

Linkage between insulin and the free radical theory of aging

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THE AGING PROCESS RESULTS in an accelerated decline of functional capacity, but the mechanisms behind this decline are unclear (21, 22). The free radical theory of aging proposes that mitochondrial production of reactive oxygen species (ROS) causes an increase in cellular damage with age (9, 10). This theory has gained strong support because it is consistent with many of the processes and degenerative diseases observed with aging (9, 22). Interestingly, reducing the energy intake of rodents by 30–50%, achieved with a calorie-restricted diet, decreases the rate of mitochondrial ROS generation (7, 8, 25) and has been repeatedly shown to increase both mean and maximum life span of a variety of animals, including rodents, teleost fish, insects, and spiders (18, 27, 28). The mechanism for the anti-aging effects of a calorie-restricted diet are not clear, but it is widely assumed that it partly results from decreased mitochondrial ROS production and subsequent reduction in oxidative damage.

In parallel, caloric restriction has been shown to decrease plasma insulin levels (6, 27), and a number of studies have demonstrated that insulin-signaling pathways are involved in determining the rate of aging. In the short-lived species *Drosophila melanogaster* and *Caenorhabditis elegans*, single gene mutations that result in reduced insulin and/or insulin-like growth factor (IGF) signaling cause increased life span as well as increased resistance to oxidative stress and other chemical stressors (17, 26). In comparatively long-lived mice, transgenic inactivation of the IGF-1R gene (IGF-1r) leads to increased stress resistance and increased longevity in female IGF-1r(+/-) mice, who live 33% longer than wild-type females (12), with energy metabolism, nutrient uptake, physical activity, fertility, and reproduction being unaffected. Interestingly, life span is not significantly increased in male IGF-1r(+/-) mice, which have higher glucose levels than females in the fed state and a stronger glucose response after a glucose tolerance test.

Although decreased mitochondria ROS production and decreased plasma insulin are both effects of calorie restriction, it has not been known whether insulin is mechanistically linked to mitochondrial oxidant production. In this issue of the *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, Lambert and Merry (14) provide the first evidence that caloric restriction decreases the mitochondrial proton motive force by increasing the mitochondrial proton leak and that this leak is dependent, at least in part, on plasma insulin levels. Mitochondrial proton leak, which is thus far not well understood, normally accounts for up to 30% of cellular O₂ consumption at rest (23, 24). Importantly, aging increases the proton leak in mouse hepatocytes (11), and long-term caloric restriction reduces the aging-induced proton leak in mouse skeletal muscle mitochondria (13).

In their study, Lambert and Merry (14) isolated liver mitochondria from three groups of male rats: ad libitum-fed, caloric-restricted, and caloric-restricted rats supplemented for 2 wk with insulin delivered from a miniosmotic pump implanted subcutaneously. Calorie restriction was achieved by limiting dietary intake such that body weights were maintained at 55% of the age-matched, ad libitum-fed rats. For each treatment group, the investigators then measured mitochondrial H₂O₂ production (an indicator of ROS production) and both mitochondrial membrane potential and O₂ consumption during state III respiration (oxidative phosphorylation) and state IV respiration (oxidative phosphorylation inhibited). This allowed them to perform modular metabolic control analyses to determine whether differences in mitochondrial bioenergetics existed between the treatment groups. In this type of analysis, developed for mitochondrial bioenergetics largely by Brand and colleagues (19, 23), the mitochondrial protonmotive force, Δp , is modeled as being produced by the flux of protons through substrate oxidation, J_S , and consumed by the fluxes of protons through substrate phosphorylation, J_P (i.e., ADP to ATP), and proton leak activity, J_L . Therefore, if Δp is affected by calorie restriction, this effect must be due to changes in the proton fluxes J_S , J_P , and J_L .

Lambert and Merry (14) found that calorie restriction decreased mitochondrial H₂O₂ production rate with no change in mitochondrial respiration rate (O₂ consumption), consistent with previous studies (2, 7, 8, 25), but observed a decreased mitochondrial membrane potential. The modular metabolic control analysis indicated that at a given Δp , mitochondria from calorie-restricted rats had decreased J_S and increased J_P and J_L , with the largest factor being the change in J_L . Therefore, calorie restriction appears to reduce the protonmotive force and possibly ROS generation, specifically by increasing the proton leak. Perhaps most remarkably, insulin treatment for 2 wk inhibited the effects of calorie restriction on mitochondrial ROS production, membrane potential, Δp , J_S , J_P , and J_L . The changes in Δp and ROS production were only partly inhibited by insulin, but this may be because insulin supplementation did not bring the plasma levels up to that of the rats fed an ad libitum diet (doing so apparently would have caused hypoglycemia).

This appealingly straightforward study is the first to single out insulin as a major player in determining the rate of ROS production. It is tempting to speculate that the antiaging effects of calorie restriction are due largely to decreased insulin levels and the resulting decreased Δp and ROS production. In further support of this, in long-lived IGF-1r(+/-) mice (12), the stress-regulated p66 Shc is underphosphorylated in IGF-1R deficiency, suggesting a plausible mechanism connecting IGF signaling to oxidative stress. However, extension of life span by calorie restriction in mammals is associated with many other changes, at least some of which may not be linked to insulin or ROS production (2, 5, 7, 16, 20). Interestingly, caloric restriction in both rats and rhesus monkeys results in a marked increase in insulin sensitivity within 5 days (6, 15).

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This effect occurs before significant losses in body fat and is not caused by increased expression of GLUT-4 (insulin regulatable glucose transporter protein in skeletal muscle). Instead, calorie restriction enhances the cell signaling mechanisms to stimulate the translocation of GLUT-4 from the interior of the cell to the surface membranes, making the transporters more accessible to extracellular glucose and thereby increasing glucose transport rate (6, 15).

In mammals, the inability to synthesize or respond appropriately to insulin results in diabetes, which is a disease with many features of premature aging (22). Diabetes is associated with increased advanced glycation end products and lipoxidation end products, specifically in long-lived proteins in the extracellular space (3, 4). These end products, which potentially impair tissue function, also accumulate in tissues with age (3, 4, 22). Furthermore, long-term calorie restriction reduces the age-associated rise in glycation of hemoglobin, plasma proteins, and glycoxidation end products in skin collagen (4), suggesting that differences in glycoxidation chemistry contribute to the increased longevity seen with calorie restriction.

The study by Lambert and Merry (14) gives renewed importance to determining the mechanism and regulation of the mitochondrial proton leak pathway and raises a number of other intriguing future research questions. These include determining which mitochondrial electron transport complex is affected by the insulin treatment (1), clarifying the effects of insulin level and duration of calorie restriction on mitochondrial energetics, defining the signaling pathway by which insulin regulates both oxidant production and the components of mitochondrial bioenergetics and determining whether short-term calorie restriction has similar effects on mitochondrial energetics (8).

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