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PREFACE

The 8th World Congress on Developmental Origins of Health and Disease has just ended in Singapore (November 2013). It attracted nearly 1000 scientists from 52 countries. The diversity and number of participants signifies the importance of this topic. The "fetal origins hypothesis" was proposed in 1989 by Prof. David Barker, working at the University of Southampton. The fetal origins hypothesis suggests that fetal undernutrition in the first and second trimester of gestation leads to disproportionate growth and programs later coronary heart disease in adulthood. In 1995, *British Medical Journal* coined the term "Barker hypothesis." The notion that prepregnancy diet and fetal and postnatal growth (all influenced by nutrition) can contribute to long-term health outcomes is a major shift in nutritional paradigm. The Barker hypothesis may be the single most important medical observation of the twenty-first century. That adult disease may have its roots in early life, particularly related to the diet consumed and nutrition, enables us to potentially shape our health destiny by dietary interventions. Here is an example of close synergy between food, nutrition, and health. It testifies the need to better understand the mechanistic, metabolic, physiological, and nutritional consequences of early programming. *Advances in Food and Nutrition Research* is at the forefront of publishing papers on the interface of food and nutrition. In the years to come, we will see how dietary manipulation early in life can imprint and shape our health trajectory.

C. J. HENRY
Singapore and Oxford

CONTENTS

CHAPTER ONE

Antioxidants in Food: Content, Measurement, Significance, Action, Cautions, Caveats, and Research Needs

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Contents

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Abstract

There are a multitude of antioxidants in foods, especially in foods of plant origin. Higher intake of antioxidant-rich foods is clearly associated with better health and functional longevity. The specific agents and mechanisms responsible are not yet clear, but there is convincing evidence that including more plant-based, antioxidant-rich foods, herbs, and beverages in the diet is effective in promoting health and lowering risk of various age-related diseases. The content of some individual antioxidants, such as vitamin C, in food can be measured, but it is not feasible to attempt to measure each antioxidant separately, and methods have been developed to assess the "total antioxidant content" of foods. One of the most widely used methods is the ferric reducing/antioxidant power (FRAP) assay, which is relatively simple, quick, sensitive, and inexpensive to perform. There are many published studies that have used the FRAP assay, and these have generated a very large database of total antioxidant content of foods that can help guide food choices for increased antioxidant intake. The FRAP assay has also been used to assess the bioavailability of antioxidants in foods and to investigate the effects of growing conditions, storage, processing, and cooking method on the total antioxidant content of food. The test can be employed as a quality control check device, and to detect adulteration of food. Furthermore, in a modified form (FRASC), the assay can measure ascorbic acid content almost simultaneously with the total antioxidant content of the sample. In this chapter, basic concepts of oxidation and the role of antioxidants, as well as the types and action of different antioxidants in foods will be reviewed briefly, and the underpinning concepts and evidence for health benefits of increased intake of dietary antioxidants will be discussed, with some focus on vitamin C, and also in the context of our evolutionary development. The basic concepts and limitations of measuring "total antioxidant content" of food will be presented. The FRAP assay and the modified version FRASC will be described, and the total antioxidant content (as the FRAP value) of a range of foods will be presented. Finally, issues of bioavailability and redox balance will be discussed in relation to the biological significance and molecular action of antioxidants in foods, some caution and caveats are presented about overcoming biological barriers to absorption of antioxidant phytochemicals, and research needs to further our understanding in the important area of food, antioxidants, and health will be highlighted.

1. INTRODUCTION: ANTIOXIDANTS IN FOOD—THEIR ROLE AND IMPORTANCE

1.1. Basic concepts of oxidation and the role of antioxidants

In chemical terms, oxidation is the addition of oxygen to or the removal of hydrogen or an electron from a molecule. A biological antioxidant can be defined as a substance that prevents the oxidation of an important biomolecule, such as a fatty acid, DNA, or a protein, by a reactive oxygen species (ROS) (Ames, 1998; Halliwell & Gutteridge, 2007). ROS are partially reduced or "energized" forms of oxygen (Table 1.1). Some ROS contain an unpaired electron in an orbital and are sometimes referred to as "free radicals"; others, such as hydrogen peroxide and singlet oxygen, are nonradical species, but their reactivity is nonetheless greater than ground-state molecular oxygen, allowing them to oxidize biomolecules they interact with. Some ROS have important physiological functions (Ames, 1998; Halliwell & Gutteridge, 2007). For example, nitric oxide is a vasodilator, hydrogen peroxide is a key intracellular signaling molecule, and superoxide and hydrochlorous acid play an important role in our innate immune defense (Wink et al., 2011). However, an excess of ROS can lead to uncontrolled or indiscriminate oxidation of important biomolecules. This deleteriously affects their structure and function and is known as "oxidative stress" (Ames, 1998; Halliwell & Gutteridge, 2007). The imbalance that results in oxidative stress can be caused by excessive production of ROS, or to a deficit in the antioxidants that oppose ROS and their damaging oxidative effects (Fig. 1.1).

