



Oxidative Damage and Aging

Giacinto Libertini

www.r-site.org/ageing

www.programmed-aging.org

giacinto.libertini@tin.it

Framingham Heart Study [1] and, afterwards, many other studies documented [2] that the risk of coronary heart disease is positively related to:

[Modifiable risk factors]

Hypercholesterolemia

Low HDL cholesterol level

Hypertension

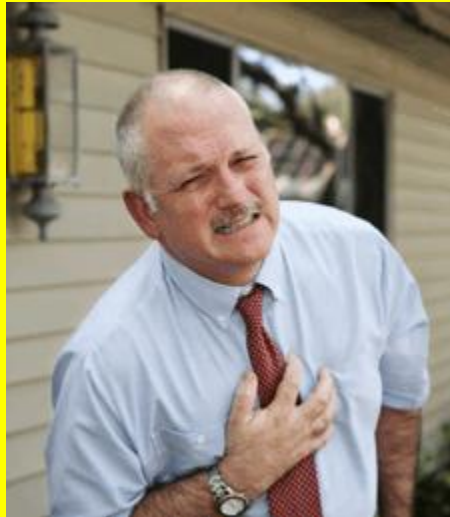
Glucose intolerance (Diabetes)

Cigarette smoking

[Not modifiable risk factors]

Age

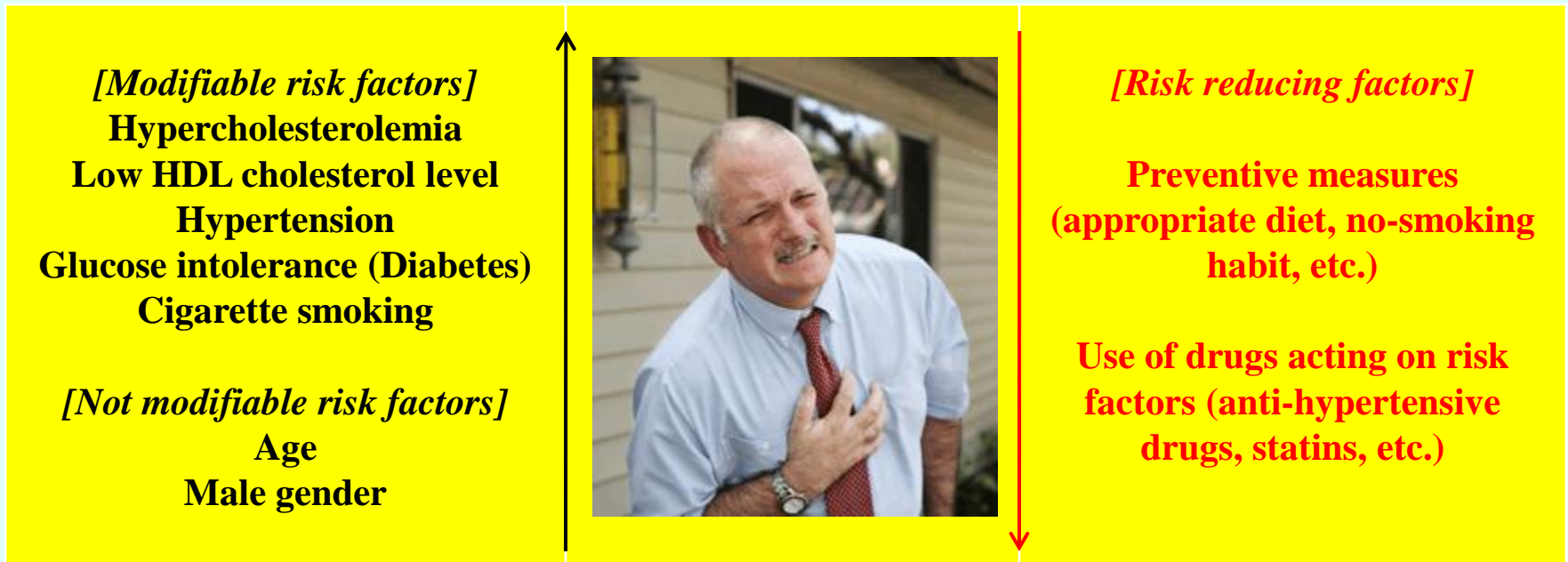
Male gender



[1] Wilson PW et al. (1987) Coronary risk prediction in adults (the Framingham Heart Study) *Am J Cardiol.* 59, 91G-94G.

