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ScienceDirect
Molecular Aspects of Medicine 27 (2006) 455–470
MOLECULAR ASPECTS OF MEDICINE
www.elsevier.com/locate/mam

Review

The involvement of macroautophagy in aging and anti-aging interventions

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Abstract

Macroautophagy is a process that sequesters and degrades organelles and macromolecular constituents of cytoplasm for cellular restructuring and repair, and as a source of nutrients for metabolic use in early starvation. Extensive evidence has been reported that macroautophagy process declines with increasing age. This impairment, probably due to ad libitum feeding, may cause accumulation of altered structures leading to the age-related decline in cell functions. It has been suggested that caloric restriction (CR) and disruption of insulin-like signals contrast the process of aging by prolonged stimulation of macroautophagy. According to this hypothesis, it is shown that life-long weekly administration of an anti-lipolytic drug decreases glucose and insulin levels, stimulates autophagy and intensifies anti-aging effects of submaximal CR.

Keywords: Aging; Anti-aging interventions; Caloric restriction; Insulin; IGF1; MTOR; Anti-lipolytic drug; Proteolysis; Protein; Mitochondria

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0098-2997/$ - see front matter
doi:10.1016/j.mam.2006.08.003

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1. Introduction

Aging is characterized by a progressive accumulation of damaged macromolecules, organelles and cytomembranes which may account for the age-associated malfunctioning of many biological processes. The inefficiency and failure of maintenance, repair and turnover pathways may be the main cause of damage accumulation during aging (Sohal et al., 1994; Grune, 2000). Turnover of cellular components is based on a homeostatic balance between synthesis and degradation: eukaryotic cells are equipped with several degradation system, a major of which is the process of macroautophagy.

Macroautophagy, generally referred to as macroautophagy, is a universal, dynamic process which takes place in all eukaryotic cells, that involves a rearrangement of subcellular membranes to sequester cytoplasm and organelles for delivery to the lysosome or vacuole, where the sequestered cargo is degraded and recycled (Yorimitsu and Klionsky, 2005).

The primary roles of macroautophagy are baseline turnover of intracellular proteins and organelles, production of amino acids in nutrient emergency, and regression of retired tissues. These functions guarantee rejuvenation and adaptation to adverse conditions, and even underlie dynamic processes such as development/metamorphosis (Mizushima, 2005).

The major role of macroautophagy is starvation response. In fact, macroautophagy deficient yeasts succumb readily to starvation (Tsukada and Ohsumi, 1993) and in mammalian, macroautophagy is induced in almost all organs in response to nutrient starvation (Mizushima et al., 2004) in order to maintain an amino acid

pool for gluconeogenesis and for synthesis of proteins essential to survival (Yoshimori, 2004).

Extensive research on mammalian cells during the last three decades showed that macroautophagy and lysosomal proteolysis are induced by lower amino acid and insulin levels during fasting, whereas the higher (postprandial) levels of insulin fully suppress macroautophagy in the physiological range of plasma amino acid concentration (Mortimore and Poso, 1987; Mortimore et al., 1989; Seglen and Bohley, 1992).

Macroautophagy is also required for normal turnover of cellular components particularly in response to starvation (Mortimore and Poso, 1987). Indeed, the bulk degradation of long-lived proteins (90% of cellular protein) is mediated by macroautophagy and the process is the only mechanism for the degradation of membranes and organelles (Pfeifer, 1978), including mitochondria (Rodriguez-Enriquez et al., 2004) and peroxisomes (Locci-Cubeddu et al., 1985).

Macroautophagy has been demonstrated to play an important role in the degradation of excess or injured organelles (Yoshimori, 2004). In addition incubation of primary cells and cell lines in the absence of serum promotes macroautophagy of mitochondria with deleterious mtDNA mutations but spares their normal counterparts (Gu et al., 2004).

In conclusion macroautophagy is a cell-mechanism involved in both the survival during starvation and the turnover of macromolecules, cytomembranes and organelles (Bergamini et al., 2004a,b).

