Dossier: Aging and age-related diseases

The anti-ageing effects of caloric restriction may involve stimulation of macroautophagy and lysosomal degradation, and can be intensified pharmacologically

E. Bergamini *, G. Cavallini, A. Donati, Z. Gori

Centro di Ricerca di Biologia e Patologia dell’Invecchiamento, University of Pisa, Scuola Medica, Via Roma 55, 56126 Pisa, Italy

Abstract

Caloric restriction (CR) and a reduced growth hormone (GH)–insulin-like growth factor (IGF-1) axis are associated with an extension of lifespan across taxa. Evidence is reviewed showing that CR and reduced insulin of GH–IGF-1 axis may exhibit their effects at least partly by their common stimulatory action on autophagy, the cell repair mechanism responsible for the housekeeping of cell membranes and organelles including the free radical generators peroxisomes and mitochondria. It is shown that the life-long weekly administration of an anti-lipolytic drug may decrease glucose and insulin levels and stimulate autophagy and intensify anti-ageing effects of submaximal CR.

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1. Ageing and senescence

The postreproductive phase of life of virtually all multicellular species is characterized by an inexorable, progressive decrease in physiological capacity and reduced ability to respond to environmental stresses, that lead to diminished homeostasis and increased organicis vulnerability to disease [1,2]. The nature of the causal mechanisms that initiate the deleterious alterations underlying this phenomenon, often referred to as ‘senescence’ or the ‘aging process’ remain controversial. A currently popular hypothesis postulates that a progressive accumulation of macromolecular oxidative damage and not completed housekeeping is the fundamental underlying cause of senescence-associated deleterious alterations [1]. Oxidative stress is involved in ageing and many acute and chronic diseases as a major pathogenic factor [3].

May be, in this finite, imperfect world nothing is perfect, including health. In this perspective, the rate of the process of ageing of any individual species may be dictated by the gap between real life and perfection, and senescence could be defined as a chronic, degenerative, not curable disease with a life-long incubation time, and age-associated diseases might be viewed as complications. This is an optimistic view, which implies that ‘ageing’ though not curable might be preventable like any other disease. Hypothesis is in line with current knowledge: ageing appears to be a phenotypic event like any other disease, which is influenced by genetic and environmental factors; interventions in genetic and nutritional factors were found to extend longevity. Evidence will be reviewed showing that both types of approach may act by the same pathophysiological mechanism, and that the beneficial effects may be intensified pharmacologically.

2. The genetic control of longevity

Genes exert a powerful control on lifespan, as indicated by the enormous difference among species, ranging from a few days to more than 100 years. In mammals, genetic factors account for only about 35% of the intraspecies variance in longevity [4]. A rapidly growing body of evidence shows that at least some of the mechanisms of genetic control of longevity might have been conserved from yeast to mammals and that insulin–insulin-like growth factor (IGF) signalling pathway is causally linked to ageing across taxa [5]. The insulin/IGF-1 signalling pathway (where IGF-1 is insulin-like growth factor-1) influences longevity (acting during adulthood to relatively old age), reproduction and diapause in many organisms [6]. In worms, flies and in the transgenic mice, mutations in the insulin signalling pathway reduce...
growth and size of the organism and, curiously, prolong longevity.\(^7,8\) Studies in \textit{C. elegans} demonstrate that disruption of the daf-2 signalling pathway extends longevity\(^9,10\) similarities with the insulin-like signalling in flies and yeast, and mammalian insulin-like growth factor I (IGF-1) signalling cascade raise the possibility that modifications to the IGF-1 signalling could also extend lifespan in mammals\(^11\). Long-standing growth hormone (GH)/IGF-1 deficiency affects several parameters of the ageing process without impairing lifespan, and prolongs longevity in animal models\(^12\) whereas high GH/IGF-1 levels accelerate death\(^13\). GH/IGF-1 deficient dwarf mice do live significantly longer than their wild counterparts\(^12\). Heterozygous knockout \(Igfr1\) (+/-) mice are not dwarf and live on the average 26% longer than their wild-type littermates\(^14\) but an optimal level of hormone appears to be required to maximize survival\(^15\). On the other hand, transgenic mice that overexpress GH exhibit a drastic reduction in lifespan\(^16\). In invertebrates and mammals, insulin and IGFs use similar signalling pathways and both are importantly related to the regulation of ageing and lifespan and may interact with each other\(^17\). Ageing may impair insulin and IGF-1 signalling but does not affect IGF-1 signalling in hepatic tissues\(^18\).

