

Towards an Understanding of the Anti-Aging Mechanism of Caloric Restriction

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Abstract: Accumulation of oxidatively altered cell components may play a role in the age-related cell deterioration and associated diseases. Caloric restriction is the most robust anti-aging intervention that extends lifespan and retards the appearance of age-associated diseases. Autophagy is a highly conserved cell-repair process in which the cytoplasm, including excess or aberrant organelles, is sequestered into double-membrane vesicles and delivered to the degradative vacuoles. Autophagy has an essential role in adaptation to fasting and changing environmental conditions. Several pieces of evidence show that autophagy may be an essential part in the anti-aging mechanism of caloric restriction: 1. The function of autophagy declines with increasing age; 2. The temporal pattern of the decline parallels the changes in biomarkers of membrane aging and in amino acid and hormone signalling. 3. These age-dependent changes in autophagy are prevented by caloric restriction. 4. The prevention of the changes in autophagy and biomarkers of aging co-varies with the effects of caloric restriction on life-span. 5. A long-lasting inhibition of autophagy accelerates the process of aging. 6. A long-lasting stimulation of autophagy retards the process of aging in rats. 7. Stimulation of autophagy may rescue older cells from accumulation of altered mtDNA. 8. Stimulation of autophagy counteracts the age-related hypercholesterolemia in rodents. It is suggested that the pharmacological intensification of suppression of aging (P.I.S.A. treatment) by the stimulation of autophagy might prove to be a big step towards retardation of aging and prevention of age-associated diseases in humans.

Keywords: Aging, autophagy, caloric restriction, antilipolytic drug.

INTRODUCTION

Life span may be determined by the balance between cellular damage resulting from metabolic events occurring within the cell and counteracting molecular responses that can repair the damage. The hypothesis is that as much as 1-2% of the used oxygen might generate oxygen radicals at the mitochondrial and peroxisomal level, which may hit and alter DNA, protein, cell membranes and organelles as well as extracellular components [1, 2]. At an older age, accumulation of altered macromolecules and membranes may impair cell functioning; accumulation of altered mitochondria and peroxisomes may boost the yield of ROS per unit of produced energy and accelerate the aging process [3].

Repair mechanisms responsible for cell maintenance are shown in Table 1. Laboratory animals are stabled in small cages and have a sedentary life and food continuously available. Endocrine and metabolic impediments (e.g. high glucose, amino acids and insulin levels) may prevent full *in vivo* long lasting activation of autophagy in these ad libitum fed (AL) [4], (but not in caloric restricted, CR) animals [5]. Alteration may contribute to the accumulation of deleterious macromolecules and altered membranes and organelles in cells, and lead to the progressive age-associated decline in the function of most physiological organs and systems including anti-aging cell repair mechanism and start a vicious cycle [6].

AUTOPHAGY

Autophagy is a transport pathway leading from cytoplasm to lysosomes required for cellular homeostasis [7]. It keeps the balance between biosynthetic and catabolic processes by regulating the turnover rate of cellular components. At least three types of autophagy, macroautophagy, microautophagy and chaperon-mediated autophagy, have been described, and macroautophagy is thought to play a major role in intracellular degradation [8]. We hereafter use the term autophagy as a synonym for macroautophagy.

Autophagy is a non stop life-sustaining renewal process, which can be further stimulated by different stressors. Such stressors may be associated either with increased damage to cellular components requiring repair (reparative autophagy), or with a request for essential end products of lysosomal degradation, as it occurs under starvation, when cells partially digest themselves to generate energy and provide their anabolic machinery with new building blocks [8, 9]. Amino acids, the final products of autophagic protein degradation, act as a negative feedback regulator for the process *in vitro* [10]. Hormones appear to control the rate of autophagy *in vivo*: insulin inhibits the process, whereas glucagon stimulates it [11].

Autophagy was reported to play an important role in the degradation of excess or injured organelles including peroxisomes [12, 13], mitochondria [14, 15] and the endoplasmic reticulum [16]. Recent evidence shows that altered mitochondria may be degraded selectively [17].