Experimental evidence was shown that impairment of macroautophagy is involved in the process of aging: in this review we focus on the consequences of macroautophagy impairment and on the interventions that may contrast aging by stimulation of macroautophagy.

2. The aging process

Aging is a progressive deterioration of physiological function that impairs the ability of an organism to maintain homeostasis and consequently increases the organism's susceptibility to disease and death (Harman, 2001).

Many theories of aging are based on the concept that damage, either due to normal toxic by-products of metabolism or inefficient repair/defensive systems, accumulates throughout the entire lifespan and causes aging.

The oxidative damage theory of aging postulates that the age-dependent accumulation of oxidative damage to macromolecules causes a progressive functional deterioration of cells, tissues and organ systems that manifests as functional senescence and culminates in death (Harman, 1956). The hypothesis is that as much as 1–2% of the used oxygen might generate oxygen radicals, endogenously produced by mitochondria and peroxisomes, which are casually involved in the determination of the rate of aging (Barja, 2002; Honda and Honda, 2002; Sohal, 2002).

The glycation hypothesis of aging (Cerami, 1985) also views senescence as a consequence of fuel use. However, in this case focus is not on oxygen but on carbohy-

drate fuel: high glucose diet (Folmer et al., 2002) and elevated extra and intracellular glucose concentrations result in an oxidative stress (Bonnefont-Rousselot et al., 2000; Ceriello, 2000) by several mechanisms including glucose autoxidation, protein glycation and formation of advanced glycation endproducts, and the polyol pathway (Bonnefont-Rousselot et al., 2000).

A major target of oxidative or non-oxidative damage are cellular and extracellular protein. Increase in the concentration of damaged proteins seems particularly important because it would lead to the malfunction of virtually all biological processes. An increase in the concentration of damaged intracellular proteins as well as an increase in the concentration of inactive or partially active forms of various enzymes in aging organisms is well-documented (reviewed in Stadtman, 1988; Rattan, 1996; Rosenberger, 1991). There is experimental evidence that certain proteolytic activities, a major of which is macroautophagy, decrease with age (reviewed in Ward, 2002; Cuervo and Dice, 1998). The age-dependent decrease in the rate of protein turnover may be the main cause of the increase in the concentration of damaged proteins with age (Ryazanov and Nefsky, 2002).

Mitochondrial theory of aging, a variant of free radical theory of aging, proposes that accumulation of damage to mitochondria and mitochondrial DNA (mtDNA) leads to aging of humans and animals (Miquel et al., 1980). This theory states that electrons leaking from the electron transfer chain (ETC) play the major role in the generation of ROS (reactive oxygen species). The ensuing state of oxidative stress results in damage to ETC components and mtDNA (mitochondrial DNA), thus increasing further the production of ROS. Ultimately, this 'vicious cycle' leads to a physiological decline in cell function and aging (Alexeyev et al., 2004).

It has been supported by the observation that mitochondrial function declines (Yen et al., 1989; Shigenaga et al., 1994) and mtDNA mutation increases (reviewed in Wei and Lee, 2002; Alexeyev et al., 2004) in tissue cells in an age-dependent manner. Recent reports indicate that, when injurious cellular stresses occur, cells protect themselves using macroautophagy to remove damaged mitochondria and mutated mtDNA (Rodriguez-Enriquez et al., 2004).

Reports of aging-related changes in peroxisomal function raised the hypothesis that peroxisomes may have a significant role in the aging process (reviewed in Perichon et al., 1998) probably through the generation of reactive oxygen species (ROS). In addition, the age-related changes in peroxisomal fatty acid oxidation activity may alter membrane lipid composition and thereby contribute to decline in cell function. Redundant, damaged or non-functional peroxisomes are degraded by macroautophagy-related processes (Kim and Klionsky, 2000; Locci-Cubeddu et al., 1985).

