Does high antioxidant capacity indicate low oxidative stress?

Costantini, David; Verhulst, Simon

Published in:
Functional Ecology

DOI:
10.1111/j.1365-2435.2009.01546.x

Publication date:
2009

Functional Ecology

British Ecological Society

Functional Ecology 2009, **23**, 506–509 doi: 10.1111/j.1365-2435.2009.01546.x

FORUM

Does high antioxidant capacity indicate low oxidative stress?

David Costantini[1]* and Simon Verhulst[2]

[1]*Via Colle Fontana Vecchia 64, 00038 Valmontone (Roma), Italy; and* [2]*Behavioural Biology, University of Groningen, Kerklaan 30, 9751 NN Haren, Groningen, The Netherlands*

In this contribution, we make the point that information on antioxidant capacity by itself is not sufficient to make inferences about oxidative stress. To illustrate this general statement, we discuss six potential scenarios, which demonstrate that inferring variation in oxidative stress using antioxidants alone may lead to erroneous conclusions.

Metabolic activity is a primary source of free radicals, which are unavoidable by-products of ATP synthesis. Free radicals, as well as non-free radical pro-oxidants, are prone to react with any molecules around, causing oxidative damage. Organisms have evolved multiple defence lines to prevent oxidative damage, ranging from antioxidant enzymes to low molecular weight antioxidants, and also specific cellular components that repair oxidatively damaged molecules. A disturbance in the balance between pro-oxidants and antioxidants in favour of the former, leading to oxidative damage, gives rise to an increase in oxidative stress (Sies 1991; Halliwell & Gutteridge 2007). Here we define oxidative stress as the rate at which oxidative damage is generated. Implicit in this definition is that oxidative stress is a continuous variable that is unlikely to ever be exactly zero since pro-oxidants are continually produced and some oxidative damage is always generated. Persistent oxidative stress may give rise to pathological conditions and is increasingly implicated as a contributing factor to several human pathologies (over 150 disorders), cellular senescence, and aging (Beckman & Ames 1998; Hulbert *et al*. 2007; Furness & Speakman 2008). Recent studies show that levels of pro-oxidants and antioxidants may also have relevant ecological and evolutionary roles and may help understand functional interactions among life-history traits (von Schantz *et al*. 1999; Costantini 2008; Monaghan *et al.* 2009).

Several methods have been applied to measure antioxidant capacity of a tissue or single classes of antioxidants (Prior & Caro 1999; Yeum *et al*. 2004), usually with the aim to quantify oxidative stress. However, we would like to emphasise in this note that comparing antioxidant capacity by itself may not be sufficient to make inferences about differences in levels of oxidative stress. Although recognized by many, this point has also been overlooked, in that conclusions on variation in oxidative stress have often been based on variation in antioxidant levels alone. Moreover, most commonly, antioxidant enzymes are measured as indicative of antioxidant capacity without consideration of the multiple array of non-enzymatic antioxidants (e.g. thiols, vitamins C and E), which may be very important to neutralize ROS. As a consequence, both the potential of antioxidant machinery and the effects of a stressor are underestimated. An evaluation of the implicit assumption that information on antioxidant capacity alone allows inferences on oxidative stress therefore seems useful at this time, given the growing interest in oxidative stress in evolutionary ecology.

To evaluate what can be inferred regarding oxidative stress from variation in antioxidant capacity alone we discuss six potential scenarios for co-variation between antioxidants, pro-oxidants, and the resulting oxidative stress. For each scenario we compare two states (e.g. experimental groups), *A* and *B*, which differ in antioxidant levels or in single classes of antioxidants (below we will refer to antioxidant capacity alone, but the points apply equally to measurements of single classes of antioxidants). To emphasize that the examined relationships are variable in the real world we illustrate the scenarios with examples from the literature. See Fig. 1 for a schematic illustration of the scenarios. Note that the axes in Fig. 1 have no scales because our aims are restricted to a qualitative evaluation.