In conclusion, autophagy is an anti-aging cell repair mechanism which is under the permissive control of the nutrition state (see Table 1). This tight coupling between nutrition and cell repair led to propose the hypothesis that auto-

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Table 1. Anti-Aging Cell Repair Mechanisms

Mechanism	Decline with Aging	Prevention by Antiaging Intervention
<i>Molecular level</i>		
DNA repair	not proved	not proved
Protein repair		
- stress proteins	yes	yes
- proteasomal proteolysis	yes	yes
- chaperone-mediated autophagy	yes	not proved
Membrane lipid repair	not proved	PUFA supplementation?
<i>Subcellular level</i>		
Autophagy	yes	yes (caloric restriction)
		Intensified by antipolytic drugs and rapamycin
<i>Cell and Tissue level</i>		
Apoptosis	yes	yes (same as autophagy)

phagy might be the anti-aging mechanism responsible for the anti-aging effect of caloric restriction [18]. More recently, a genetic evidence has been provided that autophagy is required for dietary restriction to extend *C. elegans* life span, and identify autophagy as a potential integration point in the regulation of *C. elegans* life span by dietary restriction and insulin signalling [19, 20, 21].

ANTI-AGING EFFECTS OF CALORIE RESTRICTION (CR)

Throughout history, numerous societies have touted the health benefits of food limitations, including the Ancient Greeks and Romans [22]. In more recent time, McCay and co-workers found that feeding rats with a diet containing 20% indigestible cellulose [23] or caloric-restricted diet [24] dramatically extended mean and maximum life span.

Since then, hundreds of papers showed that calorie restriction is the most effective way of extending the median and maximum life span and have positive effects on health span in different organisms, from invertebrates to mammals [25]. Observational studies suggest that CR also has beneficial effects on human longevity [26, 27]. Epidemiological studies showed that caloric intake is correlated with the incidence of several age-associated chronic diseases, including cardiovascular disease, cancer, diabetes, as well as neurodegenerative disorders [28, 29, 30, 31, 32, 33]. Studies of Okinawan centenarians support the view that a low-calorie can increase prospects for good health and longevity in humans [34].

Duration and level of CR have an important influence on the anti-aging effects. In rodents, treatment is highly effective when initiated in young age and decelerates the rate of aging by a 50% [35]. With regard to levels, maximum benefit is obtained by a 40% CR (DR) and an every-other-day ad libitum feeding (EOD) [36]; and the beneficial effects of a 10% CR or fasting one day a week were smaller but still significant [37, 38, 39].

The shapes of survival curves indicate that both DR and EOD extends both median length of life and maximal survival [40]. Extension of median life span does not necessarily mean that aging processes have been slowed since prevention of premature death would increase the median length of life even the aging processes were not affected. Extension of maximum survival shows that the effect of CR on longevity is both robust and reproducible, and intimately connected to the basic, universal mechanism(s) of biological aging.

THE MECHANISM(S) OF THE ANTI-AGING EFFECTS OF CALORIE RESTRICTION

Work of many laboratories in widely diverse species has contributed to provide important clues about the effects of CR but does not clarify the mechanism(s) underlying these anti-aging effects [25, 41]. CR has remarkable effects on glucose, lipid and amino acid metabolism and influences many endocrines functions, like insulin, glucagon and glucocorticoid secretion [25, 42]; and may counteract age-dependent alterations in cells signalling [18, 43, 44, 45]. CR protects rodents and worms from oxidative stress without affecting the metabolic rate per unit of lean body mass [46], and enhances cell repair mechanisms at the molecular [47, 48] and cellular levels [44].

Loss of function mutations in genes coding for components of cellular insulin-like signalling pathway are known to increase the longevity in diverse species [49] and it has been suggested that CR may exhibit its effects on life span extension partly through the reduced GH-IGF-1 axis [50]. It has been proposed very recently that the effectiveness of the response to glucagon might play a role in the anti-aging effect of CR [51].

When the current knowledge of the physiological actions and gene expression effects of CR has been combined with the findings on genetic manipulations that extend life span of vertebrate and invertebrate species, four hypotheses have been proposed: attenuation of oxidative damage; alteration of the glucose-insulin system; alteration of the growth hor-

mone-IGF-1 axis; and, the beneficial actions resulting from the response of an organism to a low-intensity biological stressor (hormesis) [25].

The proposal that autophagy is the hormetic cell repair process essential for keeping cells “clean”[52], which is involved in the mechanism of aging and anti-aging dietary intervention [3, 53], may compose these hypothesis in a single frame. In this perspective, it is essential to remember that CR procedures (a daily restriction of food intake or an every-other-day ad libitum feeding) involve a meal-eating pattern followed by many hours of fasting, and that this pattern of feeding has a remarkable, cyclic effect on the pattern of insulin and glucagon secretion and on the regulation of autophagy (Fig. 1).