Among most the likely targets of oxidative stress is the lipid–protein structured biological membrane complex for several reasons. For instance, most cellular activities-associated with the membrane involve reactive species production; lipids are exquisitely susceptible to oxidation and proteins contain various redox-sensitive moieties. Several investigators, including Spiteller (2002), postulate that the deterioration of membrane integrity is the underlying cause of the aging process. Earlier,

Zs-Nagy (1994) proposed the essentiality of the cellular membrane in maintaining intracellular homeostasis during aging. Many age-related membrane alterations are reported including lipid peroxidation caused by oxidative stress (Matsuo et al., 1993) and accumulation of membrane lipid dolichol that may be responsible for the age-related impairment of membrane functions (e.g. transmembrane signaling) (Dolfi et al., 2003). High levels of autophagic function has been involved in maintenance of juvenile levels of dolichol, probably by renewal of cytomembranes (Marino et al., 1998).

3. Intervention in aging: caloric restriction and disruption of insulin-like signaling

Caloric restriction (CR) has a positive effect on the median and maximum lifespan and health span of rodents and various invertebrate – protozoa, flies, water fleas, nematodes, rotifers and spiders- and vertebrate species– fish, hamsters, dogs (Masoro, 2002). CR refers to the reduction in caloric intake while maintaining essential nutrient requirements. Traditionally, experimental mammalian models of caloric restriction involve a reduction in caloric intake by 40% of the ad libitum diet throughout the lifespan of the animal, which results in a 30–40% increase in maximum lifespan (Weindruch et al., 1986). CR also delays the age of onset and/or the rate of progression of most age-associate diseases (Masoro, 1999, 2002). The extension of lifespan and the prevention of disease are secondary to the hormonal, physiological and biochemical adaptations that occur in response to CR. Compared to ad libitum-fed rodents of the same age, the physiologic processes of old rodents maintained on a CR regimen are more like those of young rodents (Masoro, 2002).

It is well established that CR retards the age-related accumulation of oxidatively damaged molecules in rodents (reviewed in Yu, 1996). This effect may be due to either a decreased rate of generation of reactive oxygen species, or to increased efficiency of protective processes, or to an increase in repair activity, or to a combination of these processes. Indeed, CR alters gene expression to favor expression of genes involved in cell repair, protein synthesis and turnover, stress resistance and glucose metabolism (Lee et al., 1999, 2000).

CR is known to decrease the level of damaged protein in mice (Sohal et al., 1994) and rats (Aksenova et al., 1998; Youngman et al., 1992). This effect could be due to the decrease in protein degradation with increasing age (Van Remmen et al., 2001). Although both autophagic and proteasomal proteolysis decline during aging, macroautophagy only is maintained at juvenile levels in caloric restricted rats (Donati et al., 2001b).

Many studies are consistent with the notion that mitochondrial oxidative damage is a consequence of aging and that caloric restriction's effect on increasing longevity may be due in part to reducing that oxidative damage. Weindruch et al. (1980) reported that CR increases the efficiency of mitochondrial electron transport and oxidative phosphorylation in isolated mouse mitochondria. In rats CR decreases mitochondrial oxygen production at complex I and lowers mtdna oxidative damage

(Sanz et al., 2005). In addition, CR prevents oxidative damage induced by peroxisome proliferation in mouse liver (Qu et al., 2000).

Pieri et al. (1991) proposed that the anti-aging action of CR may be due to the protection of the physiological properties of cellular membranes. Indeed, CR has been found to retard many of the age-associated alteration of cell membranes [e.g. accumulation of dolichol (Parentini et al., 2005)] which may be responsible for many cellular function such as cell signal transduction.

Recent evidence suggests that CR may exhibit its effects to lifespan extension partly through the reduced GH-IGF-1 axis (Bonkowski et al., 2006).

CR has been found to reduce plasma insulin, insulin-like growth factor-1 (IGF-1) and glucose concentrations. Masoro et al. (1992) reported that CR increased glucose effectiveness or insulin responsiveness or both and proposed that the lifetime maintenance of low levels of glucose and markedly low levels of insulin played a major role in the life extending and related actions of CR.

