antioxidants also decreased TBARS levels, an indicator of ROS formation, indicating that ROS have been required for the beneficial effect [13]. In this context antioxidants can also remove beneficial oxidative modifications from a target molecule.

In contrast, damage from exhaustive exercise, which causes more severe oxidative stress in muscle cells, may be prevented by the intake of antioxidants, i.e., before a marathon run or a competition. The general conclusion on this issue was: a mild physical stress is per se not damaging at all. It only occasionally can become a problem if a completely untrained and thus poorly adapted person starts to do exercise up to exhaustion. This implies that we have to understand the threshold at which a physiological situation turns into a pathophysiological one. Certainly, the definition of individual thresholds could become a critical issue.

Despite substantial progress communicated, it was equally evident at the meeting that we are far from having a comprehensive picture of ROS-related mammalian biology. Many details on interaction of peroxides with cellular targets have been unraveled, but regulatory circuits, as they have paradigmatically been established for yeast [4], remain to be characterized for mammals. Meaningful redox regulation of a biological phenomenon at least requires independent ways of activation and termination, each being subject to distinct sensors that transduce their message to distinct target molecules, in separate loops of the regulatory network. In this regard, the identification of off-signals and related targets appears to be a most neglected area. Further, regulation requires specificity of effectors and targets, an aspect that is inadequately considered when ignoring the distinct reactivity of individual ROS, as well as the distinct responses of regulatory target proteins. To this end, a provocative statement of a pioneer of the club possibly may merit consideration. Leopold Flohé warned that we will not likely make progress in understanding redox regulation (1) if we keep relying on redox potentials or redox equilibria in biological systems which are far from any equilibrium but rather a steady state determined by rate constants of distinct redox-active messengers and their regulatory proteins and the competing degrading enzymes; (2) if we continue dividing the chemical world into just two categories, antioxidants and oxidants, thus ignoring individual reactivities; and (3) if we promote recommendations such as 'five times a day fruits and vegetables' without discriminating between potatoes, straw berries and spinach and between individual bioactive compounds therein. May be we should indeed take Albert Einstein's advice: "Make everything as simple as possible, but not simpler" a bit more serious.

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