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Review

The role of macroautophagy in the ageing process, anti-ageing intervention and age-associated diseases

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Abstract

Macroautophagy is a degradation/recycling system ubiquitous in eukariotic cells, which generates nutrients during fasting under the control of amino acids and hormones, and contributes to the turnover and rejuvenation of cellular components (long-lived proteins, cytomembranes and organelles). Tight coupling between these two functions may be the weak point in cell housekeeping. Ageing denotes a post-maturational deterioration of tissues and organs with the passage of time, due to the progressive accumulation of the misfunctioning cell components because of oxidative damage and an age-dependent decline of turnover rate and housekeeping. Caloric restriction (CR) and lower insulin levels may slow down many age-dependent processes and extend lifespan. Recent evidence is reviewed showing that autophagy is involved in ageing and in the anti-ageing action of anti-ageing calorie restriction: function of autophagy declines during adulthood and is almost negligible at older age; CR prevents the age-dependent decline of autophagic proteolysis and improves the sensitivity of liver cells to stimulation of lysosomal degradation; protection of autophagic proteolysis from the age-related decline co-varies with the duration and level of anti-ageing food restriction like the effects of CR extending lifespan; the pharmacological stimulation of macroautophagy has anti-ageing effects. Besides the involvement in ageing, macroautophagy may have an essential role in the pathogenesis of many age-associated diseases. Higher protein turnover may not fully account for the anti-ageing effects of macroautophagy, and effects of macroautophagy on housekeeping of the cell organelles, antioxidant machinery of cell membranes and transmembrane cell signaling should also be considered.

Keywords: (Macro)autophagy; Lysosomal degradation; Ageing; Anti-ageing intervention; Age-associated diseases

Contents

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1. Macroautophagy

Macroautophagy is a universal, dynamic process which takes place in all eukariotyc 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 (Kanazawa et al., 2003; Wang & Klionsky, 2003). The basic machinery for macroautophagy is identical between yeast and mammalian cells (Reggiori & Klionsky, 2002). Macroautophagy has also been demonstrated in plants and shown to be involved in the response of plant cells to shortage of nutrients (Aubert et al., 1996; Chen, Liu, Chen, Wu, & Yu, 1994). Several signaling pathways control macroautophagy and protein synthesis and cell survival (macroautophagy is intimately associated with the execution of cell proliferation and cell death programs (Petiot, Pattingre, Arico, Meley, & Codogno, 2002) through the action of various kinases, phosphatases and guanosine triphosphatases Klionsky & Emr, 2000). The process is important to maintain a well-controlled balance between anabolism and catabolism in order to have normal cell growth and development under the regulation of extracellular conditions like the concentration of nutrient(s) and hormones. Genetic evidence was produced showing that autophagy genes are essential for dauer development and life-span extension in *Caenorhabditis elegans* (Melendez et al., 2003). Extensive research on mammalian cells in the last three decades showed that macroautophagy is activated by lower amino acid and insulin levels during fasting, to produce nutrients from endogenous sources, and that higher (post-prandial) levels of insulin fully suppress macroautophagy in the physiological range of plasma amino acid concentration (Mortimore & Poso, 1987; Mortimore, Poso, & Lardeux, 1989; Seglen & Bohley, 1992). Formation of autophagic vacuoles is also controlled by intracellular stress situations, including aggregation of misfolded proteins and accumulation of altered organelles, is involved in cell remodeling and atrophy, and is the only mechanism for the degradation of altered membranes and cell organelles, including mitochondria (e.g. Stevens & Lowe, 2000) and peroxisomes (Locci Cubeddu, Masiello, Pollera, & Bergamini, 1985; Yokota, 2003). In conclusion, the degradation pathway that permits the cell to eliminate unwanted or unnecessary organelles and to recycle the components for re-use (Kim & Klionsky, 2000) is under a permissive control by cell nutrition. The tight coupling between nutrition and cell repair may be the Achilles' heel of well fed people living in affluent western societies, who are prone to accelerated ageing and age-associates diseases. It was proposed that macroautophagy plays an essential role in ageing and in the anti-ageing action of calorie restriction (CR) (Bergamini, 1992; Bergamini & Gori, 1995) as well as in cell death and age-associated diseases, including neurodegenerative diseases and some form of cancer (Klionsky & Emr, 2000).