Sonntag et al. (1992) showed that IGF-1 plasma levels were markedly lower in rats and mice submitted to caloric restriction. In addition GH-IGF1 deficient dwarf mice were found to exhibit life extension (Brown-Borg et al., 1996; Bartke et al., 2001; Flurkey et al., 2001).

A rapidly growing body of evidence show that insulin-IGF signaling pathway is causally linked to aging across taxa (Bartke, 2001). The loss of function mutations of insulin-like signaling system result in life extension in *Caenorhabditis elegans* (Kenyon et al., 1993) and *Drosophila melanogaster* (Clancy et al., 2001; Tatar et al., 2001). Nematodes and fruit flies do not have separate receptors for insulin and IGF1, whereas mammals, during evolution developed two separate hormonal systems. Heterozigous knock-out mice for the IGF1 receptor live 26% longer than the wild type (Holzenberger et al., 2003). Bluher et al. (2003) reported that mice in which the insulin receptor is ''knocked out'' only in the adipose tissue exhibits life extension and decreased body mass.

Since there is no clear evidence that changes in insulin and IGF1 levels may cause any acute increase in oxidative stress or other type of damage, it is conceivable that the pro-aging effect of hormones might be secondary to inhibition of macroautophagy and cell-repair function. Indeed, in vivo macroautophagy is inhibited by high levels of insulin (Del Roso et al., 2003); IGF1 may act by the same mechanism: we have found recently that it may suppress autophagic proteolysis in isolated liver cells incubated in vitro (unpublished). Similar results were obtained by Gu et al. (2004) on cultured cells.

4. Macroautophagy in aging and anti-aging intervention

Function of macroautophagy declines with increasing age. By the use of a perfused rat liver preparation with no added amino acid, Ward reported that the maximum rate of macroautophagic degradation of long-lived protein is paramount by age 6 months and then shows a significant and progressive age-related decline (Ward, 1988).

Similar data were obtained with isolated rat liver cells by Donati et al. (2001a). These authors showed that changes in regulation of macroautophagic proteolysis by the addition of amino acid in the incubation medium paralleled the decline in the maximum rate of proteolysis, whereas sensitivity to insulin and glucagon decline dramatically (Bergamini et al., 2004a).

On the other hand, experiments with overnight-fasted rats and in vivo induction of macroautophagy by the injection of an anti-lipolytic drug (Del Roso et al., 2003) showed that regulation of macroautophagy is impaired dramatically at a very early age: the macroautophagic proteolysis response of the perfused liver decreased remarkably between ages 2 and 6 months and was almost negligible at older ages. Electron microscopy evidence was obtained that the rate of formation rate and of the elimination of autophagic vacuoles decrease in older liver cells (Pollera et al., 1990; Bergamini and Kovacs, 1990; Terman, 1995). Data may give support to the hypothesis that the increase in amino acid and insulin levels by ad libitum feeding may suppress functioning of macroautophagy and membranes and cell organelles maintenance at a very young age; thus, dysregulation of macroautophagy might be the consequence of age-related decline in the in vivo functioning of macroautophagy itself.

Lysosomes function too may play a role in the aging-related decline in macroautophagic proteolysis. Lysosomes of postmitotic cells gradually accumulate an undegradable, polymeric material called lipofuscin or age pigment which probably represents a product of oxidative attack on proteins and lipids. It was hypothesized (Terman and Brunk, 2004) that lipofuscin accumulation may greatly diminish lysosomal degradative capacity by preventing lysosomal enzyme from targeting to functional autophagosomes. Furthermore, the age-related decline of macroautophagy also correlates with increase of membrane lipid dolichol in all rodent tissues, indicating a probable alteration in membrane functions and transmembrane signaling (Marino et al., 1997, 1998).

