Total Antioxidant Capacity: Appraisal of a Concept¹,²

Helmut Sies*

Institute of Biochemistry and Molecular Biology I, Heinrich-Heine-University Düsseldorf D-40225, Düsseldorf, Germany

Abstract

In this contribution, I discuss the applicability of total antioxidant capacity (TAC) data obtained from plasma to human health issues and the use of TAC data for dietary items in epidemiological applications. Against the background of knowledge that major antioxidant defense is enzymatic, the use of the term “total” is not appropriate. Because dietary phytochemicals undergo uptake and metabolism, extrapolation to health effects requires direct molecular information, not a global parameter that uses an arbitrarily selected prooxidant source. Suitable alternatives are given in measuring functional biomarkers (surrogate endpoints). Although using TAC may be helpful in comparing different food items, the extrapolation to their contribution of antioxidant defense in vivo and, further, to health issues, should be discouraged, with the possible exception of the gastrointestinal tract. This is of particular importance because dietary phytochemicals and other small molecules have nonantioxidant activities. Direct assay of urate, ascorbate, and tocopherol, the major small-molecule contributors to TAC, is recommended. J. Nutr. 137: 1493–1495, 2007.

Introduction

The concept of “total antioxidant capacity” (TAC), which originated from chemistry and then was applied to biology and medicine, and further to nutrition and epidemiology, needs critical appraisal, because there are serious limitations that preclude meaningful application to in vivo conditions. This article briefly describes 1) strategies of antioxidant defense, 2) development of assay systems, and 3) applications in vitro vs. in vivo—all of which lead to 4) the problem of “comparing apples with oranges” in applying in vitro dietary composition data to in vivo plasma and tissue status.

Strategies of antioxidant defense

Cells, tissues, and body fluids are equipped with powerful defense systems that help counteract oxidative challenge. To maintain a steady-state of metabolites and functional integrity in the aerobic environment, antioxidant defense is organized at three principal levels of protection: prevention, interception, and repair (1). Matching the diversity of prooxidants, the antioxidant armamentarium comprises a widespread array of systems (antioxidant network).

Enzymes. Foremost, this system ranges from the classical enzymes, superoxide dismutases, glutathione peroxidases, and catalases to ancillary enzymes quenching or inactivating reactive intermediates, such as quinone oxidoreductases, and conjugation enzymes, such as glutathione S-transferases and UDP-glucuronosyl transferases, to name only a few. Further, there are essential backup systems required to maintain a steady-state, e.g., enzymes of NADPH supply or regenerating enzymes, such as glutathione disulfide reductase or thioredoxin reductase. Likewise, transport systems that export detoxication products, e.g., enzymes of NADPH supply or regenerating enzymes, such as glutathione disulfide reductase or thioredoxin reductase.

Adaptation. Regulation of the capacities of these antioxidant enzymes in response to changing levels of oxidative stress is a prerequisite for efficient defense. Short-term and long-term adaptation and cell specialization in these functions, as well as in the control of the activity of prooxidant enzymes such as NAPDH oxidases and nitric oxide synthases, have been elucidated in recent years. The molecular mechanism of the response to different redox states by the Keap1-Nrf2 system has recently...
been identified (2), a redox switch in fine-tuning the cellular response against oxidative and xenobiotic stress; dietary components are known to act via this mechanism.

**Small molecules, micronutrients, vitamins, and trace elements.** The contribution of small molecules to antioxidant defense occurs at several levels (1). One provides building blocks for the enzymes mentioned above, e.g., cysteine or its precursors for thiol groups in proteins and for the major intracellular thiol, glutathione, or selenium as part of the active-site selenocysteine in glutathione peroxidases and thioredoxin reductases (3). Small molecules also can take part directly in reactions of antioxidant character if located at strategically suitable sites and if present at appropriate concentrations. The latter applies, for example, to urate and ascorbate in the blood plasma, the former to α-tocopherol in the LDL in the plasma or in biological membranes of cells and subcellular compartments. It might be worth mentioning that defense by small molecules against the hydroxyl radical would require very high concentrations (e.g., 100 mmol/L mannitol as routinely used in vitro), so that defense against the hydroxyl radical by compounds occurring in the submicromolar range (e.g., melatonin) is not biologically feasible. Small molecules may become more important vis-à-vis the enzyme systems in specialized compartments. The stratum corneum of the skin is one example: it is composed of dead cells, which provide a shield from photooxidative stress.