[2] Wayne R et al. (eds) (1998) *Hurst's The Heart, Arteries and Veins* - 9th edit. McGraw-Hill, New York.

Moreover, the risk of coronary heart disease was lowered by [1]:



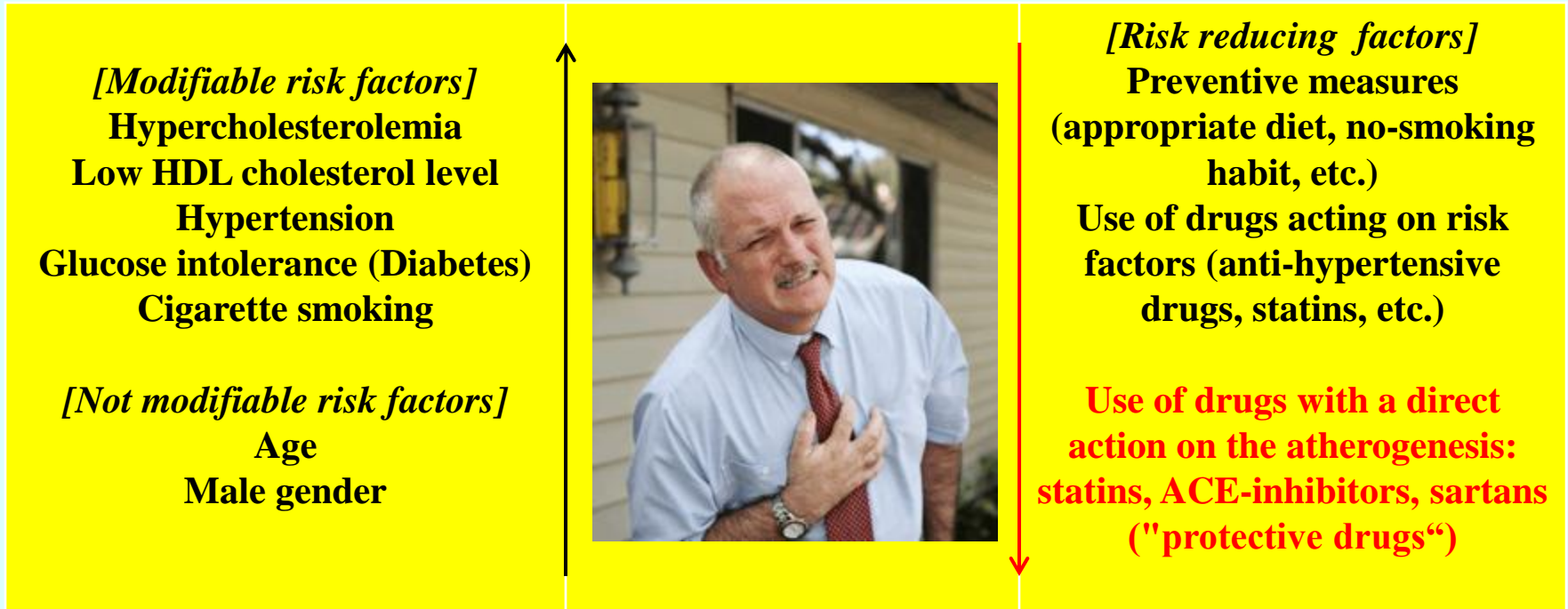
The general interpretation of these data seemed pacific and easy:

- 1) Modifiable risk factors increase oxidative damage (or cause other damages) while preventive measures and drugs avoid or reduce these harms.
- 2) Aging, as a consequence of cumulative oxidative damage (and/or of other damages), was necessarily the cause of age-related cardiovascular increasing risks, not reducible with preventive measures and drugs.

[1] Wayne R et al. (eds) (1998) *Hurst's The Heart, Arteries and Veins* - 9th edit. McGraw-Hill, New York.

Later, statins [1], ACE-inhibitors and sartans [2] (“protective drugs”), were shown to be effective in reducing the risk even without acting on risk factors, namely with a direct action on atherogenesis.

These new data were compatible with the above-said general interpretation.