By a different experimental approach, with a loss-of function mutation in the insulin-like signaling, Melendez et al. (2003) showed that macroautophagy genes are essential for lifespan extension in *C. elegans*. Disruption of macroautophagy gene apg7-1 makes Arabidopsis thaliana hypersensitive to nutrient limiting conditions and displays premature leaf senescence (Doelling et al., 2002).

Caloric restriction can protect from the age-dependent impairment of macroautophagy. Ward (1988) showed that caloric restriction produced a greater levels of maximum rate of liver macroautophagic proteolysis at any age from 6 months on. With older isolated liver cells, caloric restriction was shown to prevent both the decline in maximum rate of macroautophagic proteolysis and the changes in amino acid and hormones regulation (Donati et al., 2001b). Furthermore, caloric restriction prevents the early age-dependent decrease in the in vivo induction of macroautophagy (Del Roso et al., 2003). It has been reported that the beneficial effects of caloric restriction on liver macroautophagy correlate with effects on life-expectancy and depend on duration and intensity of the treatment (Cavallini et al., 2001). These findings may support the hypothesis that macroautophagy is involved in the anti-aging mechanism of caloric restriction.

5. Macroautophagy as an anti-aging cell mechanism

Macroautophagy may be involved in preventing the "waste" accumulation within cells by its effective function on the turnover of cell components and disposal of damaged protein or organelles. If stimulated effectively, this cell housekeeping function may prevent any age-related physiological decline [e.g. immunosenescence (Cuervo et al., 2005)] and pathologies [e.g. neurodegenerative diseases (Komatsu et al., 2006)].

5.1. Protein

It is now generally accepted that protein degradation declines with age (Ward, 1988, 2002). The drastic increase in half-life during aging (e.g. Sharma et al., 1979; Prasanna and Lane, 1979) may increase chances of protein molecules to undergo posttranslational modification (Ryazanov and Nefsky, 2002). Two major mechanisms are involved in protein degradation: the proteasome system, which is responsible for the degradation of short-lived and damaged protein and macroautophagy, which is involved in degradation of long-lived proteins (Mortimore et al., 1989). Proteasomal proteolysis may not decrease (Ward, 2002) whereas autophagic proteolysis decreases remarkably with increasing age (reviewed in Cuervo et al., 2005). In liver cells, the age-related accumulation of protein carbonyl derivatives correlates temporally with the decline in macroautophagic proteolysis; anti-aging caloric restriction prevents the age-related accumulation of damaged protein, and decline in macroautophagic proteolysis, and has no effect on proteasomal activity (Vittorini et al., 1999). Perhaps, the dramatic age-dependent impairment of autophagic proteolysis may overload the extralysosomal machinery, thus causing failure and accumulation of altered proteins in liver cells. Furthermore, altered proteins may aggregate to form aggresomes. Macroautophagy is responsible for aggresome disposal: impairment may be involved in the accumulation of aggresomes in neurodegenerative diseases (Ravikumar et al., 2002; Fortun et al., 2003).

5.2. Mitochondria

It is well known that mitochondria are degraded via a form of macroautophagy which has been named mitophagy (Lemasters, 2005). Mitochondria are frequently encountered within autophagic vacuoles at the electron microscopy observation (Klionsky and Emr, 2000). Older cells are lower in autophagy and are known to accumulate altered mitochondria that are rich in mutated mtDNA (Wei et al., 1998). Somatic mutation may be a primary cause of mitochondrial functional decline and of ROS hyper-production (Miquel et al., 1980). Evidence with genetically modified yeast (Jin, 2006) and mice (Komatsu et al., 2005) support the hypothesis that an age-related decrease in macroautophagy may promote accumulation of damaged mitochondria. Mitophagy may be a selective process that affects dysfunctional mitochondria only (Jin, 2006; Lemasters, 2005).

It may be interesting to mention here that caloric restriction (Sanz et al., 2005) and stimulation of macroautophagy (Gu et al., 2004) can prevent accumulation of altered mitochondria in older cells.

