

Nutrition Discussion Forum

Evidence in support of a concept of reductive stress

It is generally believed that free radical damage to biological systems is exerted by the oxidative action of hydroxyl radicals. Some researchers have, however, observed that many degenerative diseases are associated with a hypoxic state that results in an increased NADH:NAD⁺ ratio, leading to a reductive cytosolic environment (Ido *et al.* 1997). Yet a number of natural and synthetic substances shown to be beneficial in such pathological states have been paradoxically termed 'antioxidants' despite the fact that they exist in oxidized form, e.g. lipoic acid. It has been recently hypothesized (Ghyczy & Boros, 2001) that biomolecules with a positively charged N or S atom and a bound methyl group, termed electrophilic methyl group, react with NADH thus ameliorating reductive stress. In this letter, preliminary data are presented in support of the contention of Ghyczy & Boros (2001) that reductive rather than oxidative stress is of clinical importance.

An increasing interest in oxidative stress in diabetes mellitus (Lipinski, 2001) led to a search for a diagnostically useful method to measure the antioxidative potential of plasma. To generate hydroxyl radicals, ascorbic acid and cupric chloride were used. Unexpectedly, when these two substances were added in equimolar proportion to normal human plasma, a precipitate was formed containing mostly aggregated fibrinogen. To further investigate this phenomenon, a solution of purified fibrinogen was used and was found to form insoluble precipitate with ascorbic acid–cupric chloride mixture, however, at a concentration 10-fold lower than needed in plasma. The factor responsible for the inhibition of fibrinogen aggregation in plasma was then identified as human serum albumin. Although human serum albumin has been suggested to be an antioxidant due to the presence of one -SH group, all other thirty-four cysteines exist in the oxidized form as disulfides. Hence, this protein is rather unlikely to act as an effective antioxidant agent.

To elucidate a mechanism by which human serum albumin inhibits hydroxyl radical-induced fibrinogen aggregation, a spectrophotometric experiment was done in which ascorbic acid–cupric chloride was added to a solution of NAD and optical density recorded at 340 nm. A rapid increase in optical density proved that the hydroxyl radicals generated in this system have a reducing potential that was inhibited by the addition of human serum albumin. Molar proportions of the reagents used in the present experiment revealed that one molecule of human serum albumin neutralized fifteen hydroxyl radicals, indicating that about one-half of disulfide bonds in this protein underwent reduction. It was previously observed that hydroxyl radical modification of human serum albumin

led to the formation of higher molecular mass complexes (Davies & Delsignore, 1987), and that limited reduction with dithiothreitol caused its aggregation and coprecipitation with other plasma proteins (Lipinski & Egyud, 1992). Consequently, human serum albumin can be considered as a sacrificial antireductive protein which when modified by hydroxyl radicals gives a signal to proteolytic degradation and elimination from the circulation.

In conclusion, in view of the findings reported by Ghyczy & Boros (2001) and the results presented in this present letter, the concept of oxidative stress and antioxidants needs to be revised. Not only electrophilic methyl group biomolecules, but many other substances containing reducible groups may fall into a category of antireductants rather than antioxidants. For example, highly unsaturated eicosapentanoic acid also inhibited hydroxyl radical-induced fibrinogen aggregation. In addition, reductive addition of hydroxyl groups to double bonds of pyrimidines, pyrazines, and other aromatic rings may explain beneficial action of water soluble vitamins in free radical-induced stress (Moorthy & Hayon, 1976). Finally, similar reductive mechanisms may be involved in the formation of hydroxy derivatives of nucleic acids, e.g. 8-hydroxy-2'-deoxyguanosine, generally albeit speculatively, believed to be a result of oxidative stress (Park & Floyd, 1994).

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Evidence in support of a concept of reductive stress – Reply by Ghyczy & Boros

We are writing to agree with Dr B Lipinski's perceptive comments (Lipinski, 2002) and to suggest that they are of wide relevance. In particular, the importance of reductive stress should be more widely recognized. The prevailing view is extraordinarily blinkered. In PubMed (2001) there are 8957 citations to oxidative stress and none to reductive stress. This reflects not reality, but the mistaken but widely held ideas that oxidative stress is common, that it is the main source of biological free radicals, and that it is (or should be) susceptible to correction with antioxidants. In fact, extensive clinical and experimental work over the past 30 years has failed to reveal a single abnormal clinical state which could be confidently ascribed to oxidative stress or, more importantly, which has convincingly benefited from antioxidants. One reason is probably the initial difficulty of grasping the concept of reductive stress: another the relative paucity of experimental methods for demonstrating and measuring it.

Reductive stress can be defined as an abnormally increased electron pressure or 'reducing power' and it can occur either as a result of pathological processes leading to an excess of high-energy reducing electrons (as in NADH), a failure of mechanisms available for dealing with this rise in electron pressure, or, occasionally perhaps, a combination of both. It is probably not only more common than oxidative stress but it is also the main source of reactive oxygen species. We have suggested in our previous paper (Ghyczy & Boros, 2001): (1) that biomolecules with electrophilic methyl groups are potential electron acceptors and probably the main natural defences against reductive stress. Biomolecules which possess such electrophilic methyl groups are *S*-adenosylmethionine, betaine, carnitine, choline, glycerylphosphocholine, phosphatidylcholine, which have a trimethylnitrogen moiety. Of course there may be others. The manner in which they exercise this vital function is now under investigation.

However, the benefits of a such nutrients are being

recognized. Most importantly, in 1998, the National Academy of Sciences, USA, determined that choline is an essential vitamin (United States Food and Drug Administration, 2001), and the US Food and Drug Administration has recently agreed to allow new food labels to indicate choline content with phrases such as: 'a good source of choline' (United States Food and Drug Administration, 2001) Our preliminary results suggest that electrophilic methyl group-containing biomolecules, such as choline, may fulfil the criteria for a new class of functional food for the control of intracellular redox balance.

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