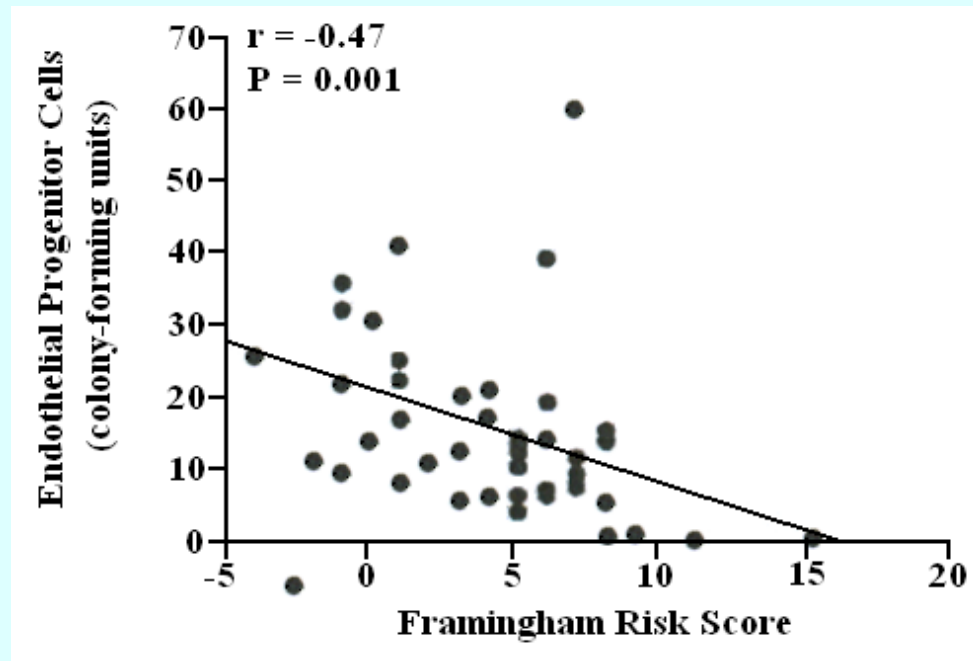


[1] Davidson MH (2007) Overview of prevention and treatment of atherosclerosis with lipid-altering therapy for pharmacy directors. *Am. J. Manag. Care* 13, S260-9.

[2] Weir MR (2007) Effects of renin-angiotensin system inhibition on end-organ protection: can we do better? *Clin. Ther.* 29, 1803-24.

But, this peaceful picture was challenged by the results of Hill et al. [1] and of other Authors that have confirmed and widened them (e.g.: [2]):

They showed that the number of circulating Endothelial Progenitor Cells (EPC) is significantly negatively related to the Framingham Risk Score.



[1] Hill JM et al. (2003) Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N. Engl. J. Med.* 348, 593-600.

[2] Werner N et al. (2005) Circulating endothelial progenitor cells and cardiovascular outcomes. *N. Engl. J. Med.* 353, 999-1007.

Moreover:

"the levels of circulating EPC were a better predictor of vascular reactivity than was the presence or absence of conventional risk factors.

In addition, EPC from subjects at high risk for cardiovascular events had higher rates of *in vitro* senescence than cells from subjects at low risk." [1]

The age-related decline of EPC, hinted by Hill et al. ($P=.07$) was confirmed by other studies (e.g.: [2]; $P=0.013$).

Statins, ACE-inhibitors and sartans are associated with higher values of EPC [2].

[1] Hill JM et al. (2003) Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N. Engl. J. Med.*. 348, 593-600.

[2] Xiao Q, et al. (2007) Endothelial progenitor cells, cardiovascular risk factors, cytokine levels and atherosclerosis--results from a large population-based study. *PLoS One*. 2: e975.