Suppose that *B* has higher antioxidant capacity than *A*. How can we interpret this result? Does this mean that *B* is subjected to less oxidative stress than *A*? Suppose that *A* and *B* do not differ in pro-oxidant production (Fig. 1a). In this case, *B* has more antioxidants per unit of pro-oxidants and, consequently, attains a lower level of oxidative stress (this is the intuitive interpretation commonly encountered of a difference in antioxidant capacity). This pattern could arise for any number of reasons that affect the antioxidant machinery of *A* or *B* without affecting pro-oxidants. Two-week-old calves (*Bos taurus*) had the same level of pro-oxidants as 3-week-old calves, but higher plasma antioxidant capacity as measured using the FRAP method, lower oxidative damage (lipid peroxidation), and, consequently, lower oxidative stress (Gaál *et al*. 2006). Now, suppose that, in addition to having higher antioxidant levels, the levels of pro-oxidants are also higher in *B*. In the case shown in Fig. 1b, the higher free radical production in *B* is associated with over-expression of the antioxidant response, resulting in lower oxidative stress in *B*. Chickens (*Gallus gallus domesticus*) showed at 1 h after supplementation of stress hormones (inducer of pro-oxidant

*Correspondence author. E-mail: david.costantini@uniroma1.it

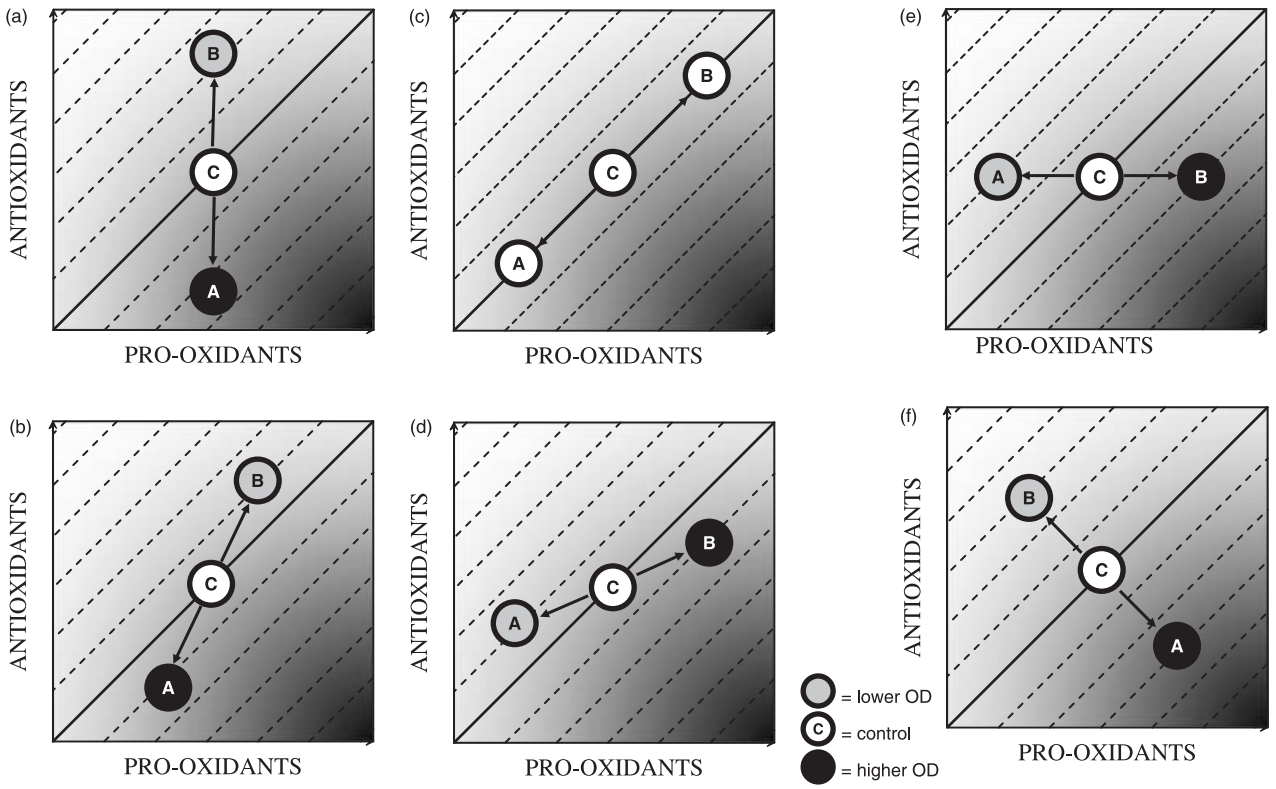

Fig. 1. Relationship between pro-oxidants, antioxidants and oxidative stress. Oxidative stress, defined as the rate at which oxidative damage is generated, increases when antioxidant levels decrease for a given level of pro-oxidants. Hence oxidative stress increases from top left corner to bottom right corner as indicated by background shading (darker indicates higher oxidative stress level). Dashed lines are 'iso-oxidative stress' lines, in that all combinations of anti- and pro-oxidant levels on the line yield the same level of oxidative stress. The solid line represents combinations of anti- and pro-oxidants with an equal level of oxidative stress as the C-group. In a–f different hypothetical situations are compared with respect to possible ways that two (experimental) groups (a) and (b) could differ from a control (c) group. See main text for further explanation.

production) a decrease in oxidative damage (lipid peroxidation) and an increase of plasma antioxidant capacity (Lin *et al.* 2004), resulting in a lower oxidative stress. Two-week-old calves (*B. taurus*) had higher levels of free radicals than their mothers at calving, but also higher plasma antioxidant capacity as measured using the FRAP method and antioxidant capacity was such that oxidative damage (lipid peroxidation) and so, oxidative stress, were lower in calves (Gaál *et al.