Accumulation of damaged mitochondria and biological garbage (for example, lipofuscin in lysosomes) caused by decline in macroautophagy may be part of a vicious cycle leading to a further impairment of autophagic function and accumulation of altered mitochondria (Brunk and Terman, 2002).

5.3. Peroxisomes

Peroxisomes are degraded by the autophagic machinery both in yeast (Veenhuis et al., 1983) and in mammals (Locci-Cubeddu et al., 1985; Iwata et al., 2006). Process was named pexophagy: both pexophagy and mitophagy are induced by stimuli that mimic a severe nutrient deprivation (Locci-Cubeddu et al., 1985). Pexophagy may be involved both in the degradation of excess peroxisomes when nutritional substrates are changed, and in the selective degradation of abnormal peroxisomes in cells from patients with Zellweger disease (Heikoop et al., 1992). A decline in peroxisome turnover rate with age may increase oxidative stress and alterate membrane lipid composition.

5.4. Cytomembranes

The aging-related decline of macroautophagy in ad libitum fed rats may be associated with deterioration of cell membranes. A significant accumulation of the membrane lipid dolichol was seen in rat tissues after age 6 months (Cavallini et al., 2003; Kalen et al., 1989; Marino et al., 1998); the aging-related accumulation of dolichol was significantly retarded by calorie restriction (Cavallini et al., 2003; Marino et al., 1998); retardation correlated with the effect on life-expectancy and on the function of macroautophagic proteolysis (Cavallini et al., 2001, 2002; Dolfi et al., 2003). The accumulation of dolichol in older tissues may reflect an age-related derangement of free radicals metabolism in membranes (Bergamini et al., 2004b). ROS are physiologically important mediators in biological signaling (Droge, 2003), and their constitutive alteration in older cell membranes may be associated with dysregulation of cell signaling (Yeo and Park, 2002; Yoon et al., 2002); perhaps oxidized lipids and their electrophilic decomposition products may directly modify signal transduction proteins (Levonen et al., 2004). Ten years ago, a signal transduction theory of aging was proposed (Bergamini and Gori, 1995).

6. Pharmacological anti-aging interventions by the stimulation of macroautophagy

6.1. Pharmacological intensification of suppression of aging (PISA) by stimulation of macroautophagy

The administration of an anti-lipolytic agent to fasting rats causes a sudden decline of free fatty acids and glucose and an increase in the glucagon/insulin

ratio leading to an intensification of autophagic proteolysis, as shown by an increase in liver autophagic compartment and a release of valine in plasma (Pollera et al., 1990; Bergamini and Kovacs, 1990; Bergamini et al., 1993). Treatment increases the expression of LC3, a well known marker of autophagosomes (Donati et al., in press). This defense mechanism against nutrient deprivation provides a convenient (i.e. a safe, highly reproducible and timable) physiologic model to study the effects of hormone (low insulin-high glucagon)-induced macroautophagy in liver cells (Bergamini et al., 1993; Bergamini et al., 1993). Treatment causes a significant degradation of selected liver cell organelles including peroxisomes and, to a minor extent, mitochondria (Locci-Cubeddu et al., 1985). Very recent results show that treatment can rescue older liver cells from the age-related accumulation of oxidative damage in the mtDNA in less than 6 h (Donati et al., in press). These latter results invite to conclude that the age-related changes in mtDNA and function are a likely consequence of an age-dependent failure in the regulation of macroautophagy, impairing recognition and/or disposal of a small number of severely injured mitochondria.

A life-long pharmacological stimulation of macroautophagy can intensify the anti-aging effect of caloric restriction. For instance, a life-long weekly treatment with anti-lipolytic agents may enhance the beneficial effects of a mild (10%, fasting 1-day-a-week) calorie restriction on two parameters that are known to correlate with life-expectancy: the age-related changes in liver macroautophagy and the accumulation of dolichol in the liver tissue (Donati et al., 2004). In conclusion, a way was found to pharmacologically intensify the beneficial effects of a mild dietary restriction in humans (Bergamini et al., 2004a).

