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Passive smoking reduces and vitamin C increases exercise-induced oxidative stress: Does this make passive smoking an anti-oxidant and vitamin C a pro-oxidant stimulus?

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ABSTRACT

The current interpretative framework states that, for a certain experimental treatment (usually a chemical substance) to be classified as "anti-oxidant", it must possess the property of reducing (or even nullifying) exercise-induced oxidative stress. The aim of the study was to compare side by side, in the same experimental setup, redox biomarkers responses to an identical acute eccentric exercise session, before and after chronic passive smoking (considered a pro-oxidant stimulus) or vitamin C supplementation (considered an anti-oxidant stimulus). Twenty men were randomly assigned into either passive smoking or vitamin C group. All participants performed two acute eccentric exercise sessions, one before and one after either exposure to passive smoking or vitamin C supplementation for 12 days. Vitamin C, oxidant biomarkers (F_2-isoprostanes and protein carbonyls) and the non-enzymatic antioxidant (glutathione) were measured, before and after passive smoking, vitamin C supplementation or exercise. It was found that chronic exposure to passive smoking increased the level of F_2-isoprostanes and decreased the level of glutathione at rest, resulting in minimal increase or absence of oxidative stress after exercise. Conversely, chronic supplementation with vitamin C decreased the level of F_2-isoprostanes and increased the level of glutathione at rest, resulting in marked exercise-induced oxidative stress. Contrary to the current scientific consensus, our results show that, when a pro-oxidant stimulus is chronically delivered, it is more likely that oxidative stress induced by subsequent exercise is decreased and not increased. Reversely, it is more likely to find greater exercise-induced oxidative stress after previous exposure to an anti-oxidant stimulus. We believe that the proposed framework will be a useful tool to reach more pragmatic explanations of redox biology phenomena.

1. Introduction

In chemistry, oxidants are defined as elements or compounds in an oxidation–reduction (redox) reaction that accept electrons from another species, whereas reductants are defined as elements or compounds that donate electrons to another species. For their property to act as electron donors, reductants in biology are referred to as "anti-oxidants", whereas oxidants retain their "chemical" name, unless the substance in question is not a free radical per se (albeit it can promote oxidation), in that case referred to as "pro-oxidants". Considering the very high rate of redox reactions and the inherent complexity of living biological organisms, monitoring redox reactions in vivo is essentially infeasible. To overcome this obstacle, redox biologists invented the term "oxidative stress" to follow the effects of pro-oxidants and anti-oxidants in living biological organisms. Due to the elusive nature of free radicals and their difficulty to be directly assessed, a common practice in redox biology is to measure the levels of redox biomarkers (both oxidant and anti-oxidant). Most researchers use the term "oxidative stress" to indicate "an increase in the level of reactive species and/or oxidant biomarkers" [1]. In this context, relevant review and opinion papers have been published, providing methodological guidelines for redox biomarkers assessment and recommendations on data interpretation on the basis of which alterations in redox biomarkers indicate oxidative stress (e.g.,

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[1]). These theory-based interpretative frameworks assume a stimulus (ranging from a chemical substance to physical activity) as a pro-oxidant, if it increases the levels of oxidant biomarkers and/or decreases the levels of anti-oxidant biomarkers, or as an anti-oxidant, if it does the opposite.

Recently, a central concept has emerged supporting that, to define the mechanisms regulating health and organismal performance, monitoring the responses to a homeostatic challenge is more informative than static homeostatic measures (e.g., [2]). Many studies have used either pro-oxidant (e.g., [3]) or anti-oxidant (e.g., [4]) stimuli to test the robustness and elasticity of functions involved in maintaining redox homeostasis. Acute exercise is probably the most commonly used physiological stimulus generating an oxidative stress response [1]. It is very common that an acute exercise session is performed before and after a redox treatment (e.g., an anti-oxidant supplement) to facilitate the disclosure of a redox effect in vivo (e.g., [5]). The current framework states that, for a certain experimental treatment (usually a chemical substance) to be classified as "anti-oxidant", it must possess the property of reducing (or even nullifying) exercise-induced oxidative stress, compared to that appeared before the treatment (e.g., [6]).