1.2. Antioxidants: Types and action

In an oxygenic environment, the threat of oxidative stress is ubiquitous and continuous for all organisms, but the photosensitizing plants that are responsible for our oxygenic environment are exposed to particularly high

Table 1.1 Types of ROS and relative reactivity

ROS	Redox couple	$E^{\circ\prime}$ (mV)
Hydroxyl radical	$HO^{\bullet}$, H^{+}/H_2O	+2310
Alkoxyl radical	$RO^{\bullet}$, H^{+}/ROH	+1600
Peroxyl radical	$ROO^{\bullet}$, $H^{+}/ROOH$	+1000
Glutathiyl radical	$GS^{\bullet}/GS^{-}$	+920
Tocopheroxyl radical	$TO^{\bullet}$, H^{+}/TOH	+480
Hydrogen peroxide	H_2O_2, H^{+}/H_2O, $HO^{\bullet}$	+320
Ascorbate radical	$Asc^{\bullet -}$, $H^{+}/AscH^{-}$	+282
Superoxide radical	$O_2/O_2^{\bullet -}$	−330

One-electron reduction potentials at pH 7.0.
Source: Halliwell and Gutteridge (2007).

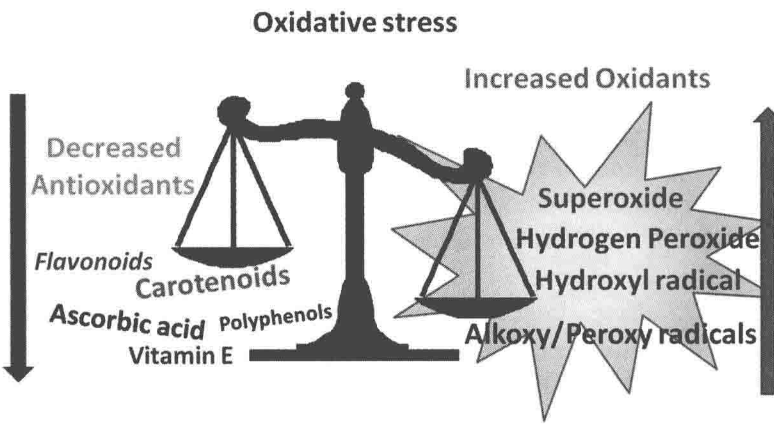

Figure 1.1 The concept of antioxidant/oxidant imbalance and the development of oxidative stress. (For color version of this figure, the reader is referred to the online version of this chapter.)

intracellular levels of oxygen and ROS and have evolved specialized defense factors to deal with the high oxidant challenge and protect plant structures and tissues (Benzie, 2000). These factors are referred to as antioxidants. The best-known antioxidant is vitamin C (ascorbic acid), but there are various others, and many are unique to the plant kingdom. These include "vitamin E," a group of eight tocopherols and tocotrienols, and the very large families of flavonoids and carotenoids (Benzie, 2005; Buettner, 1993; Gey, 1998; Tam et al., 2005). Indeed, there are thousands of different antioxidants in plants, and they work in different ways (Table 1.2). Some antioxidants prevent the generation of ROS, some are enzymes that destroy ROS, some are small water-soluble molecules that act as reducing (hydrogen or electron-donating) agents to "neutralize" free radicals, and some absorb electrons or excess energy from ROS and "dissipate" this within their complex lipophilic structure (Ames, 1998; Halliwell & Gutteridge, 2007). Overall, the effect is to oppose ROS action and limit oxidative stress.

1.3. A closer look at ascorbic acid: A key antioxidant

Vitamin C (ascorbic acid) is present in high concentration in many plants (Benzie & Wachtel-Galor, 2009; Frei et al., 1989; Gey, 1998; Halliwell & Gutteridge, 2007; Halvorsen et al., 2002). The content of this water-soluble antioxidant is particularly high in fruits, but it is found in various plant parts and aids repair and growth of plants and the ripening of seeds.