* 2006). In the case shown in Fig. 1c, in both *A* and *B* the antioxidant capacity is matched to the different pro-oxidant levels, and, consequently, there is no difference in oxidative damage between the groups, although such a difference would clearly be indicated when either axis (antioxidants or pro-oxidants) would be considered in isolation. Rats (*Rattus rattus*) produce more free radicals than canaries (*Serinus canaria*), but rat lungs show slightly lower levels of lipid peroxidation together with higher activity of enzymatic antioxidants than canary lungs (López-Torres *et al.* 1993; Pérez-Campo *et al.* 1993). This case is also well-exemplified in a recent study on mice (Costantini *et al.* 2008b). Long attack latency line mice had higher serum antioxidant capacity compared to short attack latency line mice, resulting in similar levels of oxidative damage. In the case shown in Fig. 1d, *B* is exposed to higher oxidative damage because the antioxidant response of *B* to a ROS challenge, while still operating, is not sufficient to maintain oxidative damage at the same level of *A*. A study of human beings (*Homo sapiens*) showed that old men had, on the one hand, higher levels of urinary 8-hydroxy-2′-deoxyguanosine and of protein carbonyls (both markers of oxidative damage), and, on the other hand, higher activity of antioxidant enzymes (Gianni *et al.* 2004). In a study of laying hens (*G. g. domesticus*), heat exposure increased the production of free radicals and the level of lipid peroxidation and both enzymatic and non-enzymatic antioxidant systems (Lin *et al.* 2008).

Now, suppose that *A* and *B* do not differ in antioxidant capacity. Does this mean that *A* and *B* have same levels of oxidative damage? Actually, *B* may suffer higher oxidative damage when its production of free radicals is higher (Fig. 1e). This case may suggest two quite different interpretations: the antioxidant defences of *B* are still able to control pro-oxidant levels or are chronically deficient. In this latter case, the organism is unable to mount an adaptive response. A study on captive kestrels (*F. tinnunculus*) showed that, while having same serum antioxidant capacity and circulating carotenoids, oxidative damage had a 32% increase in kestrels supplemented with stress hormones (Costantini *et al.* 2008a).

Finally, what if *B* has lower pro-oxidant production than A, but still has higher antioxidants? In this case (Fig. 1f), *B* has lower oxidative damage and, so, oxidative stress than *A*. Nestling kestrels (*Falco tinnunculus*) exposed to immune response-induced free radicals have higher oxidative damage and lower serum antioxidant capacity than controls (Costantini & Dell'Omo 2006).

Given this array of scenarios (most of which can be illustrated with examples from the literature), we argue that total antioxidant capacity of a tissue or the level of a single class of antioxidants as markers of oxidative stress is not sufficient to make inferences about oxidative stress without supplemental information on oxidative damage or free radical production. Note that our aim is no more than to illustrate that each of our interpretations listed above can be valid in a particular case, and not to develop general guidelines as to how variation in antioxidant capacity should be interpreted.