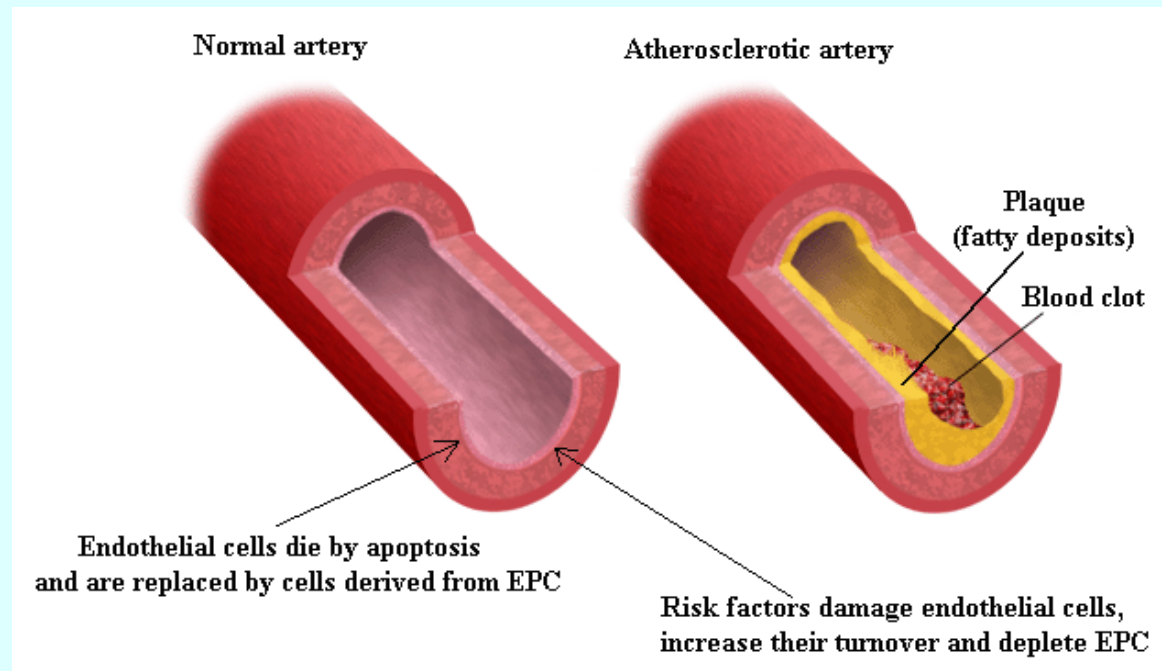
Interpretation of these data [1]

Endothelial cells manifest a continuous turnover assured by EPC, which derive from primary stem cells of bone marrow.

Excessive stress (oxidative or of other types) increases apoptosis rate of endothelial cells and quickens their turnover and this is manifested by the reduction of EPC count.

Older endothelial cells, which suffer by cell senescence, increase the probability of atherosclerosis:

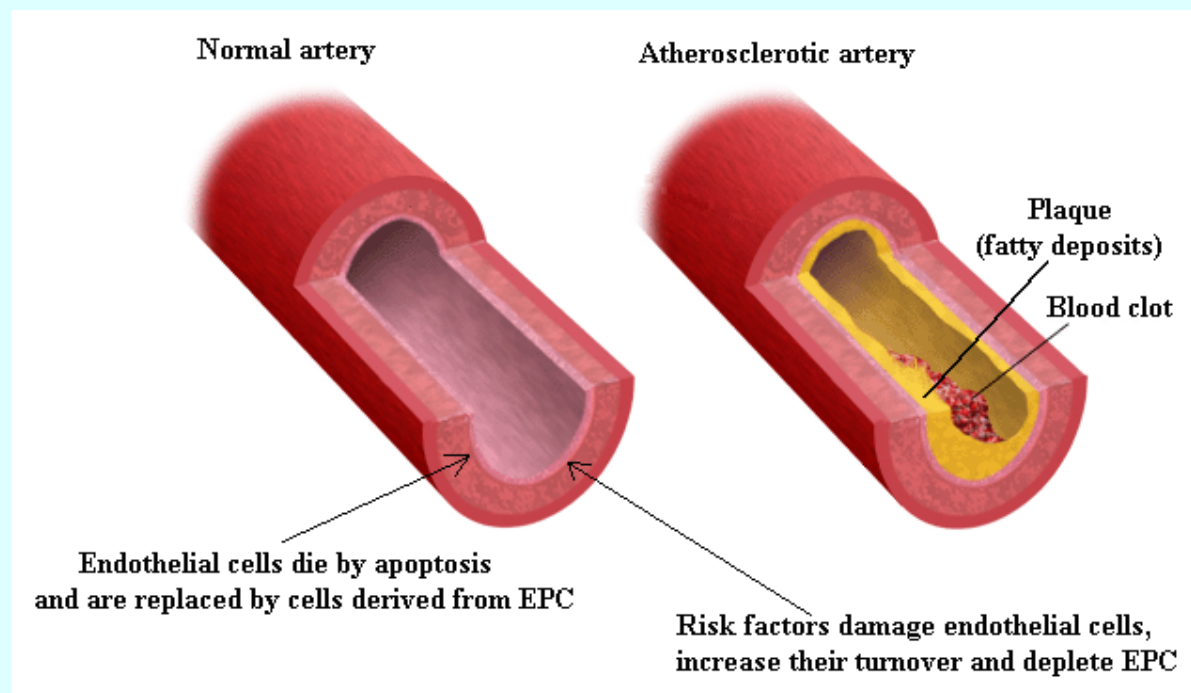
cell senescence -> endothelial dysfunction -> inflammation, plaques, blood clot, etc. ...



