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Review Article

The effects of dietary restriction on oxidative stress in rodents

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ABSTRACT

Oxidative stress is observed during aging and in numerous age-related diseases. Dietary restriction (DR) is a regimen that protects against disease and extends life span in multiple species. However, it is unknown how DR mediates its protective effects. One prominent and consistent effect of DR in a number of systems is the ability to reduce oxidative stress and damage. The purpose of this review is to comprehensively examine the hypothesis that dietary restriction reduces oxidative stress in rodents by decreasing reactive oxygen species (ROS) production and increasing antioxidant enzyme activity, leading to an overall reduction of oxidative damage to macromolecules. The literature reveals that the effects of DR on oxidative stress are complex and likely influenced by a variety of factors, including sex, species, tissue examined, types of ROS and antioxidant enzymes examined, and duration of DR. Here we present a comprehensive review of the existing literature on the effect of DR on mitochondrial ROS generation, antioxidant enzymes, and oxidative damage. In a majority of studies, dietary restriction had little effect on mitochondrial ROS production or antioxidant activity. On the other hand, DR decreased oxidative damage in the majority of cases. Although the effects of DR on endogenous antioxidants are mixed, we find that glutathione levels are the most likely antioxidant to be increased by dietary restriction, which supports the emerging redox-stress hypothesis of aging.

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Introduction

Denham Harman conceived the first iteration of the free radical theory of aging in 1956 [1]. Harman based his theory on the presumption that life span is dependent on metabolic rate—a hypothesis that has since fallen out of favor [2]. He proposed that toxic by-products of metabolism, notably the hydroxyl radical and protonated superoxide, can damage proteins and nucleic acids and lead to cancer and aging. The observations that mitochondria produce the majority of oxygen radicals in the cell and that reactive oxygen species include

Abbreviations: DR, dietary restriction; ROS, reactive oxygen species; PM, pyruvate/malate; S, succinate; AA, antimycin A; R, rotenone; Cat, catalase; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione; GST, glutathione *S*-transferase; SOD, superoxide dismutase; NADPH, nicotinamide adenine dinucleotide phosphate.

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nonradicals like hydrogen peroxide led to the development of the oxidative stress theory of aging [3]. Oxidative stress results from an imbalance in the rate of reactive oxygen species (ROS) production and detoxification. There is strong support for the oxidative stress theory of aging in invertebrate models, especially *Drosophila*, although data in rodent models are inconclusive as to whether and how oxidative stress affects the aging process [4]. Even more recently, a spinoff of the oxidative stress theory of aging, the redox-stress hypothesis, implicates redox-sensitive signaling pathways in the aging process. The redox stress hypothesis is supported by an oxidizing shift in the cellular redox balance during aging [5–8], which is regulated by the glutathione and thioredoxin systems [9–12]. This redox imbalance can lead to altered protein function and gene transcription [13].

Oxidative stress has conclusively been shown to be associated with aging and age-related diseases, including cancer [14,15], neurodegeneration [16,17], cardiovascular disease [18,19], and diabetes [20]. A number of studies suggest that dietary restriction can protect against these oxidative stress related diseases, including cancer [21], neurodegeneration [22], and cardiovascular disease [23–25]. DR also prevents a number of age-related pathologies, including loss of myenteric neurons [26], hearing loss [27], cataracts [28], insulin resistance [29,30], and skeletal muscle loss [31,32]. Although DR is generally thought to reduce oxidative stress, these data are mixed and have not been comprehensively reviewed. Three mechanisms may be responsible for the antioxidant effects of dietary restriction. Specifically, DR could reduce reactive oxygen species production, increase antioxidant enzyme activity or increase the turnover of oxidized macromolecules. These mechanisms are interrelated and often result in confounding results. For example, DR might lead to decreased expression of antioxidant enzymes, but this could be due to reduced production of reactive oxygen species [33]. Here we will review the effects of DR on ROS production, antioxidant enzyme activity, and oxidative damage and discuss potential mechanisms for how these effects are achieved.

