All animals have limited life spans, and work in a variety of organisms has established that caloric restriction results in increased longevity. For example, inbred female mice fed an ad libitum diet had an average life span of 27 months, whereas calorie-restricted mice fed a diet with 65% fewer calories had an average life span of 45 months.1 The mechanisms by which caloric restriction retards aging and promotes longevity are likely manifold, but reduced insulin action in various tissues is a well-accepted mechanism of this phenomenon. Indeed, in the nematode Caenorhabditis elegans, a loss-of-function mutation in the insulin receptor-like gene daf-2 significantly promoted longevity.2 In mice, generalized overexpression of the insulin/insulin-like growth factor signaling inhibitor Klotho resulted in animals that had significantly increased longevity and reduced insulin sensitivity.3 In addition, tissue-specific targeted disruption of the insulin receptor gene in the adipose tissue of mice led to significantly increased life span.4

Insulin receptor stimulation leads to the activation of the intracellular lipid kinase phosphoinositide 3-kinase (PI3K). A loss-of-function mutation in the age-1 gene that encodes a PI3K also promotes increased longevity in C elegans.5 Activated PI3K that is localized at the internal surface of the plasma membrane generates phosphatidylinositol-3', 4', 5'-phosphate (PIP3). PIP3 activates various kinases and ion channels, including phosphatidylinositol-dependent kinase 1, which, in turn, activates Akt family members among other kinases.6,7 A loss-of-function mutation in the C elegans pdk1 gene resulted in significantly increased life span in worms.8 Furthermore, overexpression of pdk-1 relieved the requirement for AGE-1 PI3K signaling. Therefore, caloric restriction, reduced insulin receptor, reduced PI3K, and reduced PDK1 activity all promote longevity in animals, but the role of these manipulations in cardiac aging is unclear.

In mammals, pancreatic insulin release in response to caloric intake leads to insulin receptor binding on the surface of insulin-responsive cells.6 Insulin-receptor liganding, in turn, leads to receptor tyrosine kinase activity, with receptor transphosphorylation and phosphorylation of insulin receptor substrate 1.6,7 Phosphorylated insulin receptor substrate 1 recruits PI3K to the internal surface of the plasma membrane, where it generates PIP3. PIP3 activates phosphatidylinositol-dependent kinase 1, which, in turn, activates Akt family members among other kinases.7 Akt2 promotes glut4 vesicle translocation and fusion with the plasma membrane, which results in increased glucose uptake by cardiomyocytes.7 Akt family members also indirectly promote activation of target of rapamycin protein kinase complex 1 (TORC1), a central regulator of protein synthesis and inhibitor of autophagy (Figure).9,10 Furthermore, Akt family members phosphorylate and inhibit the transcriptional activity of FoxO proteins.11 FoxO transcription factors promote autophagy, fatty acid oxidation, and apoptosis in certain circumstances (Figure).12 Clearly, insulin signaling regulates a variety of important facets of cardiomyocyte physiology, and the relationship between insulin signaling and cardiac aging may be complex.

In this issue of Circulation, Inuzuka et al13 examine the role of PI3K signaling in cardiac aging. Transgenic mice with cardiomyocyte-specific overexpression of a dominant negative form of PI3Ka (p110α) were compared with wild-type mice with the identical genetic background. In previous work, these dnPI3K mice were shown to have smaller hearts and to be resistant to exercise-induced cardiac hypertrophy.13 In the present study, dnPI3K mice were shown to have significantly higher survival rates than wild-type mice when they were followed for 20 months. Furthermore, 20- to 24-month-old dnPI3K mice were demonstrated to have increased cardiac function as determined by cardiac catheterization. Interestingly, at 3 months of age, wild-type mice had superior cardiac function compared with dnPI3K mice in terms of dP/dTmax and −dP/dTmin. Therefore, antagonizing PI3K signaling in heart decreases cardiac function at younger ages but somehow promotes function at older ages.