[1] Hill JM et al. (2003) Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N. Engl. J. Med.* 348, 593-600.

... In old individuals, with or without excessive stress, EPC are reduced because of EPC stem cell exhaustion by telomere shortening: diseases derived from compromised blood circulation are a common end to the life of healthy old individuals with no particular risk factor [1].

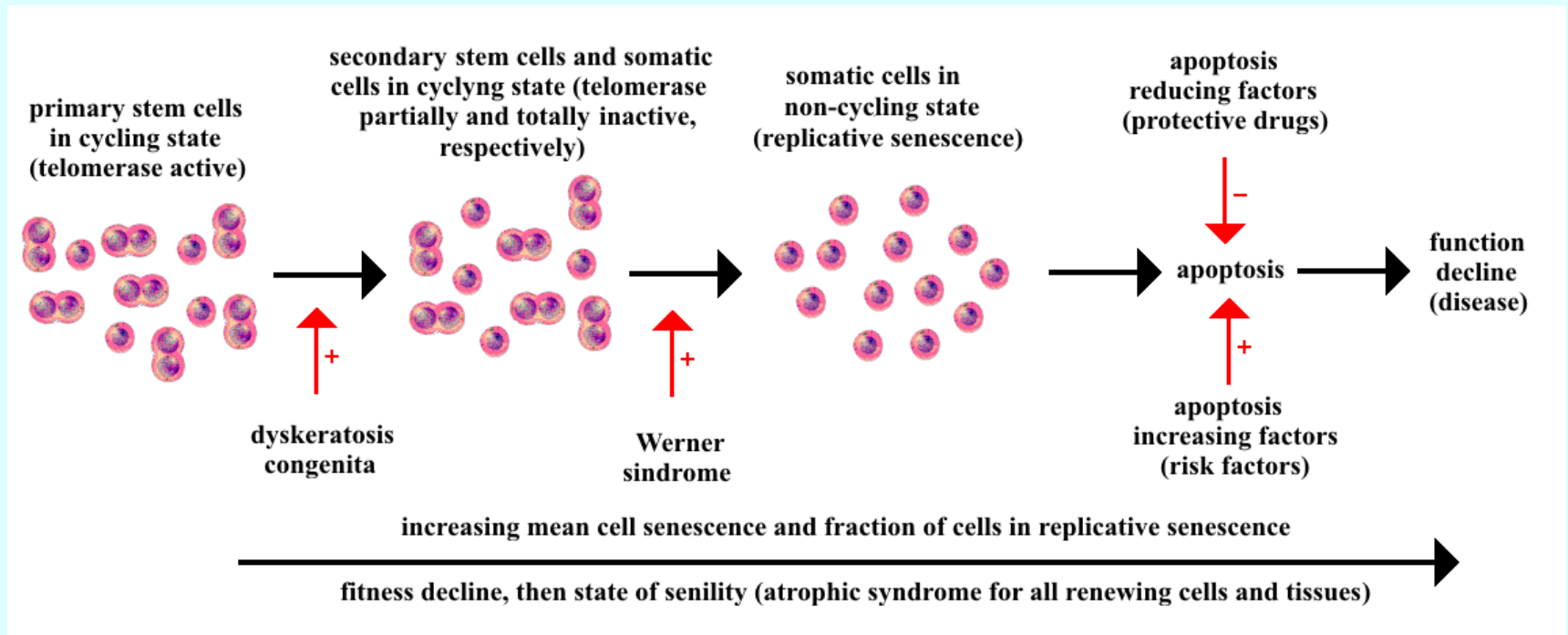
Some genetic diseases (as *Dyskeratosis congenita* and Werner syndrome) increase apoptosis rate and cell turnover, so accelerating atherogenesis [2].



[1] Tallis, RC, Fillit, HM & Brocklehurst, JC (eds) (1998) *Brocklehurst's Textbook of Geriatric Medicine and Gerontology* (5th ed.) Churchill Livingstone, New York.

[2] Marciniak, R & Guarente, L (2001) Human genetics. Testing telomerase. *Nature*, 413, 370-2.