Table 1.2 Plant-derived antioxidant types, action, and sources

Antioxidant	Action	Mechanism	Sources
Ascorbic acid (vitamin C)	Scavenging of ROS	Sacrificial interaction by replaceable or recyclable substrates to scavenge and destroy ROS	Fruits and vegetables, particularly strawberries, citrus, kiwi, Brussels sprouts, cauliflower, some Chinese green vegetables
"Vitamin E" (α, β, δ, γ) isomers of tocopherols and tocotrienols	Quenching of ROS and chain breaking	Absorption of electrons and/or energy	Green leafy vegetables (e.g., spinach); nuts, seeds, especially wheat germ; vegetable oils, especially sunflower
Carotenoids (hundreds)	Chain breaking	Chain breaking at low partial pressures of oxygen, complements action of vitamin E	Orange/red fruits and vegetables (e.g., carrot, tomato, apricot, melon, pumpkin), green leafy vegetables, peppers
Flavonoids (large range of different types)	Scavenging of ROS	Sacrificial interaction	Berries, apples, onions, tea, red wine, some herbs (parsley, thyme) citrus fruits, grapes, cherries

Source: Halliwell and Gutteridge (2007).

Most animals, fish, and reptiles can synthesize vitamin C in liver or kidney, but humans have lost the ability to make ascorbic acid, even though we have retained an absolute requirement for it (Benzie, 2000, 2003; Benzie & Strain, 2005; Frei et al., 1989). Ascorbic acid is known to be needed for collagen synthesis in humans, and severe deficiency results in scurvy, which is nowadays rare but cases are nonetheless still reported (Doll & Ricou, 2013). Humans must obtain a regular and adequate dietary supply to maintain plasma and tissue levels, and the best dietary sources are fruits and vegetables (Frei et al., 1989). In the past, the daily recommended intake of vitamin C was set at a level (~30 mg/day) that would prevent overt deficiency (Newton et al., 1983). However, accumulated epidemiological and experimental evidence indicates that the role of ascorbic acid in the human body is not restricted to collagen synthesis and that it has an important role in health maintenance overall (Benzie, 1999). Ascorbic acid is recognized as a major player in human antioxidant defense (Benzie, 1999, 2000). Current dietary recommendations are for at least 70 mg/day for women, 100 mg/day for men, with higher levels advised in pregnancy and for smokers (Carr & Frei, 1999). These levels of intake are recommended to attain "optimal" status for health, though this has not been clearly defined. Human cells, especially white blood cells, and some organs, most notably the eye, contain millimolar concentrations of ascorbic acid (Choy et al., 2001, 2003). Plasma ascorbic acid concentrations in the fasting state range quite widely, from <10 (worryingly low) to ~100 μmol/l in apparently healthy adults (Choi et al., 2004). Tissue and plasma levels are maintained by, and so reflect, dietary intake of vitamin C, and the plasma concentration of ascorbic acid can act as a marker of fruit and vegetable intake (provided no supplements are used) (Choi et al., 2004). A concentration <10 μmol/l indicates very low intake and severe deficiency, even if signs of scurvy are not clearly present (Levine et al., 2011). Plasma levels at 1–2 h after ingestion of a large dose (1 g or more) of vitamin C can approach 200 μmol/l, but peak levels are limited by restricted gastrointestinal absorption of a large dose and by urinary loss of ascorbic acid when the plasma concentration exceeds the renal threshold of ~100 μmol/l (Levine et al., 2011). Therefore, most of a single, large dose is not retained in the body, and regular, modest doses (500 mg or less) are likely to be more effective in enhancing the ascorbic acid status of the body. In large prospective trials, it has been shown that healthy people with higher fasting plasma concentrations of food-derived ascorbic acid (i.e., not from supplements) have significantly lower risk of incident diabetes, cancer, stroke, heart failure, and all cause mortality in the ensuing years (Fig. 1.2; Harding et al., 2008; Pfister et al., 2011). It has been estimated that for every 20 μmol/l increase in fasting plasma ascorbic acid concentration, there is a 9% relative reduction in risk of incident heart failure and a 29% decrease in risk of incident type 2 diabetes (Harding et al., 2008; Pfister et al., 2011). Plant-based food is the primary source of ascorbic acid in human plasma. However, it must be noted that the protective agent may not be vitamin C itself. It could be something very closely associated with vitamin C in plant-based foods, or it could be that the health benefits are due to the interactive effects of the various antioxidants present in plant-based foods.