Altogether, these studies show that different components of the redox system may respond differently to a challenge. Consequently, variation in antioxidant levels does not necessarily mirror the variation in oxidative stress that organisms experience. This variation is such that measuring total antioxidant capacity of a certain tissue or a single class of antioxidants alone may lead to interpretations regarding oxidative stress that are opposite to the correct interpretation (compare e.g. Fig. 1a,c, where the difference in antioxidants is similar, but the oxidative stress differs in opposite direction in the two situations). We are aware that the system may also be more complicated than those listed in our examples. In fact, species may greatly differ in their tolerance to stressors generating oxidative stress. For example, naked-mole rats have similar levels of pro-oxidants and antioxidants to those of mice and suffer higher oxidative damage, but naked-mole rats live substantially longer than mice (Hulbert *et al.* 2006). The low proportion of unsaturated lipids in cell membranes, combined with the higher susceptibility of unsaturated fatty acids to oxidative damage, has been suggested to explain the naked-mole rat paradox (Hulbert *et al*. 2006). Thus interpretation of interspecific variation in antioxidant capacity has additional complications, over and above the complexities in our comparisons between experimental groups that are (on average) similar in genetic background and physiology.

Although we see problems in making inferences regarding oxidative stress on the basis of information on antioxidant capacity alone, we like to stress that this does not mean that we consider data on antioxidant capacity uninteresting. Information on pro- and antioxidants will be required to understand how a particular level of oxidative stress arises, which is in itself an interesting issue. In this context experimental studies that manipulate anti- and pro-oxidants are of particular value (see e.g. Zhang *et al*. 2004; Galván & Alonso-Alvarez 2008) to further elucidate and examine the processes determining the level of oxidative stress. For example, it would be interesting to determine whether high antioxidant levels reflect a large reserve of unused antioxidants or up-regulated antioxidant production in response to oxidative stress. This is relevant in that individuals may differ in the levels of antioxidants (e.g. dietary antioxidants) stored in tissues, as well as in expression of genes modulating the redox system.

In conclusion, information on antioxidant capacity by itself is not sufficient to make inferences about oxidative stress. So, we suggest that a marker of antioxidant capacity should always be associated with at least a marker of oxidative damage when the aim is to make inferences about oxidative stress. A discussion of the merits of various markers of oxidative damage falls outside the scope of this article, but has been extensively discussed elsewhere (e.g., Prior & Cao 1999; Dotan *et al.* 2004; Yeum *et al*. 2004; Monaghan *et al.* 2009).

Acknowledgements

We thank the associate editor and three anonymous referees for valuable comments and constructive criticisms. S.V. was supported by an NWO Vici-grant. David Costantini dedicates this article in the memory of his mother, Nadia Macciocchi.

References

Beckman, K.B. & Ames, B.N. (1998) The free radical theory of aging matures. *Physiological Reviews*, **78**, 547–581.

Costantini, D. (2008) Oxidative stress in ecology and evolution: lessons from avian studies. *Ecology Letters*, **11**, 1238–1251.

Costantini, D. & Dell'Omo, G. (2006) Effects of T-cell-mediated immune response on avian oxidative stress. *Comparative Biochemistry and Physiology Part A*, **145**, 137–142.

Costantini, D., Fanfani, A. & Dell'Omo, G. (2008a) Effects of corticosteroids on oxidative damage and circulating carotenoids in captive adult kestrels (*Falco tinnunculus*). *Journal of Comparative Physiology B*, **178**, 829–835.

Costantini, D., Carere, C., Caramaschi, D. & Koolhaas, J.M. (2008b) Aggressive and non-aggressive personalities differ in oxidative status in selected lines of mice (*Mus musculus*). *Biology Letters*, **4**, 119–122.

Dotan, Y., Lichtenberg, D. & Pinchuk, I. (2004) Lipid peroxidation cannot be used as a universal criterion of oxidative stress. *Progress in Lipid Research*, **43**, 200–227.