1.1. The ageing process

Ageing denotes a post-maturational deterioration of cells and organisms with the passage of time that underlies an increased vulnerability to challenges and a decreased ability to survive (Masoro, 1999). With advancing age, organisms exhibit a spectrum of alterations in morphological and physiological characteristics, that may be deteriorations directly due to ageing processes, while others may be secondary compensatory responses. Longitudinal studies showed that physiological deteriorative age-dependent changes occur in most physiological systems (Shock, 1962). Changes in function of organs and systems may be accounted in part by the accumulation of altered DNA and protein; by alteration in membranes and transmembrane signalling (Bergamini & Gori, 1995); and by alteration in extracellular components (e.g. accumulation of glycated proteins) and tissue blood-supply (Masoro, 1999).

Oxidative damage and not completed housekeeping may be the fundamental underlying cause of the progressive senescence-associated accumulations of deleterious macromolecular and membrane alterations (Troen, 2003; Weindruch & Sohal, 1997). 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 causally involved in the determination of the rate of ageing, at least in homeothermic vertebrates (Barja, 2002; Honda & Honda, 2002; Sohal, 2002). Oxidative stress is also involved in many acute and chronic diseases as a major pathogenic factor and in the pathophysiology of a number of

Table 1
Anti-ageing cell repair mechanisms

Mechanism	Effects of malfunctioning	Moment-to-moment regulation by nutrition
Molecular level		
DNA repair	Accumulation of altered DNA	No
Proteolysis	Accumulation of altered protein	No (proteasomal) Yes (autophagic)
Fatty acid replacement	Changes in phospholipid	No
Subcellular level		
Autophagy	Abnormal accumulation of membrane lipids (dolichol); accumulation of altered organelles (mitochondria, levels) peroxisomes) and increased oxidative stress	Yes (by amino acids, insulin, IGF-1 plasma)
Cell and tissue level		
Apoptosis	Accumulation of defective cells in tissues	No

chronic age-associated diseases including cancer, atherosclerosis, diabetes, cataracts and neurodegenerative diseases (Cotran, Kumar, & Collins, 1999). The glycation hypothesis of ageing (Cerami, 1985) also views senescence as a consequence of fuel use. However, in this case focus is not on oxygen but on carbohydrate fuel: high glucose diet (Folmer, Soares, & Rocha, 2002) and elevated extra- and intra-cellular glucose concentrations result in an oxidative stress (Bonnefont-Rousselot, Bastard, Judon, & Delattre, 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). Incidentally, diabetes may enhance oxidative stress and decrease antioxidant enzyme activities and lead to premature ageing (Koo & Vaziri, 2003; Singal, Bello-Klein, Farahmand, & Sandhawalia, 2001). Not completed housekeeping (that is inadequate ability to repair or get rid of altered macromolecules, cell membranes and organelles and replace them with new) is an essential concause of ageing. Table 1 summarizes the mechanisms responsible for cell repair and (on a longer time scale) ageing, and shows the primary role of macroautophagy and regulation by nutrition.

1.2. Intervention in ageing: the effects of caloric restriction and physical exercise

Caloric restriction has been documented to have a positive effect on the median and maximum life span and health span of rodents and various invertebrate—protozoa, flies, water fleas, nematodes, rotifers and spiders—and vertebrates species—fish, hamsters, dogs (Masoro, 2002). In fact, research spanning more than 60 years has shown that diet restriction is the only nutritional intervention that consistently extends the median and maximum lifespan and health span of animals (Masoro, 2002) and may counteract age-related changes in tissue function (e.g. Payne, Dodd, & Leeuwenburgh, 2003). There are ongoing long-term studies on the effects of caloric restriction on non-human primates in several US laboratories; none of these studies has been carried on long enough to yield information on the effect of restriction on longevity of these monkeys thus far, but preliminary evidence suggests that CR will have beneficial effects on morbidity and mortality (Mattison, Lane, Roth, & Ingram, 2003). CR is also the most potent, broadly acting cancer-prevention regimen in experimental carcinogenesis models (Hursting, Lavigne, Berrigan, Perkins, & Barrett, 2003). CR also delays the age of onset and/or the rate of progression of most age-associated diseases (Masoro, 1999, 2002); reduces metabolic rate and oxidative stress, alters neuroendocrine and sympathetic nervous system function (Heilbronn & Ravussin, 2003); decreases blood sugar and insulin levels and increases sensitivity to insulin of peripheral tissues (Masoro, 2002; Yamaza, Chiba, Higami, & Shimokawa, 2002) by enhancing Akt2 phosphorylation (McCurdy, Davidson, & Cartee, 2003); stimulates macroautophagy and turnover of membranes, organelles and protein, and thus renewal, into old age in several tissues (Hagopian, Ramsey, & Weindruch,