Noteworthily, the appropriateness of the current framework in redox biology is mainly based on intuition and theoretical considerations. To our knowledge, original data on a direct in vivo comparison between a supposed pro-oxidant and/or anti-oxidant stimulus are lacking. In human research, the most frequently used anti-oxidant stimulus is probably vitamin C. This is mainly because consumption of vitamin C above the recommended values is considered safe and its antioxidant capacity in vitro is undisputed, although pro-oxidant effects of vitamin C in vivo have also been described [7]. On the other hand, smoking is probably the most well investigated pro-oxidant stimulus in humans [8,9]. However, for ethical reasons, the use of smoking as an oxidant stimulus is restricted to smokers, thus limiting its applicability in research. Passive smoking may be an appropriate replacement for smoking but, to our knowledge, no study has yet investigated the effects of passive smoking on oxidative stress in comparison to an anti-oxidant stimulus. Thus, it becomes apparent that the lack of direct and well controlled in vivo comparisons between pro-oxidants and anti-oxidants before and after an exercise stimulus is raising serious doubts whether the current framework is appropriate for studying human redox responses, either at rest or after exercise. Therefore, the aim of the present study was to compare side by side, in the same experimental setup, redox biomarkers responses to an identical acute eccentric exercise session (considered a potent pro-oxidant stimulus; [10]), before and after chronic passive smoking or vitamin C supplementation. A main objective of this paper is to propose another interpretative framework within which researchers can assess more realistically the alterations in redox biomarkers after exercise.

2. Materials and methods

2.1. Participants

Twenty untrained men were randomly assigned into either passive smoking (22.6 ± 0.9 years; 73.1 ± 1.6 kg; mean ± SEM) or vitamin C group (22.8 ± 1.1 years; 72.2 ± 1.4 kg). The participants were asked to recall whether they had participated in regular exercise or in unaccustomed and/or heavy exercise in the 3 months before the study entry. Individuals who reported participation in such activities were precluded from the study. Smoking and consumption of nutritional supplementation the last 3 months before the study initiation were also exclusion criteria to participate in the present investigation. Volunteers were instructed to abstain from any strenuous exercise during their participation in the study as well as for 5 days prior and 2 days following the exercise session. A written consent was obtained from all participants. The procedures were in accordance with the Helsinki declaration of 1975, as revised in 2000, and approval was received from the institutional review board (016/12-05-2013).

2.2. Study design

An overview of the study design is shown in Fig. 1. All participants performed the first acute isokinetic eccentric exercise bout with the knee extensors of one leg. Plasma, erythrocytes and urine were collected at immediately before (day 0) and 48 h post-exercise (day 2), since previous studies have shown that oxidative stress peaks 1–3 days after eccentric exercise [1]. Fourteen days after, participants in the pro-oxidant group were exposed to passive smoking for 1 h, while the participants in the anti-oxidant group received oral supplementation of 1 g of vitamin C in a single dose at the time point "pre" (ascorbic acid; Lamberts Health Care Ltd., Kent, United Kingdom). Before and 1 h after exposure to passive smoking, or before and 1 h after supplement consumption plasma, erythrocytes and urine were collected. During the next 12 days, participants in the pro-oxidant group were exposed to passive smoking for 1 h daily, while the participants in the anti-oxidant group received oral supplementation of 1 g vitamin C daily. Supplements were taken every 8 h in capsules containing 333 mg of vitamin C, in order to achieve high and sustained blood concentration throughout the day [11]. After the 12 days of exposure to passive smoking or vitamin C supplementation, all participants performed the second acute isokinetic eccentric exercise bout with the knee extensors of the other leg. Plasma, erythrocytes and urine were collected at immediately before (day 14) and 48 h post-exercise (day 16). Evaluation of oxidant biomarkers (F_2-isoprostanes and protein carbonyls), the non-enzymatic antioxidant (glutathione) and vitamin C was performed at all sample collection

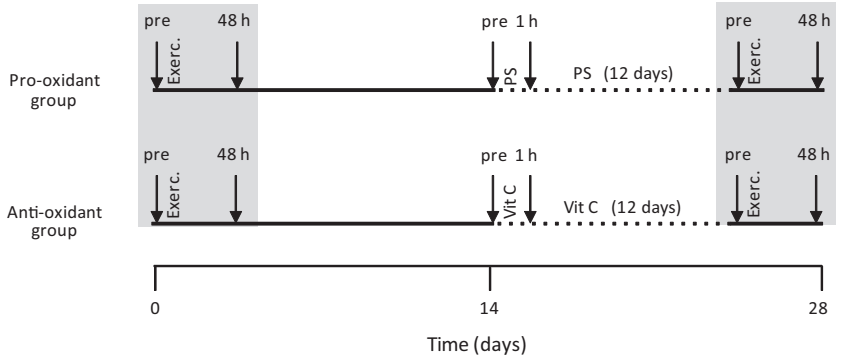

Fig. 1. Study design. Arrows indicate the time of body fluids collection. Exerc., exercise; PS, passive smoking; Vit C, vitamin C.