Furness, L. & Speakman, J.R. (2008) Energetics and longevity in birds. *Age*, **30**, 75–87.

Gaál, T., Ribiczeyné-Szabó, P., Stadler, K., Jakus, J., Reiczigel, J., Kövér, P., Mézes, M. & Sümeghy, L. (2006) Free radicals, lipid peroxidation and the antioxidant system in the blood of cows and newborn calves around calving. *Comparative Biochemistry and Physiology Part B*, **143**, 391–396.

Galván, I. & Alonso-Alvarez, C. (2008) An intracellular antioxidant determines the expression of a melanin-based signal in a bird. *PLoS One*, **3**, e3335.

Gianni, P., Jan, K.J., Douglas, M.J., Stuart, P.M. & Tarnopolsky, M.A. (2004) Oxidative stress and the mitochondrial theory of aging in human skeletal muscle. *Experimental Gerontology*, **39**, 1391–1400.

Halliwell, B.H. & Gutteridge, J.M.C. (2007) *Free Radicals in Biology and Medicine*. 4th ed. Oxford University Press, Oxford.

Hulbert, A.J., Faulks, S.C. & Buffenstein, R. (2006) Oxidation-resistant membrane phospholipids can explain longevity differences among the longest-living rodents and similarly-sized mice. *Journal of Gerontology*, **61**, 1009–1018.

Hulbert, A.J., Pamplona, R., Buffenstein, R. & Buttemer, W.A. (2007) Life and death: metabolic rate, membrane composition, and life span of animals. *Physiological Reviews*, **87**, 1175–1213.

Lin, H., Decuypere, E. & Buyse, J. (2004) Oxidative stress induced by corticosterone administration in broiler chickens (*Gallus gallus domesticus*) 2. Short-term effect. *Comparative Biochemistry and Physiology Part B*, **139**, 745–751.

Lin, H., De Vos, D., Decuypere, E. & Buyse, J. (2008) Dynamic changes in parameters of redox balance after mild heat stress in aged laying hens (*Gallus gallus domesticus*). *Comparative Biochemistry and Physiology Part C*, **147**, 30–35.

López-Torres, M., Pérez-Campo, R., Cadenas, S., Rojas, C. & Barja, G. (1993) A comparative study of free radicals in vertebrates – II. Non-enzymatic antioxidants and oxidative stress. *Comparative Biochemistry and Physiology Part B*, **105**, 757–763.

Monaghan, P., Metcalfe, N.B. & Torres, R. (2009) Oxidative stress as a mediator of life history trade-offs: mechanisms, measurement and interpretation. *Ecology Letters*, **12**, 75–92.

Pérez-Campo, R., López-Torres, M., Rojas, C., Cadenas, S. & Barja, G. (1993) A comparative study of free radicals in vertebrates – I. Antioxidants enzymes. *Comparative Biochemistry and Physiology Part B*, **105**, 749–755.

Prior, R.L. & Cao, G. (1999) *In vivo* total antioxidant capacity: comparison of different analytical methods. *Free Radical Biology and Medicine*, **27**, 1173–1181.

Sies, H. (1991) *Oxidative Stress II. Oxidants and Antioxidants*. Academic Press, London.

von Schantz, T., Bensch, S., Grahn, M., Hasselquist, D. & Wittzell, H. (1999) Good genes, oxidative stress and condition-dependent sexual signals. *Proceedings of the Royal Society of London B*, **266**, 1–12.

Yeum, K.J., Russell, R.M., Krinsky, N.I. & Aldini, G. (2004) Biomarkers of antioxidant capacity in the hydrophilic and lipophilic compartments of human plasma. *Archives of Biochemistry and Biophysics*, **430**, 97–103.

Zhang, H.J., Doctrow, S.R., Xu, L., Oberley, L.W., Beecher, B., Morrison, J., Oberley, T.D. & Kregel, K.C. (2004) Redox modulation of the liver with chronic antioxidant enzyme mimetic treatment prevents age-related oxidative damage associated with environmental stress. *FASEB Journal* Express Article doi:10.1096/fj.04-1629fje.

Received 19 September 2008; accepted 21 January 2009
Handling Editor: Peeter Hõrak