2003; Spindler, 2001) and counteracts age-dependent alterations in cell signaling (Ando, Higami, Tsuchiya, Kanematsu, & Shimokawa, 2002; Hansen, 2001; Ikeyama et al., 2003). With regard to mechanism(s), CR may make cells more resistant to stress and damaging agents (Van Remmen, Guo, & Richardson, 2001) directly or indirecly counteracting the age-related increase in oxidative stress (Zou, Jung, Kim, Yu, & Chung, 2004), and may be enhancing function of reactive oxygen free radical scavengers (Srekumar et al., 2002) and of cell repair mechanisms at the molecular (Hulbert, 2003; Rao, 2003) subcellular (Lee, Yu, & Herlihy, 1999; Mayhew, Renganathan, & Delbono, 1998) and cellular levels (Ando et al., 2002).

Physical exercise too has been documented to have a positive effect on life span (Holloszy & Kohrt, 1995). Exercised voluntarily running rats are lower in weight and live longer than freely eating sedentary controls, without any increase in maximum lifespan, and may live as long as paired-weight (calorie restricted) sedentary controls. On the other hand, for unknown reasons exercise can sometimes result in increased early mortality rate in food-restricted rats. The effects of exercise extending lifespan may not be attributed to lower oxidative stress (exercising animals take up and use more oxygen than untrained individuals and may generate higher amounts of oxygen free radicals (Di Meo & Venditti, 2001), and enhanced autophagy and better cell repair may be involved instead (evidence was produced that strenuous exercise induced autophagy and autophagic vacuoles contained mitochondria at various stages of degradation (Salminen & Vihko, 1984). Individuals who exercise vigorously on a regular basis have lower blood glucose levels and a reduced plasma insulin response to glucose and an increased sensitivity to insulin (Kahn et al., 1990; Rodnick, Henriksen, James, & Holloszy, 1992). Nothing is known yet about the effects of moderate physical exercise on macroautophagic degradation.

1.3. Genetic intervention in ageing

Genes exert a powerful control on life span and may account for about 35% of the intraspecies variance in longevity of mammals (Finch & Tanzi, 1997). Genetic modification of an antioxidant defense may cause a progeric phenotype (Petric & Rachubinski, 2004). A rapidly growing body of evidence shows that insulin-IGF signaling pathway is causally linked to ageing across taxa (Bartke, 2001). Genetic interventions disrupting the insulin-GH/IGF-1 signaling pathways reduce growth and size of the organism and prolong longevity (Brown Borg, Borg, Meliska, & Bartke, 1996; Kimura, Tissenbaum, Liu, & Ruvkun, 1997; Tatar, Bartke, & Antebi, 2003), whereas high GH/IGF-1 levels accelerate death (Laron, 2002). Growth hormone (GH)/IGF-I deficient dwarf mice do live significantly longer than their wild counterparts (Brown Borg et al., 1996); heterozigous knockout IGF-1r (+/−) mice are not dwarf and live on the average 26% longer than their wild-type littermates (Holzenberger et al., 2003); an optimal level of hormone appears to be required to maximise survival (Shimokawa et al., 2002); transgenic mice that overexpress GH exhibit a drastic reduction in life-span (Steger, Bartke, & Cecim, 1993). Insulin and insulin-like growth factors use similar signaling pathways and both are importantly related to the regulation of ageing and life span and may interact each-other (Dominici, Hauck, Argentino, Bartke, & Turyn, 2002). Disruption of the insulin receptor in the adipose tissue (FIRKO mice) extends longevity, shrinks mice fat pad and causes a percent wise increase in lean body mass, and does not decrease (it may rather increase) energy expenditure per unit of body weight (Blueher, Kahn, & Kahn, 2003). Since there is no clear-cut evidence that changes in insulin levels may cause any acute increase in free radical production, it is conceivable that the pro-ageing effect of the hormone might be secondary to inhibition of macroautophagy and cell-repair function(s). IGF-1 may act by the same mechanism: we have found recently that it may suppress autophagic proteolysis of isolated liver cells incubated in vitro (unpublished).