To determine whether inhibiting PI3K activity in heart blocks cardiac aging, a variety of markers for aging were examined by Inuzuka et al.13 In particular, the accumulation of lipofuscin and of senescence-associated β-galactosidase activity was examined and was shown to be reduced in 20- to 24-month-old dnPI3K ventricular tissue compared with wild-type tissue. The presence of intracellular lipofuscin is a widely accepted biomarker of cellular aging. Lipofuscin, first described ~100 years ago, is a heterogeneous insoluble material composed of highly oxidized and cross-linked proteins and lipids and also includes heavy metals.14 Lipofuscin accumulates in the perinuclear area of cells, fluoresces at predictable wavelengths, and is thought to represent materials from damaged organelles such as mitochondria and lysosomes. Lipofuscin accumulates in the heart and other organs of aging rats and is known to decrease proteasomal and lysosomal degradation.14 Caloric restriction is known to reduce the lipofuscin content of mammalian brain tissue.15 The presence of senescence-associated β-galactosidase activ-
ity is another well-accepted marker of cellular aging. This activity is defined as β-galactosidase activity that is present at pH 6.0. Senescence-associated β-galactosidase activity is derived from the gene GLB1 that encodes lysosomal β-D-galactosidase.16 This activity is due to the fact that lysosomal β-D-galactosidase is expressed at higher levels in senescent cells.

In addition to the evaluation of lipofuscin and senescence-associated β-galactosidase activity, Inuzuka et al found that the expression of several inflammatory marker genes, including interleukin-1β, plasminogen activator inhibitor-1, and tumor necrosis factor-α, was reduced in aged dnPI3K ventricular tissue.13 Furthermore, the expression of the cell cycle inhibitors p16 and p19 was reduced in dnPI3K ventricular tissue. In addition, markers of oxidative stress, such as thiobarbituric acid reactive substances, were reduced in aged dnPI3K cardiac tissue. Therefore, several markers of tissue aging were reduced in the hearts of dnPI3K mice at 20 to 24 months of age.

To determine the mechanism underlying reduced cardiac aging in dnPI3K mice, Inuzuka et al examined autophagy in heart tissue.13 In autophagy, damaged or excess cytosolic components of cells are degraded by evolutionarily conserved catabolic pathways.17 In macroautophagy, double-membrane vesicles form in the cytosol to sequester damaged or excess organelles or unfolded proteins, and these autophagosomes subsequently deliver materials to the lysosome for degradation.17 Therefore, autophagy and lipofuscin accumulation are largely opposing processes in cells. In both young and old dnPI3K cardiac tissue, the expression of various marker genes of autophagy, including ATG4, Beclin1, and Gabarap, was increased. Chloroquine-stimulated autophagic flux was significantly increased in young and old dnPI3K cardiac tissue as measured by determining protein levels of the conjugated form of microtubule-associated protein light chain 3.13 The increased rate of autophagy in dnPI3K cardiac tissue suggests that this may be a causal factor in the diminished cardiac aging that was observed. In support of this theory, administration of rapamycin, an inhibitor of TOR and activator of autophagy, to aging wild-type mice decreased lipofuscin accumulation in the myocardium.

The precise relationship between reduced insulin signaling, increased autophagy, reduced lipofuscin accumulation, and reduced cardiac aging in dnPI3K mice is not established conclusively by the work of Inuzuka et al.13 In particular, it is not clear whether Akt family members play an important role in cardiomyocyte aging or whether TOR or FoxO family members are specifically involved in this process. For example, it is unclear whether PI3K-stimulated TORC1 activation, PI3K-stimulated FoxO nuclear exclusion, or some other downstream effect of PI3K action is the key mediator of reduced cardiac autophagy and lipofuscin accumulation (Figure). Another unresolved issue is the relationship between reduced insulin signaling in dnPI3K mice and the expression and activity of sirtuin proteins such as SIRT3 that are known to antagonize cardiac aging.18

The study of cardiac aging remains in its early stages. The ability of caloric restriction and reduced insulin signaling to prolong the lives of invertebrate and vertebrate animals is well established. PI3K appears to be an important intracellular mediator of the effects of caloric restriction and insulin on cardiac aging. The precise signaling proteins and transcription factors that act downstream of PI3K in cardiac aging remain unclear. The clinical implications of the present work support the hypothesis that caloric restriction will inhibit cardiac aging in humans. Furthermore, pharmacological agents that block insulin signaling pathway components, such as rapamycin, may also inhibit cardiac aging. Obviously, antagonizing insulin signaling in the myocardium has numerous possible deleterious consequences, such as causing acute contractile and diastolic dysfunction, and therefore much additional research needs to be performed.

Sources of Funding
This work was supported by National Institutes of Health grants HL057278, HL076670, and HL91913.
Disclosures

None.

References


Key Words: Editorials ▪ aging ▪ free radicals ▪ insulin ▪ myocardium ▪ autophagy
New Insights Into Cardiac Aging
Anthony J. Muslin

Circulation. 2009;120:1654-1656; originally published online October 12, 2009;
doi: 10.1161/CIRCULATIONAHA.109.905356

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
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