These concepts may be generalized in the following scheme (concepts from [1]; figure from [2]):



[1] Marciniak R & Guarente L (2001) Human genetics. Testing telomerase. *Nature*, 413, 370-2.

[2] Libertini G (2009) Prospects of a Longer Life Span beyond the Beneficial Effects of a Healthy Lifestyle, Ch. 4 in *Handbook on Longevity: Genetics, Diet & Disease*, Nova Science Publishers Inc., New York.

An important concept:

Oxidative damage (+ other damaging factors) are important in atherogenic process and in aging but the key actor is the **progressive failure of cell turnover caused by cell duplication limits**, which are determined by the genetic regulation of telomere-telomerase system.

The scheme proposed for endothelial cells and atherogenesis is likely valid for other organs and tissues and for the whole organism.

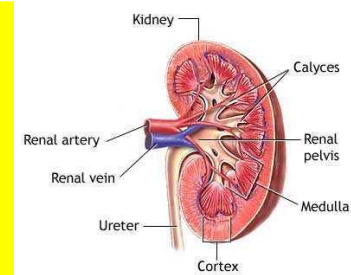
E.g.:

Apoptosis is well documented, in healthy organisms, for glomerular cells [1], alveolocytes type II [2], pancreatic β cells [3, 4], etc.

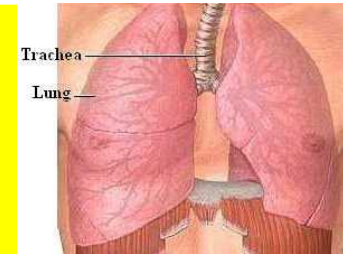
This means that these cells have turnover, and so ...

- [1] Cardani R & Zavanella T (2000) Age-related cell proliferation and apoptosis in the kidney of male Fischer 344 rats with observations on a spontaneous tubular cell adenoma. *Toxicol. Pathol.* 28, 802-806.
- [2] Sutherland LM et al. (2001) Alveolar type II cell apoptosis. *Comp. Biochem. Physiol.* 129A, 267-285.
- [3] Bonner-Weir S (2000) Islet growth and development in the adult. *J. Mol. Endocrinol.* 24, 297-302.
- [4] Cerasi E et al. (2000) [Type 2 diabetes and beta cell apoptosis] [Article in French]. *Diabetes Metab.* 26, 13-6.

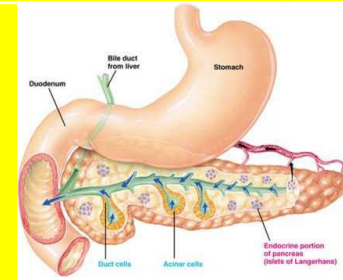
- for glomerular cells: microalbuminuria, a marker of renal damage and also a good marker of atherogenesis, is corrected by "protective drugs" [1]



-for alveolocytes type II: the decline in lung function in smokers is reduced by statins, which are among the "protective drugs" [2]



-for pancreatic β -cells: diabetes in the case of a wrong diet. The risk of diabetes is reduced by "protective drugs" [3, 4]



[1] Weir MR (2007) Microalbuminuria and cardiovascular disease. *Clin J Am Soc Nephrol.* 2, 581-90.

[2] Alexeeff SE et al. (2007) Statin use reduces decline in lung function: VA Normative Aging Study. *Amer. J. Respir. Crit. Care Medic.* 176, 742-7.

[3] McCall KL et al. (2006) Effect of angiotensin-converting enzyme inhibitors and angiotensin II type 1 receptor blockers on the rate of new-onset diabetes mellitus: a review and pooled analysis. *Pharmacotherapy* 26, 1297-306.

[4] Ostergren J (2007) Renin-angiotensin-system blockade in the prevention of diabetes. *Diabetes Res. Clin. Pract.* 78, S13-21.

This means a general view where [1-4]:

- the organism is in continuous renewal (turnover) of its cells;**
- aging is the consequence of the progressive slackening of this turnover;**
- many diseases are the effect of the acceleration of the physiologic turnover of some cell types and the consequent exhaustion of renewal capacities;**
- many risk factors and many drugs contrasting these factors act by increasing or reducing, respectively, this turnover acceleration.**

**Aging can be described as the progressive atrophy
of each tissue and organ**

[1] Fossel MB (2004) *Cells, Aging and Human Disease*. Oxford University Press, New York.