1.4. Macroautophagy in ageing and anti-ageing interventions

It is now generally accepted that protein degradation declines with age but that lysosomal enzyme activity may increase (Ward, 2000) and proteasome degradation may not be altered (Ward, 2002). There are experimental observations suggesting that age may have strong effects on both the chaperone-mediated autophagic (Cuervo & Dice, 2000) and macroautophagic processes (Cuervo, 2003). Macroautophagy, which did not receive much attention as an anti-ageing

cell repair mechanism so far, appears to have a very important role in the process of ageing and may be the best candidate-mediator of the anti-ageing effects of caloric restriction (Bergamini & Gori, 1995; Bergamini et al., 1998) and other interventions which are associated with hypoinsulinemia. The age-dependent changes in the function of macroautophagy were studied in vivo on male Sprague Dawley rats fed ad libitum or calorie restricted (Del Roso et al., 1991a,b). Rats were fasted overnight and autophagy was induced by the injection of an antilipolytic drug (Bergamini et al., 1993). In the rats fed ad libitum, the in vivo-induced autophagic-proteolytic response was paramount in 1-month-old rats; was high but delayed in 2-month-old rats; decreased remarkably in 6 month-old rats and was almost negligible in older rats (Del Roso et al., 1991a,b, 2003). Parallel changes were observed in the effects of treatment lowering glucose and insulin plasma levels (Del Roso et al., 2003). Data gave support to the hypothesis that ad libitum feeding accelerates the rate of ageing by raising amino acid and insulin plasma levels and suppressing macroautophagy and membrane maintenance (Del Roso et al., 2003). The age-related changes in the regulation of autophagic proteolysis by amino acids and hormones were studied in vitro with isolated liver cells (Donati et al., 2001a,b). The maximum rate of proteolysis (no amino acid added in the medium) was maximum by age 6 months and declined thereafter (Donati et al., 2001a,b); percent changes in proteolysis by the addition of amino acids were not significantly affected by ageing (Fig. 1) whereas sensitivity to insulin and glucagon decreased dramatically (Fig. 2). Calorie restriction prevented the in vivo changes (Del Roso et al., 2003) and the decrease in the maximum rate of proteolysis observed with isolated liver cells incubated in vitro (Donati et al., 2001a,b and Fig. 1, the legend). With regard to the effects of hor-

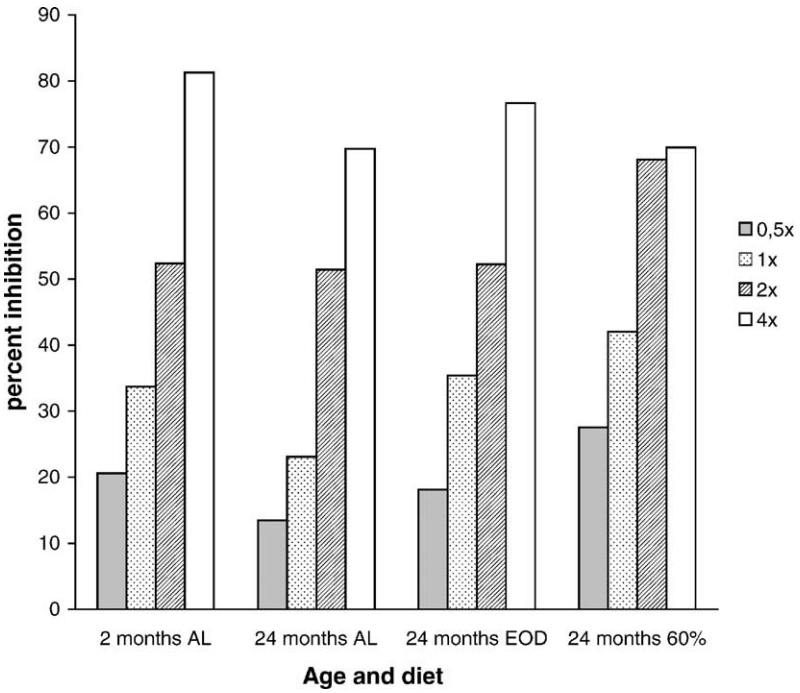

Fig. 1. Effects of ageing and of two types of anti-ageing calorie restriction (EOD, every-other-day feeding; 60, 40% caloric restriction of ad libitum feeding) on the inhibitory effect of amino acid on the autophagic proteolysis of liver cells (AL, control ad libitum fed rats). Data are given as the percent inhibition of the maximum rate of autophagic proteolysis (measured in the absence of amino acid). 0.5×, 1×, 2×, 4× are fractions/multiples of amino acid concentration in the venous plasma of fasted rats (for methods see Cavallini et al., 2001; Donati et al., 2001a,b). Means ± E.S.M. of the maximum rate of autophagic proteolysis were: 2 months AL, 37.4 ± 0.75; 24 months AL, 20.8 ± 0.99; 24 months EOD, 38.1 ± 0.91; 24 months 60%, 27.6 ± 2.10 (nmol valine/min per gram of wet tissue).