EVIDENCE THAT AUTOPHAGY MAY MEDIATE THE ANTI-AGING EFFECTS OF CR

There are several experimental observations suggesting that autophagy is involved in the process of aging and may be the best candidate mediator of the anti-aging effects of CR.

- 1) The age-related changes in the functioning of autophagy were studied *in vivo* by a pharmacological stimulation of the process [54]. Older AL rats were less susceptible to the metabolic and endocrine effects of fasting (the autophagic response decreased remarkably by age 6-months and was almost negligible in older rats) [5]. The maximum rate of liver autophagic proteolysis was studied on the *in vitro*-incubated isolated liver cells (no amino acid added in the medium) and declined beyond age 6-months [55]. The rate of formation and elimination of autophagic vacuoles decreased in older liver cells [56, 57]. All these data may support the hypothesis that the dysregulation of autophagy in older animals might be secondary to the age-related decline of the *in vivo* functioning of autophagy at younger age. Decline might perhaps account for later accumulations of altered organelles and membranes, and malfunctioning.
- 2) The age-related decline of autophagy was associated with a progressive increase in the levels of dolichol rat tissues after age 6-months [58, 59]. Dolichol is a fat-soluble antioxidant [60] that satisfies all proposed primary and secondary applicable criteria and the desirable features re-

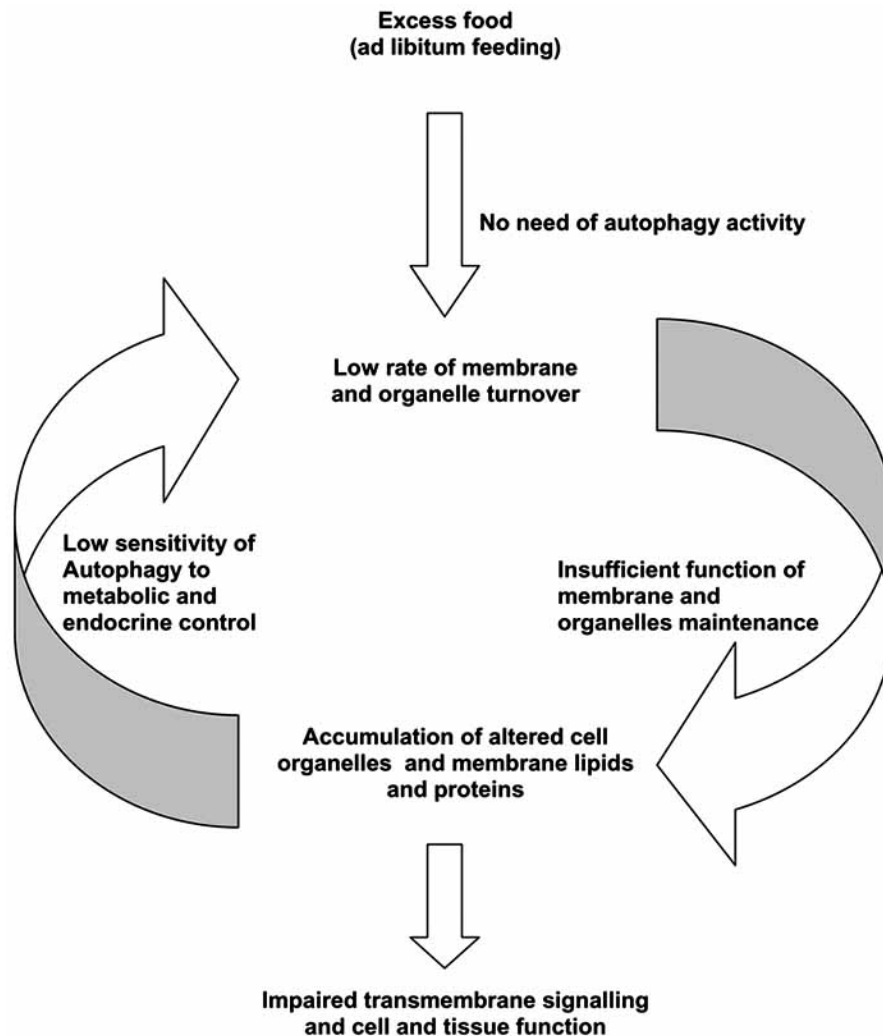


Fig. (1). Long lasting food intake in ad libitum fed animals lowers the function of Autophagy and the rate of membrane and organelles turnover. The lower quality of cell membranes and organelles and the accumulation of lypophilic waste may affect cell function and impair the sensitivity of Autophagy to its metabolic and endocrine control, and close a vicious pro-aging circle. Caloric restriction can break the circle.