The following three hypotheses provide a useful framework to test the role of oxidative stress in aging. First, oxidative damage should increase with age; second, manipulations that delay aging should attenuate the age-related change in oxidative damage; and third, specifically modulating the presumptive age determinants in old animals should reverse functional decline. For the first hypothesis, a number of studies have observed increased reactive oxygen species production and oxidative damage, although increased oxidative stress is not universal, as discussed below. For the second hypothesis, dietary restriction, reducing growth hormone, and insulin-like growth factor 1 signaling as well as reducing mammalian target of rapamycin signaling with rapamycin are common dietary, genetic, and pharmacological interventions, respectively [34–37]. Dietary restriction is the antiaging paradigm most commonly used to test the oxidative stress theory of aging, and will be discussed extensively in this review. The third hypothesis has generated the most damning evidence against the oxidative stress theory of aging. Attempts to modulate oxidative stress using mice deficient in or overexpressing antioxidant enzymes do not generally support the oxidative stress theory of aging [38]. Two exceptions to this generalization are the mitochondrially targeted catalase overexpressing mouse [39], which is long-lived, and the copper zinc superoxide dismutase deficient mouse, which is short-lived [40]. The purpose of this review is not to make a definitive statement on the role of oxidative stress in aging. Rather, our primary objective is to determine the effect of dietary restriction on oxidative stress. As an aside, we will evaluate the effects of DR on oxidative stress during aging.

Dietary restriction is the most well-studied aging intervention in rodents, although the effects of DR depend on the extent of restriction, age, sex, species, strain and duration of restriction. Dietary restriction extends life span in many, but not all, strains of mice and rats [41,42]. Protocols for dietary restriction in rodents vary in extent and duration of feeding, with reductions in food from 10 to 60% and durations from 1 week to the entire postweaning life span. If a standard protocol exists for long term DR, it would be a gradual reduction in food availability after maturation to 40% relative to animals fed *ad libitum*, and then maintaining this level throughout the study. Some studies use a nutrient supplement for the restricted group, which is considered calorie restriction, while other studies use alternate day feeding or intermittent fasting, in which animals are fed *ad libitum* on some days and fed nothing on others. Although less well studied, alternate day feeding and intermittent fasting produces many, but not all [43–45], of the beneficial effects of DR, including increasing life span [41]. The variety of restriction protocols makes comparisons among studies difficult, but in this review we comprehensively surveyed the available literature to search for trends in the effects of dietary restriction on oxidative stress.

Dietary restriction and reactive oxygen species production

For all of our analyses, we included models of DR with differing duration and percentage restriction without regard to sex, species, or strain. Because mitochondria are a major source of reactive oxygen species (ROS) production in the cell, and because other sources of ROS have been minimally studied with dietary restriction, we only included studies of mitochondrial ROS production. A systematic review of the literature uncovered 157 observations of mitochondrial ROS production using dietary restriction in rodents (Supplemental Table 1) [33,46–67]. Many reports used isolated mitochondria preparation, saturating concentration of substrates, and ambient oxygen tension (20%), which subjects mitochondria to hyperoxia relative to the *in vivo* environment. Nevertheless, analysis of ROS production in isolated mitochondria is informative as to approximate the maximal production of ROS seen *in vivo* without scavenging. For mitochondrial ROS production, 96% of observations were made in males, with 4% in either female or mixed sex populations, and 63% of observations were in rats. ROS production was more likely to be reduced with dietary restriction in mice (Fisher's exact test, $P < 0.001$). Most studies measured hydrogen peroxide production with the remaining studies measuring superoxide or other, sometimes nonspecific, reactive oxygen species. The distribution of tissues studied reveals that most studies were performed in the liver, heart, or skeletal muscle (Table 1). *In total, 62% of observed measurements reported no change with DR and 37% reported a decrease in ROS production.* Below, we will discuss the effects of DR on ROS production in different tissues and with various electron transport chain substrate and inhibitor combinations.

In Table 2, we have summarized the effects of dietary restriction on ROS production with specific substrate and substrate/inhibitor

Table 1
Percentage of studies per tissue that examined mitochondrial ROS production, antioxidant enzyme activity and oxidative damage.

Tissue	Reactive oxygen species Ć ($n=157$)	Antioxidants Ć ($n=350$)	Damage ($n=315$)
Brain	10	22	22
Heart	19	14	12
Kidney	4	13	6
Liver	52	37	35
Sk. muscle	15	5	15
Other	0	10	10

Liver, brain, heart, skeletal muscle, and kidney are the most studied tissues in analyses of dietary restriction and oxidative stress.