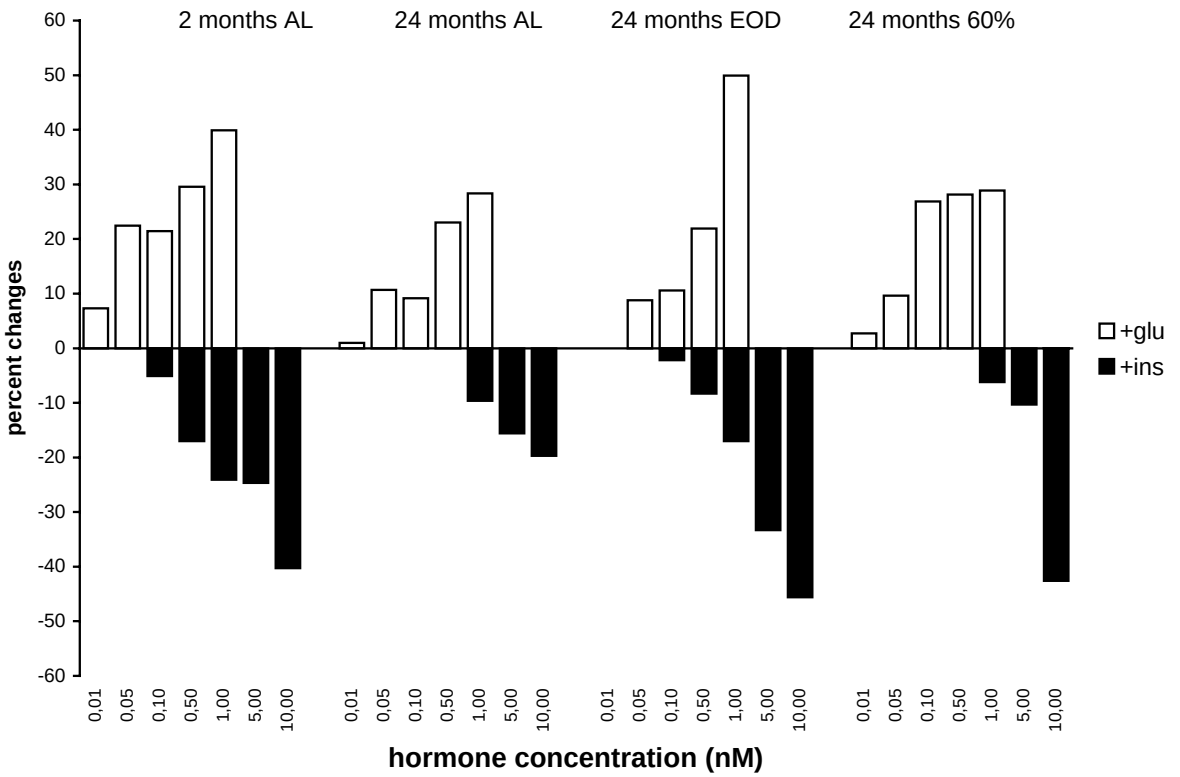

Fig. 2. Effects of ageing and of two types of anti-ageing calorie restriction (EOD, every-other-day feeding; 60, 40% caloric restriction of ad libitum feeding) on the insulin and glucagon regulation of the liver cells autophagic proteolysis (AL, control ad libitum fed rats). Data are given as the percent change of the rate of autophagic proteolysis at different concentration of hormones. Isolated liver cells were incubated with the addition of a physiological concentration of amino acid. Means ± E.S.M. of the rate of autophagic proteolysis without any added hormone were: 2 months AL, 23.5 ± 1.09; 24 months AL, 16.7 ± 1.26; 24 months EOD, 22.5 ± 1.36; 24 months 60%, 23.8 ± 1.86 (nmol valine/min per gram of wet tissue). Statistical analysis, performed on the original data (ANOVA), showed that both EOD and CR significantly prevented the ageing-related changes in function of both hormones.