6.2. Stimulation of macroautophagy by the administration of the mTOR (mammalian target of rapamycin) inhibitor rapamycin

It is well-known that TOR pathway signalling play a central role in inhibiting macroautophagy in response to nutrients, especially amino acids (Abeliovich, 2003). Administration of the antibiotic rapamycin to eukaryotic cells results in physiological responses that mimic nutrient starvation and activate autophagy (Abeliovich, 2003). Data show that decreased TOR signaling by genetic disruption or pharmacological inhibition extend chronological lifespan in *Saccaromyces cerevisiae* (Powers et al., 2006) and in *C. elegans* (Vellai et al., 2003) and may prevent neurodegeneration in a mouse model of Huntington disease (Ravikumar et al., 2004). In addition decreased TOR signaling may be responsible for the extension of lifespan in *D. melanogaster* fed an amino acid deficient diet (Min and Tatar, 2006).

Although there is no experimental evidence, it can be predicted that long-lasting administration of rapamycin in mammals should mimic the anti-aging-action of caloric restriction stimulating macroautophagy via mTOR blockage.

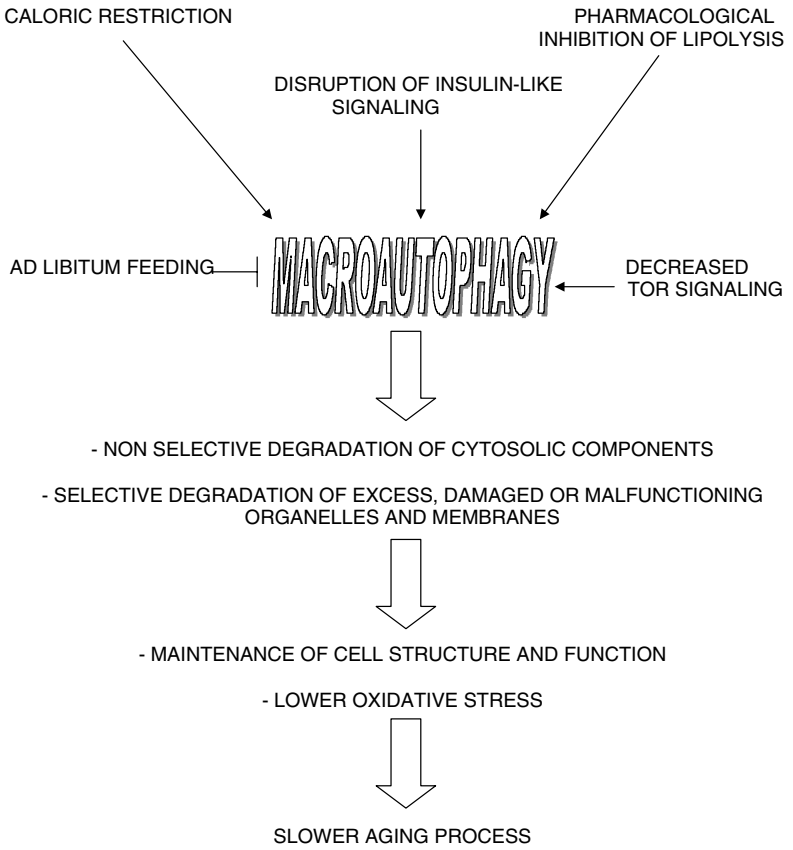

Fig. 1. Anti-aging mechanisms of macroautophagy.

7. Conclusion

Data support the hypothesis that macroautophagy has a major role in the retardation of the aging process by anti-aging interventions. According to this hypothesis, ad libitum feeding may inhibit, and caloric restriction and disruption of insulin-like signaling may intensify, macroautophagy throughout the life. Data with the PISA model show that safe pharmacological procedure are available to intensify the process (Fig. 1).

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