- quired to be qualified as a biomarker of aging of biological membranes [61]. Very interesting, dolichol accumulation appears to be associated with an increase in 3-hydroxy 3-methylglutaryl CoA reductase activity (HMGC_oA reductase), the rate-limiting enzyme of isoprenoid and cholesterol biosynthesis [62], that may be secondary to an increase in oxidative stress [63]. Better autophagic functioning in CR rats may help to maintain levels of dolichol juvenile, possibly by enhancing the turnover rate of cytomembranes [59]. Furthermore, CR prevents the age-related enhancement of HMGC_oA reductase activity [62].
- 3) CR prevents both the *in vivo* [5] and *in vitro* age-related changes [64] and preserves the juvenile amino acid and hormone regulation of autophagy [51, 64]. Two different anti-aging dietary regimens were tested, with different effects on metabolism [65] and similar effects on longevity [66] (namely, DR and EOD). Both CR regimens make animals spend a long part of their time in the state of fasting, with lower glucose and insulin levels and turned-on autophagy. The protective effects of DR and EOD may be different in part, but benefits on the regulation of autophagic proteolysis were always similar. With regard to the endocrine regulation, both types of caloric restriction fully restored a juvenile sensitivity to glucagon but DR had a smaller effect on the sensitivity to the inhibitory action insulin.
 - 4) The beneficial effects of CR on autophagy and on the levels of dolichol correlate with the effects of diet on life-expectancy, and depend on the duration and intensity of the treatment [38, 67].
 - 5) The 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 [68]. The life-long administration of chloroquine was shown to reduce life-span, to impair the function and regulation of the autophagy and to cause an earlier accumulation of dolichol in liver cells (data not published). The chronic pharmacological inhibition of autophagic proteolysis was reported to accelerate the rate of the process of aging [69]. It has been shown that inhibitors of autophagy like 3-methyl adenine (3MA) and bafilomycin A1 (Baf) may delay the clearance of mutant huntingtin fragments, expanded polyalanines tagged to enhanced green fluorescent protein and forms of α -synuclein are autophagy substrates in cell models [70, 71].
 - 6) The administration of an antilipolytic agent to fasting rats causes a sudden decrease in plasma free fatty acids and glucose levels and an increase in the glucagon/insulin ratio, and stimulates autophagy [57, 72]. A life-long weekly treatment with an antilipolytic drug licensed for human use (Acipimox) can intensify the beneficial effects of a mild CR (fasting 1-day-a-week) and prevent the age-related changes in autophagy and the accumulation of dolichol in liver cells [73]. This pharmacological intensification of suppression of aging (P.I.S.A. treatment) secondary to stimulation of autophagy currently is under investigation in humans.

- 7) Recent data show that the acute stimulation of autophagy by the injection of an antilipolytic drug can rescue older liver cells from the age-related accumulation of oxidative damage of mtDNA in less than 6 hours [17] and restore in part the urinary excretion of 8-OHdG (unpublished). Since autophagy is responsible for mitochondria and peroxisomes quality control [74], results suggest that the age-related decline of autophagy may be behind the age-dependent accumulation of defective mitochondria in mammalian cells, and eventually bear on ROS production, oxidative stress and progress of aging [75].
- 8) Preliminary data show that an every-other-day stimulation of autophagy by the injection of an antilipolytic drug fully rescues 14-month-old AL rats from the increase in total, LDL and HDL plasma cholesterol (unpublished).

CONCLUSIONS AND PERSPECTIVES

Growing evidence supports the hypothesis that autophagy has a major role in the retardation of the aging process by anti-aging interventions like CR and that the pharmacological intensification of autophagy by antilipolytic drugs licensed for clinical use may be a promising intervention for retardation of aging and age-associated diseases [76]. From the practical point of view, this treatment might open a way to make more people likely to adhere to an anti-aging regimen of dietary restriction otherwise too intensive to be endurable over an extended period involving much of human life.

Currently available data show that the pharmacological intensification of the process of autophagic degradation might be a big step towards retardation of aging and prevention and therapy of neurodegenerative diseases [77, 78]. Since the understanding of the process of autophagy in mammalian systems is still in its infancy, a major challenge remains to define and characterise the machinery regulating mammalian autophagy to identify more specific and safe targets for long-term therapy.

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