mones, response of liver cells to insulin and glucagon were significantly improved (Fig. 2). Calorie restriction prevented the age-dependent decrease in autophagic proteolysis and protection was correlated with life-expectancy (Cavallini, Donati, Gori, Pollera, & Bergamini, 2001). Evidence was produced that the ageing-related decline of macroautophagy in ad libitum fed rats was associated with a progressive deterioration of cell membranes: a significant accumulation of dolichol was seen in rat tissues after age 6 months (Cavallini et al., 2003; Kalén, Appelqvist, & Dallner, 1989; Marino et al., 1998); the ageing-related accumulation of dolichol was significantly retarded by calorie restriction (Cavallini et al., 2003; Marino et al., 1998); and the protective effect of diet correlated with the effect on life-expectancy and on the function of macroautophagic proteolysis (Cavallini, Donati, Gori, Pollera, & Bergamini, 2001, Cavallini, Donati, Gori, Parentini, & Bergamini, 2002; Dolfi et al., 2003). The accumulation of dolichol in older tissues may reflect an age-related derangement in free radicals metabolism of membranes (Bergamini et al., 2004). It is known that ROS are physiologically important mediators in biological signaling processes (Droge, 2003) and that a constitutive alteration in basal free radical metabolism of older cell membranes may be associated with dysregulation of cell signaling (Yeo & Park, 2002; Yoon, Yun, & Chung, 2002) perhaps because of a direct modification of signal transduction proteins by oxidized lipids and their electrophilic

decomposition products (Levonen et al., 2004). Ten years ago, a signal transduction theory of ageing was proposed (Bergamini & Gori, 1995).

1.5. Macroautophagy, cell death and age-associated diseases

There is evidence that the ageing-related decline in macroautophagic function may be involved in many diseases like cancer (Edinger & Thompson, 2003; Klionsky & Emr, 2000), hereditary myopathies (Nishino, 2003), heart failure (Hein et al., 2003) and neurodegenerative diseases (Ding et al., 2003) including Alzheimer Disease (Nixon, Cataldo, & Mathews, 2000) and might account for age-dependency. There is evidence that autophagy is involved in the mechanism(s) of neuron death activated by growth factors deprivation (Yu et al., 2003; Yuan, Lipinski, & Degterev, 2003) in Lewy body diseases and in Parkinson disease (Zhu, Guo, Shelburn, Watkins, & Chu, 2003). In regard to prion disease, formation of autophagic vacuoles was demonstrated in Creutzfeld-Jacob disease and scrapie-affected rodent brains (Liberski, Gajdusek, & Brown, 2002). Activation of autophagy may enhance elimination of protein aggregates (aggresomes) in nerve and Schwann cells, whereas inhibition of autophagy may prevent it (Fortun, Dunn, Joy, Li, & Notterpek, 2003). Altered processing of mutant huntingtin by autophagy and cathepsin D may contribute to the pathogenesis of Hungtington Disease (Qin et al., 2003). Lipofucsin accumulation in the retinal pigment epithelium is associated with age and various retinal diseases (Schutt, Bergmann, Holz, & Kopitz, 2003). In conclusion, it appears that failure to dispose altered proteins may induce the formation of inclusion bodies associated with intermediate filaments and ubiquitin, which are observed in many diseases; that autophagic vacuoles may be activated to eliminate inclusion bodies; and that an overload might eventually lead to autophagic cell death (Ding et al., 2003; Harada et al., 2003; Jellinger & Stadelmann, 2001).

1.6. Effects of pharmacological intervention in autophagy on the rate of ageing

Pharmacological modulation of autophagy may either accelerate or retard ageing. Autophagic-lysosomal function can be reduced by the injection of inhibitors of thiol proteases (e.g. leupeptin) and lysosomotropic agents like chloroquine, which are general lysosomal enzyme inhibitors (Ivy, Schottler, Wenzel, Baudry, & Linch, 1984). Chronic pharmacological inhibition of autophagic proteolysis may accelerate the rate of ageing: in several species and organ systems the treatment induced the formation of lysosomal aggregates closely resembling ceroid-lipofuscin (Ivy et al., 1989a,b), which strongly suggested that the 'protease-inhibitor model of ageing' is generally valid (Kitani et al., 1989). The recent observation that a pharmacological blockade of autophagy by 3-methyl-adenine (MA) for less than 2 weeks causes neonatal cultured cardiomyocytes acquire an altered phenotype different than the "senescent" phenotype and dramatically increases

