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Measurement of F_2-Isoprostanes Unveils Profound Oxidative Stress in Aged Rats

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Free radicals have been theorized to play a causative role in the normal aging process. To date, methods used to detect oxidative stress in aged experimental animals have only detected 2- to 3-fold differences or less between young and aged animals. Measurement of F_2-isoprostanes has emerged as probably the most reliable approach to assess oxidative stress status *in vivo*. Therefore, we measured levels of F_2-isoprostanes free in plasma and levels esterified in plasma lipids in young rats (3–4 months of age) and aged rats (22–24 months of age). Plasma concentrations of free F_2-isoprostanes were increased dramatically by a mean of 20.3-fold (range 4.3 to 42.9-fold) and levels esterified in plasma lipids were also strikingly increased by a mean of 29.9-fold (range 15.8 to 50.0-fold). These findings unveil profound oxidative stress in aged rats which adds considerable support for the free radical theory of aging. © 2001 Academic Press

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The tenet of the free radical theory of aging is that there is an imbalance between oxidative stress and the ability of antioxidant defenses to keep oxidative stress in check such that there is an accrual of irreparable oxidative damage to proteins, lipids, and DNA over time (1–8). The single intervention that has consistently been found to increase life-span in experimental animals is caloric restriction (9–13). Decreased mitochondrial respiration is thought to be the underlying mechanism by which caloric restriction prolongs life span. This links to the free radical theory of aging because superoxide generation is a byproduct of mitochondrial respiration; as much as 1–2% of oxygen utilized by mitochondria may be converted to superoxide (14). It is thought that free radical damage to critical biomolecules in mitochondria increases during aging resulting in enhanced superoxide generation. This notion is supported by the findings in nematodes that mutations in mitochondrial succinate dehydrogenase cytochrome b is associated with shortened life span and that administration of superoxide dismutase/catalase mimetics prolongs life span (15, 16).

Support for the free radical theory of aging has been sought by measuring a variety of markers of oxidant injury in experimental animals, including products of lipid peroxidation, protein oxidation, and DNA oxidation. However, levels using these measurements in aged animals have only been approximately 2- to 3-fold or less higher compared to levels found in young animals (3, 8, 13, 17–24). While these increases are not inconsistent with the free radical theory of aging, the small differences found do not provide compelling support for the theory. Nonetheless, the findings that caloric restriction can attenuate the small increases in markers of oxidative stress detected during aging supports a role for mitochondrial generation of free radicals underpinning the aging process. (11, 13, 20,23).

One possible reason why only small differences in markers of oxidative injury have been detected in aged compared to young animals may be due to shortcomings of the methods used to assess oxidative stress status *in vivo* (8). In this regard, measurement of F_2-isoprostanes (F_2-IsoP) has emerged over the last 10 years as probably the most reliable approach to assess oxidative stress status *in vivo* (25, 26). F_2-IsoPs are prostaglandin F_2-like compounds that are formed nonenzymatically by free radical induced peroxidation of arachidonic acid (27). IsoPs are initially formed esterified to phospholipids and subsequently released by phospholipase action (28). Thus, free F_2-IsoPs can be measured in plasma to assess total endogenous IsoP production. Levels esterified in tissues or plasma lipids can be measured to localize lipid peroxidation in key tissues or sites of interest. Therefore, we explored whether measurement of F_2-IsoPs free in the circula-

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tion and esterified to plasma lipids in young and aged rats may provide more compelling support for the free radical theory of aging than has been obtained previously using other markers of oxidant injury.

MATERIALS AND METHODS

Rats. Male Sprague–Dawley rats were purchased from Harlan Industries (Indianapolis, IN) and allowed to age to 22–24 months. Rats were maintained on standard rat chow (Teklad, Harlan SD, Indianapolis, IN) and tap water and exposed to 12-h light/12-h dark cycles. Animals were anesthetized with pentobarbital (50 mg/kg), the abdomen opened, and blood was drawn from the aorta. The animal protocols complied with the *Guideline for Care and Use of Laboratory Animals* by the NIH and were approved by the Institutional Animal Care and Use Committee of the University of Mississippi Center.