[2] Libertini G (2006) Evolutionary explanations of the “actuarial senescence in the wild” and of the “state of senility”. *The Scientific World JOURNAL* 6, 1086-108 DOI 10.1100/tsw.2006.209.

[3] Libertini G (2009a) Prospects of a Longer Life Span beyond the Beneficial Effects of a Healthy Lifestyle, Ch. 4 in *Handbook on Longevity: Genetics, Diet & Disease*, Nova Science Publishers Inc., New York.

[4] Libertini G (2009b) The Role of Telomere-Telomerase System in Age-Related Fitness Decline, a Tameable Process, in *Telomeres: Function, Shortening and Lengthening*, Nova Sc. Publ., New York.

The atrophic syndrome of a tissue or organ is characterized by [1]:

- a) reduced cell duplication capacity and slackened cell turnover (replicative senescence);
- b) reduced number of cells (atrophy);
- c) possible substitution of missing specific cells with nonspecific cells;
- d) hypertrophy of the remaining specific cells;
- e) altered functions of cells with shortened telomeres or definitively in noncycling state (cell senescence);
- f) alterations of the surrounding milieu and of the cells depending from the functionality of the senescent or missing cells
- g) vulnerability to cancer because of dysfunctional telomere-induced instability [2].



[1] Libertini G (2006) Evolutionary explanations of the “actuarial senescence in the wild” and of the “state of senility”. *The Scientific World JOURNAL* 6, 1086-108 DOI 10.1100/tsw.2006.209.

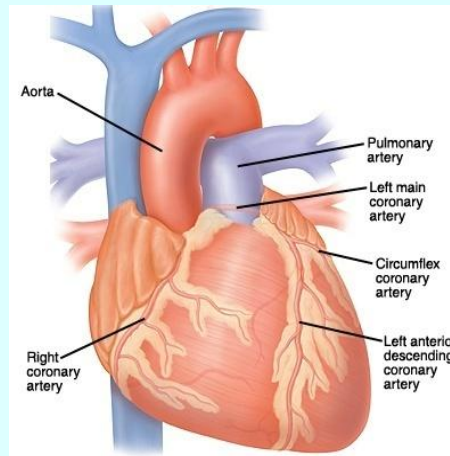
[2] DePinho RA (2000) The age of cancer. *Nature* 408, 248-54.

This view stimulates an immediate objection:
There are cells or tissues that have no turnover and so cannot be included in this scheme,
thus greatly weakening it:

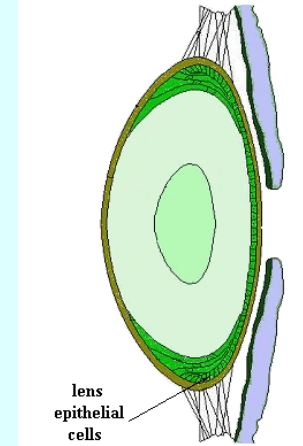
1) Muscular tissue



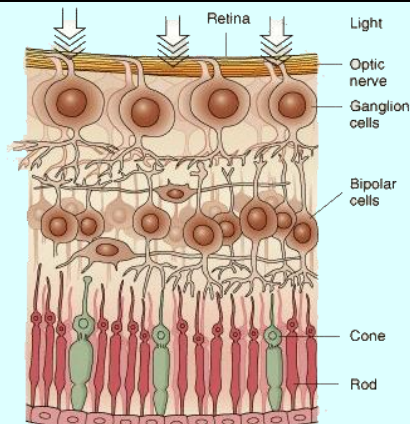
2) Heart muscle tissue



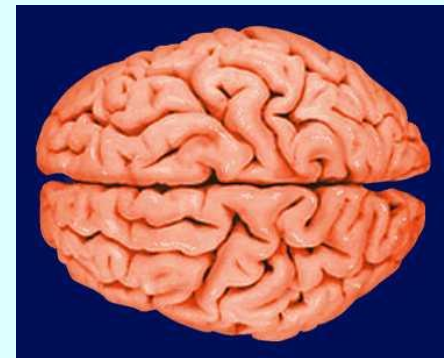
3) Eye crystalline lens



4) Photoreceptors of retina



5) Neurons of the Central Nervous System



But ...

1) Muscular tissue



Myocytes are cells with turnover!