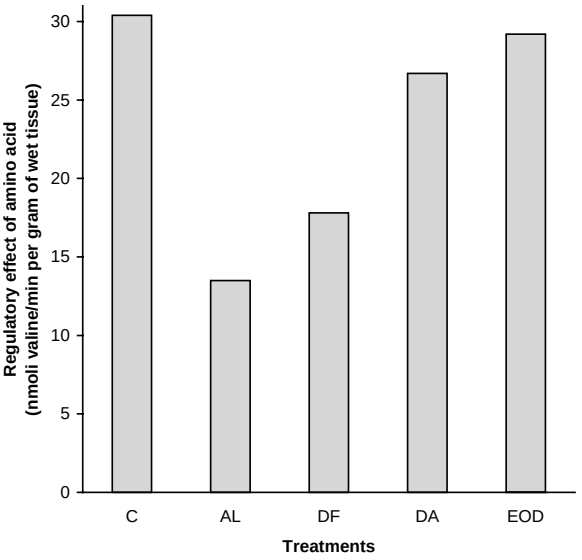

Fig. 3. Effects of ageing, caloric restriction and pharmacological intervention on the amino acid regulation of the liver cells autophagic proteolysis. Data are given as the difference between rate of autophagic proteolysis in the absence of amino acid and in the presence of maximally active amino acid concentration in the incubation medium (see Cavallini et al., 2001; Donati et al., 2001a,b for details). Liver donors were C: 2-month-old rats; AL: 24-month-old rats fed ad libitum; DF: 24-month-old rats fasted 1-day-a-week starting by age 2 months; DA: 24-month-old rats fasted like DF and given an antilipolytic drug on the day of fasting; EOD: 24-month-old rats on a severe anti-ageing caloric restriction (every-other-day ad libitum) starting by age 2 months (from Bergamini, Cavallini, Donati, & Gori, 2002a with permission). The effects of age, diet and drug were highly significant.

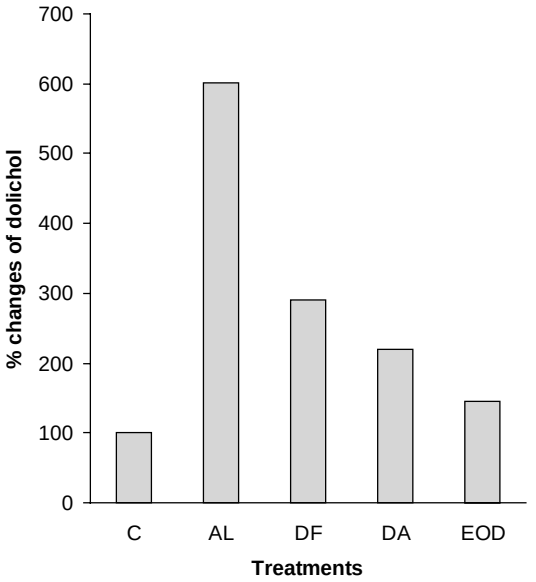

Fig. 4. Effects of ageing, caloric restriction and pharmacological intervention on the age-related accumulation of dolichol in rat liver. Data are given as percent changes in liver dolichol content (control, C: 2-month-old rats = 100). AL: 24-month-old rats fed ad libitum; DF: 24-month-old rats fasted 1-day-a-week starting by age 2 months; DA: 24-month-old rats fasted like DF rats and given an antilipolytic drug on the day of fasting; EOD: 24-month-old rats on a severe anti-ageing caloric restriction (every-other-day ad libitum), starting by age 2 months (from Bergamini et al., 2002a with permission). The effect of age, diet and drug were significant.

the rate of cell death (Terman, Dalen, Eaton, Neuzil, & Brunk, 2003) may not disprove the validity of the hypothesis, since in vivo ageing may not correlate with in vitro senescence of cultured cells (Cristofalo, Allen, Pignolo, Martin, & Beck, 1998). Furthermore, it has been known for decades that one must be cautious when extrapolating to organs in vivo observations on endocytosis obtained with primary culture of cells (Thirion & Wattiaux, 1991). Protease inhibition caused an anomalous accumulation of tau and ubiquitin immunoreactivity in brain (Ivy et al., 1989a,b). The intensification of autophagic proteolysis in fasting animals by the administration of an antilipolytic agent 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, 1994). Treatment caused a significant degradation of selected liver cell organelles including peroxisomes and, to a minor extent, mitochondria (Locci Cubeddu, Masiello, Pollera, & Bergamini, 1985). Preliminary results show that life-long weekly treatment with antilipolytic agents may enhance the beneficial effects of a mild (fasting 1-day-a-week) 10% calorie restriction on two parameters that are known to correlate with life-expectancy: the ageing-related changes in liver macroautophagy (Fig. 3) and dolichol accumulation in the liver tissue (Fig. 4) (Bergamini, Cavallini, Donati, & Gori, 2002a,b). In conclusion, a way was found to pharmacologically intensify dietary suppression of ageing (Bergamini, 2003).