**Development of assay systems: TRAP and a flurry of sequels**
A pivotal paper appeared in 1985, entitled: “*Quantitative measurement of the total, peroxyl radical-trapping antioxidant capability of human blood plasma by controlled peroxidation. The important contribution made by plasma proteins*” (4). Keith Ingold and his chemist colleagues used a thermolabile compound, 2,2′-azo-bis(2-amidinopropane) hydrochloride, to generate useful quantities of peroxyl radicals at a known and constant rate. They defined the total radical-trapping antioxidant parameter (TRAP) as a quantitative measure of the total secondary antioxidant content of a biological fluid. The main finding of this work was that urate, tocopherol, and ascorbate were the major small molecules contributing to TRAP, but that the combined contribution of these 3 antioxidants was only 27–43% of TRAP. The authors concluded that the majority of the TRAP could be accounted for by proteins, notably albumin (4).

A further publication (5) corroborated this, with contributions to TRAP from urate (35–65%), plasma proteins (10–50%), ascorbate (0–24%), and tocopherol (5–10%).

Although this was pioneering work in terms of highlighting plasma proteins, the authors of the TRAP article themselves and, unfortunately, apparently a good part of the scientific community subsequently fell into a “trap”: the assertion of “total” may have been chemically appropriate in the context of the in vitro assay, but the assay did not account for in vivo antioxidant enzyme activities in plasma. “Total,” therefore, is inappropriate.

**Application of TAC in vitro vs. in vivo**
There was a flurry of method development along the lines of TRAP: FRAP, TEAC, ORAC, etc.; some were based on hydrogen atom transfer, some on electron transfer reactions, and soon commercial kits were available, leading to numerous studies. As stated in an article on the chemistry behind antioxidant capacity assays, “it is very appealing to researchers to have a convenient method for the quick quantitation of antioxidant effectiveness in preventing diseases” (6). The term total antioxidant capacity, or TAC, emerged in an attempt to unify the concept. In a review by nutritionists, it was formulated as the “cumulative action of all the antioxidants present in plasma and body fluids, thus providing an integrated parameter rather than the simple sum of measurable antioxidants” (7). Unfortunately, against the backdrop of knowledge of enzymatic antioxidant defense (see above), these expectations are not founded, and are devoid of a molecular basis. Neither the term “total” nor the term “capacity” are applicable to the in vitro assays using an arbitrarily selected oxidant generator and assaying a sample removed from its biological context, which is characterized by enzymatic maintenance of steady state.

One other aspect may be exemplified here: large transient increases in the TAC of plasma have been observed after the consumption of flavonoid-rich foods by humans, leading to the hypothesis that dietary flavonoids play an important role as antioxidants in vivo, thereby decreasing chronic disease risk. However, flavonoids reach only very low (micromolar) concentrations in human plasma after the consumption of flavonoid-rich foods, and most are extensively metabolized in vivo, which affects their antioxidant capacity. Furthermore, fruits and vegetables contain many macro- and micronutrients in addition to flavonoids, which may directly, or through their metabolism, affect TAC of plasma. An overview of these issues (8) concluded that the large increase in plasma TAC observed after the consumption of flavonoid-rich foods is not caused by the flavonoids themselves, but is likely the consequence of the increased uric acid levels resulting from fructose metabolism.

“Comparing apples with oranges” in applying in vitro dietary composition data to in vivo plasma and tissue status
Many studies were carried out with extracts of food items, assaying the antioxidant capacity using the mentioned assays [e.g., (9)]. These may have their merits in comparing time courses (e.g., ripening) or different fruit varieties, but there is a fundamental flaw in trying to apply the numbers obtained from extracts of food items to nutrition and medicine. Uptake in the gastrointestinal tract, metabolism and excretion, and bio kinetics fundamentally modify the impact of a given food item. Furthermore, a given molecule might have health effects completely independent of any antioxidant property. If a compound or extract is active in vitro, but if it is not taken up in the gastrointestinal tract or if it is metabolized before or after uptake to products which are not redox-active, those in vitro data do not apply.

The problem of comparing apples with oranges with respect to TAC data becomes evident when plasma TAC data are combined with FFQ data, as in a recently published article (10). A potential application, however, may be given for the gastrointestinal lining before uptake or modification of the phytochemicals, which are useful in the removal of dietary prooxidants.

In conclusion, increased fruit and vegetable consumption is epidemiologically associated with a decreased incidence of cardiovascular diseases, cancer, and other chronic diseases. The beneficial health effects of fruits and vegetables have been associated with TAC, which is measurable in samples prepared from these foods. In this brief discussion, I contend that there are major potential flaws inherent in this association, and that there is little evidence to support cause-effect relations. By no means does this article dispute the potentially useful antioxidant effects of dietary micronutrients. My intent is to alert unsuspecting workers in nutrition, clinics, or epidemiology that there are pitfalls and serious limits of applicability of the TAC concept [see also (11)]. These are likely to be the reason for the numerous
“no effect” publications on dietary intervention and the TAC response. I suggest that investigators measure urate, ascorbate, and tocopherol if they are interested in nonprotein small-molecule antioxidants in human plasma.

Literature Cited