3. Nutritional intervention in ageing: the effects of CR

Caloric restriction (CR) has been documented to have a positive effect on the lifespan of rodents and various invertebrate species—protozoa, flies, water fleas, nematodes, rotifers and spiders—and vertebrates species—fish, hamsters, dogs\(^19,20\). 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\(^19,21\). There are ongoing long-term studies on the effects of CR on non-human primates in several US laboratories but 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\(^22\). Nutrition has remarkable effects on glucose, lipid and amino acid metabolism and influences many endocrine functions. Restriction of caloric intake is known to reduce blood sugar and insulin levels and to lower resistance to insulin of peripheral tissues\(^19,23\). Recent evidence invited to suggest that CR might exhibit its effects to lifespan extension partly through the reduced GH–IGF-1 axis.

4. Mechanisms of the genetic and nutritional anti-ageing intervention

It has been shown that 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\(^24\). To our knowledge, the in vivo administration or the in vitro treatment with insulin never caused any sudden increase in free radical production, which could account for a direct pro-ageing effect of the hormone. CR extends median and maximum life and health span, lowers body weight and causes a percent wise increase in lean body mass; careful physiological studies showed that it does not cause any decrease in the energy expenditure per unit of body mass\(^25\). It was shown that restriction of the caloric intake for 4 months does not decrease oxygen-free radical production\(^26\). On the whole, there is no evidence to support the hypothesis that genetic and nutritional intervention might extend longevity by decreasing oxidative metabolism and free radical generation. Therefore, the alternate hypothesis should be considered that anti-ageing interventions might extend life and health span by improving the function of the anti-ageing cell repair mechanism(s)\(^1\). There is no evidence that the cell repair mechanisms acting at the molecular level and apoptosis are primarily controlled by nutrition. Macroautophagy, which has not been given much attention as an anti-ageing cell repair mechanism so far, was proposed to be the best candidate-mediator of the anti-ageing effects of CR\(^27,28\) because it is tightly coupled with nutrition and is inhibited by higher levels of insulin. Macroautophagy has the function to serve as an important source of amino acids for gluconeogenesis and other systemic oxidative and biosynthetic reactions when exogenous substrate is not available\(^29,30\).

5. Macroautophagy

Macroautophagy is a universal, highly conserved process, which takes place in all eukaryotic cells. The process is important to maintain a well-controlled balance between anabolism and catabolism in order to have normal cell growth and development. It plays an essential role during...
starvation, cellular differentiation, cell death and ageing but also in preventing some form of cancer [31]. This degradation pathway permits the cell to eliminate unwanted or unnecessary organelles and to recycle the components for reuse [32]. The basic machinery for autophagy is identical between yeast and mammalian cells [33]. Macroautophagy has also been demonstrated in plants and shown to be involved in the response of plant cells to the shortage of nutrients [34,35]. Autophagy is involved in cell re-modelling and atrophy, and is the only mechanism for the degradation of altered membranes and cell organelles, including mitochondria (e.g. [36]). Defective autophagy may result in cell loss by type II apoptosis. Autophagy is a dynamic process involving the rearrangement of subcellular membranes to sequester cytoplasm and organelles for delivery to the lysosome or vacuole, where the sequestered cargo is degraded and recycled. The process is highly regulated through the action of various kinases, phosphatases and guanosine triphosphatases [31]. Extensive research on mammalian cells in the last three decades showed that autophagy and lysosomal proteolysis are activated by lower amino acid and insulin levels during fasting to produce nutrients from endogenous sources, and that higher (postprandial) levels of insulin fully suppress autophagy in the physiological range of plasma amino acid concentration [29,30,37]. In conclusion, it is conceivable that mammalian cells can use autophagy in order to get rid of altered membranes and cell organelles only during the time of organismic fasting.