2. Conclusions

It is well known that ad libitum feeding (i.e. overfeeding) may cause an increase in plasma glucose, in insulin secretion and in amino acid, insulin and IGF-1 plasma levels. The afore mentioned metabolic and endocrine changes may suppress macroautophagy and slow down the turnover rate of long lived protein, membrane and organelles. Longer biological life of cell macromolecules and structures may magnify the accumulation of peroxidized macromolecules and defective organelles in cells, and accelerate ageing. Accumulation of altered membranes (De Cabo et al., 2004) mitochondria (Brunk & Terman, 2002; Huang & Manton, 2004) and peroxisomes (Legakis et al., 2002; Badr & Birnbaum, 2004) might enhance free radical production and cell injury. It was proposed that increased free radical generation and alteration in membrane free-radical metabolism and cell signaling may start a vicious circle, which eventually leads to irreversibility of the cell changes (see Fig. 5 and references Bergamini & Gori, 1995). Calorie restriction, physical exercise and genetic disruptions of the insulin and GH/IGF-1 axis may lower insulin and IGF-1 levels and break the cycle: macroautophagy may be enhanced; accumulation of altered macromolecules and subcellular components may be prevented and life-span might be extended. Antilipolytic drugs and severe atrophy of the fat tissue (e.g. by genetic disruption of the fat insulin receptor Blueher et al., 2003) may cause a shortage of FFA and enhance the rate of autophagic proteolysis and glucogenesis during fasting to compensate for the missing fuel and improve

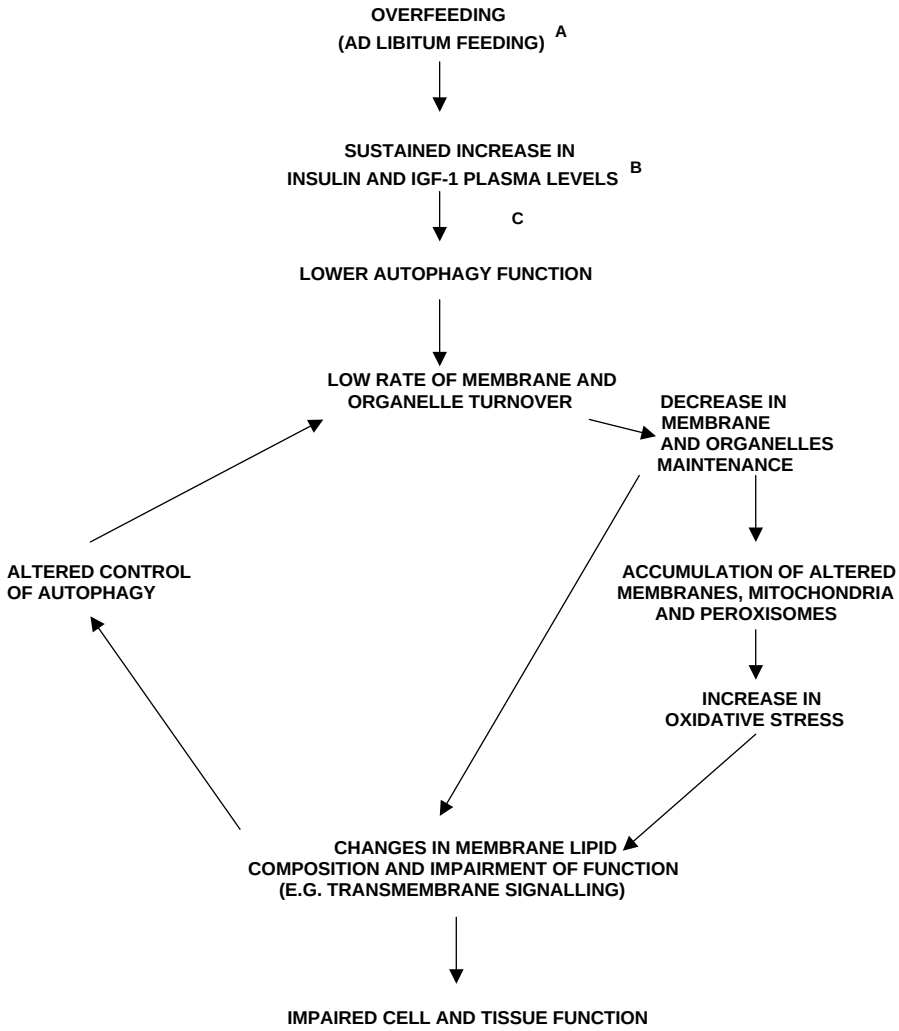

Fig. 5. (A) Prevented by caloric restriction. (B) Prevented by disruption of insulin and GH/IGF-1 axis by genetic modification. (C) Prevented in FIRKO mice and by the administration of anti-lipolytic drugs (from Bergamini, Cavallini, Donati, & Gori, 2002b with permission and modification).

cell maintenance and extend longevity as a secondary effect.

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