1.4. Human antioxidant defense and dietary influences

The human body is exposed to ROS on a continuous basis, and we have an effective antioxidant defense system that has evolved to help deal with the

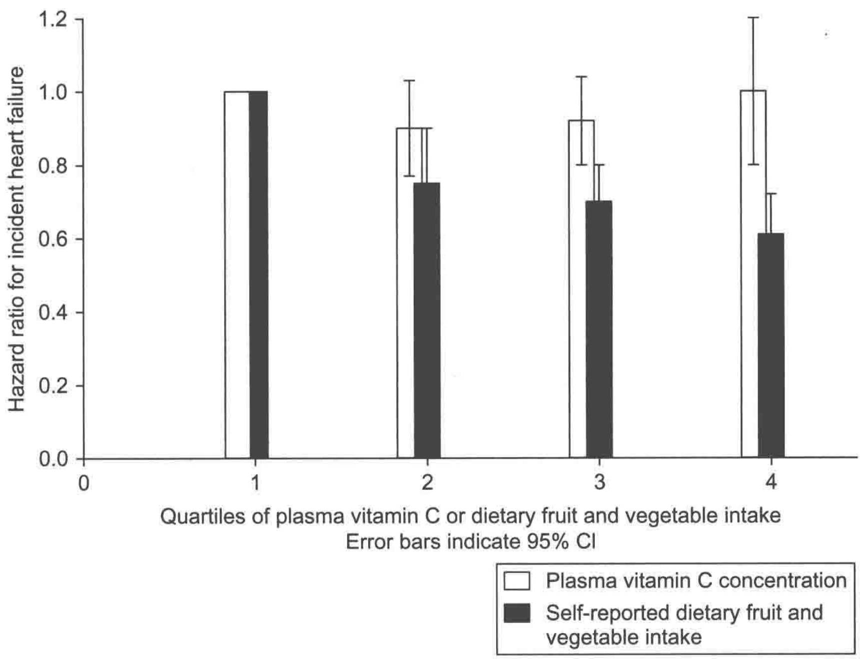

Figure 1.2 Risk for incident heart failure decreases with increased plasma vitamin C in subjects observed over a period of 16 years. *Data taken from Pfister et al. (2011).* (For color version of this figure, the reader is referred to the online version of this chapter.)

threat of oxidant challenge (Benzie, 2000, 2003). The system is complex and, as with plants, there are different types of antioxidants that prevent generation of, divert, or destroy ROS (Benzie & Wachtel-Galor, 2009). Our intrinsic antioxidant defense is highly effective but not wholly adequate, and dietary input of antioxidants is needed (Benzie, 2003; Benzie & Wachtel-Galor, 2009; Norat et al., 2014). Plant-based foods and beverages, such as fruits, vegetables, tea, coffee, spices, and herbs, are the main source of antioxidants in the human diet (Benzie & Wachtel-Galor, 2009; Halvorsen et al., 2006). It is noted that the only dietary-derived antioxidants that have been shown to be essential for human health are the water-soluble vitamin C and the lipophilic vitamin E (which in human tissues and structures is mainly in the form of α-tocopherol). Still, conceptually at least, the other antioxidants that evolved to deal with oxidant challenge in plants could have a role to play in defending human tissues from this same challenge, augmenting our endogenous antioxidant system in the prevention of oxidative stress. To what extent they do this and their contribution to health outcomes are not yet clear. Plant-derived antioxidants including the catechins and some anthocyanins, carotenoids, xanthophylls, and other members of the vitamin E family are found in human plasma (Table 1.3), and the antioxidant capacity of human plasma increases after intake of polyphenol-rich dietary agents such as tea and coffee (Benzie et al., 1999; García-Alonso et al., 2006; Hukkanen et al., 2006; Molan et al., 2008; Othman et al., 2007; Seeram et al., 2008). However, the very low (nanomolar) concentrations of the "nonessential" plant-derived antioxidants have made it difficult to study

Table 1.3 Plant-derived antioxidants and their typical concentrations in fasting plasma

	Concentration (μM)	Food source
Ascorbic acid	10–60	Fruits, particularly kiwi fruits, strawberries
Vitamin E (α-tocopherol)	16–40	Wheatgerm, sunflower seeds, nuts
Epicatechin	0.005	Tea, chocolate, wine
Epicatechin gallate	0.041	
Epigallocatechin gallate	0.016	
Epigallocatechin	Undetectable	
Catechin	Undetectable	
Quercetin	0.001	Apple, onion, red grape, leafy green vegetables
Anthocynanin	0.046	Aubergine, blackberry, blackcurrant
Lutein	0.160	Green leafy vegetables, spinach, kale
Zeaxanthin	0.050	Wolfberry, egg yolk, corn, paprika
Lycopene	0.377	Tomatoes, guava, grapefruit, watermelon
B-carotene	0.354	Sweet potato, carrots, kale, butternut squash
Phytoene	0.069	Most fruits and vegetables
Phytofluene	0.044	Most fruits and vegetables
Isoflavones	0.428	Soya, chickpea, peanut, alfalfa

Source: Benzie et al. (1993), Halliwell and Gutteridge (2007), Harding et al. (2008), and Engelmann et al. (2011).