6. Age-changes in the function of macroautophagy. The effects of CR

Lysosomal function(s) decline(s) in older animals [38]. In liver cells isolated from ad libitum fed rats, the function of autophagy declines with increasing age and the decline is prevented by CR [39,40]. The ageing-related changes in the function of autophagy correlate with the expectation of life [41]. The action of autophagy in vivo becomes weaker and weaker the older the animal starting by age 6 months in ad libitum fed animals, but retains its strength in food-restricted rats [42–44]. As expected, the ageing-related decline in autophagy may produce a fall in the maintenance of cell membranes: the lipid composition of rat tissues changes after the age of 6 months, because of a progressive age-related accumulation of the membrane lipid dolichol [45]. Rats on a caloric restricted regimen consume their given food in less than 6–8 h and spend most of the day in a state of fasting, lower concentration of glucose and insulin and higher plasma concentration of leucine, isoleucine and valine and higher rate of autophagic proteolysis. The ageing-related accumulation of dolichol is significantly retarded by calorie restriction [46], efficacy depending on level [47] and duration [48]. The age-changes in the lipid composition of cytomembranes in calorie restricted rats correlate with the function of autophagy and expectation of life [41,48].

Fig. 1. Effects of ageing, CR and pharmacological intervention on the regulatory effect of amino acids on the autophagic proteolysis of liver cells.

Pharmacological modulation of the autophagic function 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 [49]. Chronic pharmacological inhibition of autophagic proteolysis appears to accelerate the rate of the process of ageing: in several species and organ systems, the treatment induced the formation of lysosomal aggregates which closely resembled ceroid-lipofuscin [50], which strongly suggested that the ‘protease-inhibitor model of ageing’ is generally valid [51]. Protease inhibition caused an anomalous accumulation of tau and ubiquitin immunoreactivity in brain [52]. Function of autophagic proteolysis can easily be intensified in vivo by a pharmacological intervention on the physiological regulatory mechanism. The administration of anti-lipolytic agents to fasted rats may provide a convenient (i.e. an inexpensive, highly reproducible and timeable) physiologic model to study hormone (low insulin–high glucagon and corticosterone)-induced autophagy in liver cells [53,54]. Treatment caused a significant degradation of selected liver cell organelles including peroxisomes and, to a minor extent, mitochondria [55]. Preliminary results showed that life-long weekly stimulation of autophagy by the
intragastric administration of an anti-lipolytic agent licensed for human use maximized the beneficial effects of a mild (one-day-a-week, 10%) calorie restriction on two parameters that are known to correlate with life expectancy: the ageing-related changes in liver autophagy (Fig. 1) and dolichol accumulation in the liver (Fig. 2) [56].

8. Conclusions

Evidence in ad libitum fed animals suggests that overfeeding may increase plasma glucose and insulin secretion and cause a long-lasting increase in amino acid, insulin and IGF-1 plasma levels. Physiological expectation is that the aforementioned metabolic and endocrine changes could suppress autophagy and slow down turnover rate of cell protein, membrane and organelles. Longer biological life of cell macromolecules and structures may give more time for alteration and accumulation of peroxidized macromolecules and defective organelles in cells [57], and may accelerate aging. A vicious circle may be started by the alteration of membrane function, which eventually leads to irreversibility of the cell changes (see Fig. 3 and Refs. [28,29]). CR and genetic disruptions of the insulin and GH/IGF-1 axis may lower insulin and IGF-1 levels and break the cycle: enhanced autophagy can prevent the age-related accumulation of deteriorated subcellular components (including altered mitochondria) and extend lifespan. Anti-lipolytic drugs and severe atrophy of the fat tissue (e.g. by genetic disruption of the fat insulin receptor [24]) may cause a shortage of FFA and a compensatory intensification of autophagic proteolysis [54,55] and glucogenesis during fasting, which may account for beneficial effects on longevity.

References


