

Food, Life Span regulation and Cancer

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Nascimur uno modo, multis morimur ('in one way we are born, in many ways we die'), and there is probably no single way to age. Indeed, so far there is no single accepted explanation or mechanism of ageing (although more than 300 theories have been proposed), and controversy reigns on whether ageing is the expression of a specific genetic programme or the simple consequence of a lifelong accumulation of random molecular damage.

Genetic evidence suggests that metabolic factors are strong determinants of longevity, and that the adipose tissue plays a critical role in the regulation of lifespan in both invertebrates and mammals: i) fat-specific disruption of the insulin receptor gene decreases body fat and increases lifespan in mice; ii) overexpression in the fat tissue of Foxo, a key target of insulin/IGF-1 signaling that is inactivated by insulin/IGF-1, prolongs lifespan in *Drosophila* and *C. Elegans*; iii) sirtuin1 (SIRT1), the mammalian ortholog of the life-extending yeast gene silent information regulator 2 (SIR2), inhibits adipogenesis in precursor cells and reduces fat storage in differentiated adipocytes by repressing the PPAR-g nuclear receptor, a master regulator of fat cell development in the insulin/IGF1 signaling pathway. Notably, reducing food intake to induce undernutrition but not malnutrition (caloric restriction) extends the life spans of multiple species, ranging from single-celled organisms to mammals.

At the molecular level, the most important mechanisms of aging involve damage to intracellular macromolecules. It is proposed that oxidizing species (reactive oxygen species; ROS) are produced in mitochondria during aerobic metabolism, which consequently causes molecular damage and, over time, cell and tissue dysfunction, ultimately increasing the risk of disease (the free-radical or oxidative-stress hypothesis of ageing). This hypothesis is supported by a vast body of experimental evidence demonstrating that: i) aerobic organisms chronically generate ROS; ii) cells accumulate oxidative damage over time (oxidative stress); iii) ROS induce cell senescence and apoptosis; iv) ROS, senescence and apoptosis are mechanistically linked to ageing-associated degenerative diseases; v) mutations of p66^{Shc}, a gene that increases production of mitochondrial ROS, or overexpression of catalase, that increases their scavenging, prolong lifespan in mice. Despite its popularity, however, one fundamental aspect of this theory still remains puzzling: how and why are dangerous pro-oxidant species generated during oxidative metabolism?

Recent findings from our laboratory demonstrate that a fraction of mitochondrial ROS are generated by specialized enzymes and serve as signaling molecules in the adipose tissue, thus suggesting that these lifespan determinants (insulin/IGF1 signaling in the fat tissue and oxidative stress) are mechanistically related.

We demonstrated that p66Shc functions as a redox enzyme that generates mitochondrial ROS to trigger mitochondrial swelling and apoptosis. For this function, p66Shc uses reducing equivalents of the mitochondrial electron-transfer chain through the direct oxidation of cytochrome c. These findings provide evidence that the generation of ROS by mitochondria is not just the by-product of respiration, but can also be the result of specific enzymes, such as p66Shc. We then investigated the physiological function of p66Shc-generated ROS and demonstrated that insulin activates the redox enzyme-activity of p66Shc specifically in adipocytes, and that p66Shc-generated ROS regulate insulin signaling through multiple and independent mechanisms, including AKT phosphorylation, Foxo localization and regulation of selected insulin-gene targets. Deletion of p66Shc resulted in increased mitochondrial uncoupling and reduced trygliceride accumulation in adipocytes, and, *in vivo*, increased metabolic rate, decreased fat mass and resistance to diet-induced obesity. In addition, p66-null mice showed improved systemic sensitivity to insulin and impaired thermo-insulation.

These findings demonstrate that p66Shc-generated oxidative signal regulates the threshold of sensitivity to insulin in adipocytes, and the development of fat tissue *in vivo*, suggesting that regulated generation of ROS has evolved to control energy conservation. Since attenuation of p66Shc or insulin signaling in the fat tissue, as by genetic mutations, reduces adiposity and increases lifespan, these results pose the question of why the threshold of insulin sensitivity is apparently "so low" in the fat tissue under physiological conditions. We propose that the physiological size of the fat tissue is set to serve other functions, which are evolutionarily more critical than longevity. Adaptation to cold, which is altered in the lean p66Shc-null mice, could be one of such functions, or, alternatively, optimization of energy storage when food is available.

The findings of reduced adiposity and increased overall insulin sensitivity in p66Shc-null mice might have important implications for the effect of p66Shc on lifespan. Aging is associated with the development of a relative resistance of the peripheral tissues to normal amounts of insulin (insulin resistance), a pathological trait, often associated with obesity, which predisposes to diabetes and cardiovascular diseases (metabolic syndrome). In humans, these diseases strongly affect morbidity and mortality, especially among elderly. P66Shc-null mice, like the caloric-restricted mice, have a relative increase of the systemic sensitivity to insulin, suggesting that reduced oxidative stress in p66^{Shc}^{-/-} mice might increase longevity through the direct effect of reduced adiposity on insulin signalling in peripheral tissues. Notably, p66Shc-null mice are more resistant to diabetes and have reduced risk of atherosclerosis and cardiovascular damage upon HF-diet.