Measurement of F_2-IsoPs. Plasma was stored at −70°C until analyzed. F_2-IsoPs free in plasma and esterified in plasma lipids were quantified by negative ion chemical ionization gas chromatography mass spectrometry as described previously (29). The precision of the assay is ±6% and the accuracy is 96%.

RESULTS AND DISCUSSION

Shown in Fig. 1 are the levels of free circulating F_2-IsoPs measured in young (n = 12) and aged rats (n = 11). F_2-IsoP levels in the aged animals were profoundly increased by a mean of 20.3-fold (range 4.3–42.9-fold) compared to levels measured in young animals. Shown in Fig. 2 are levels of F_2-IsoPs esterified in plasma lipids in young and aged animals. Again, F_2-IsoP levels in the aged animals (n = 5) were increased dramatically by a mean of 29.9-fold (range 15.8–50.0-fold) compared to levels measured in young animals (n = 5). Importantly, we found similar increases in both free levels in the circulation and levels esterified in plasma lipids, which derive from different sources. Measurements of F_2-IsoPs esterified in plasma lipids reflect only plasma lipid oxidation whereas levels of free F_2-IsoPs in the circulation derive from hydrolysis of F_2-IsoPs from all sites in the body. The fact that both free and esterified levels of F_2-IsoPs in plasma were increased to a similar extent adds a dimension of validity to the conclusion that the aged animals are under a marked general oxidant stress. The magnitude of the increases in levels of F_2-IsoPs both free in the circulation and esterified in plasma lipids in aged rats compared to young rats is distinctively much higher than has been previously been detected with other markers of oxidative injury/stress. Data has been obtained suggesting that, at least in regard to protein carbonyls and DNA oxidation, levels of oxidative injury markers increase in a nonlinear or exponential fashion during aging (3, 22). However, the very high levels of F_2-IsoPs found in this study cannot simply be attributed to the age of the animals studied; other studies measuring other markers of protein, DNA, and lipid oxidation have only found ~2- to 3- fold increases in animals of similar age (22–24 months) or older (3, 8, 17, 19–24). With this data in hand, it will be of considerable interest in future studies to explore the time-course of increase in F_2-IsoP levels in animals that span ages from young to very old. It will also be of interest to correlate the time-course of the increases of F_2-IsoPs, which detect lipid peroxidation, with other markers of protein and DNA oxidation and with levels of antioxidant enzymes. In conclusion, the finding that plasma F_2-IsoPs levels are profoundly increased in aged rats adds considerable support to the free radical theory of aging.

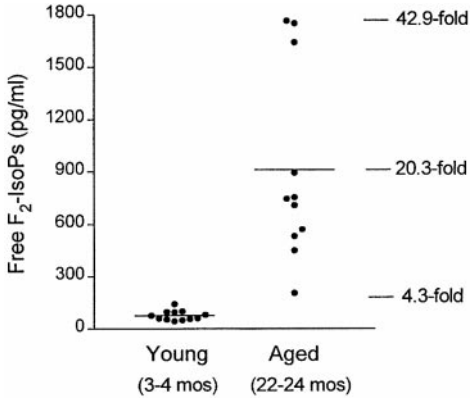

FIG. 1. Levels of free F_2-IsoPs in plasma of young and aged rats.

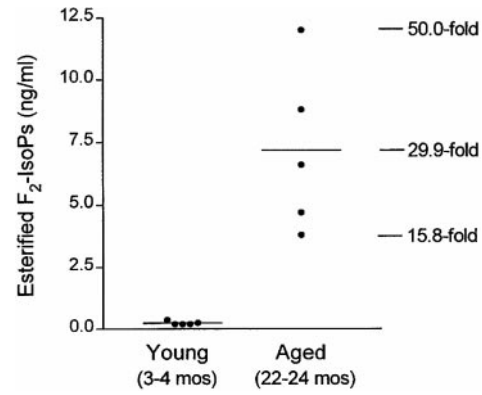

FIG. 2. Levels of F_2-IsoPs esterified in plasma lipids of young and aged rats.

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