Stem cells from muscles of old rodents divide in culture less than cells from muscles of young rodents [1];

A transplanted muscle suffers ischaemia and complete degeneration and then there is a complete regeneration by action of host myocyte stem cells that is poorer in older animals [2];

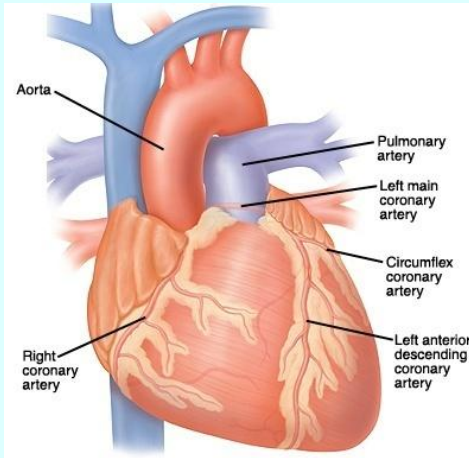
In *Duchenne* muscular dystrophy, there is a chronic destruction of myocytes that are continually replaced by the action of stem cells until these are exhausted [3].

[1] Schultz E & Lipton BH (1982) Skeletal muscle satellite cells: changes in proliferation potential as a function of age. *Mech. Age. Dev.* 20, 377-83.

[2] Carlson BM & Faulkner JA (1989) Muscle transplantation between young and old rats: age of host determines recovery. *Am. J. Physiol.* 256, C1262-6.

[3] Adams V et al. (2001) Apoptosis in skeletal muscle. *Front. Biosci.* 6, D1-D11.

2) Heart muscular tissue



Heart myocytes are cells with turnover!

“It remains a general belief that the number of myocytes in the heart is defined at birth and these cells persist throughout life ... But myocytes do not live indefinitely – they have a limited lifespan in humans and rodents. Cell loss and myocyte proliferation are part and parcel of normal homeostasis ...” [1]

“Age-associated left ventricular hypertrophy is caused by an increase in the volume but not in the number of cardiac myocytes.” [2]

“With aging, there is also a progressive reduction in the number of pacemaker cells in the sinus node, **with 10 percent of the number of cells present at age 20 remaining at age 75.**” [2]: This causes atrial fibrillation and “protective drugs”, as ACE-inhibitors, sartans and statins, are effective in the prevention of it [3, 4].

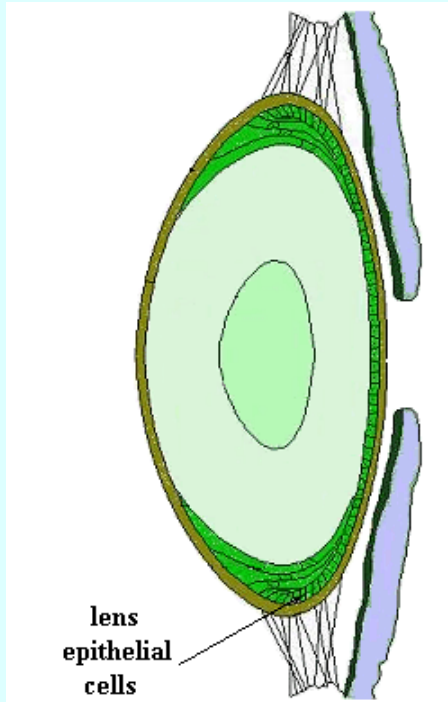
[1] Anversa P & Nadal-Ginard B (2002) Myocyte renewal and ventricular remodelling. *Nature* 415, 240-3.

[2] Aronow WS (1998) Effects of Aging on the Heart. In *Brocklehurst's Textbook of Geriatric Medicine and Gerontology*.

[3] Jibrini MB et al. (2008) Prevention of atrial fibrillation by way of abrogation of the renin-angiotensin system: a systematic review and meta-analysis. *Am. J. Ther.* 15, 36-43.

[4] Fauchier L et al. (2008) Antiarrhythmic effect of statin therapy and atrial fibrillation a meta-analysis of randomized controlled trials. *J. Am. Coll. Cardiol.* 51, 828-35.

3) Eye crystalline lens



The crystalline lens has no cell in its core, but its functionality depends on lens epithelial cells that show turnover [1].

“Many investigators have emphasized post-translational alterations of long-lived crystalline proteins as the basis for senescent ocular cataracts. It is apparent in Werner syndrome that the cataracts result from alterations in the lens epithelial cells” [2], which is consistent with age-related reduction in growth potential for lens epithelial cells reported for normal human subjects [1].

Smoke and diabetes are risk factors for cataract [3].

Statins lower the risk of cataract [4]. This has been attributed to “putative antioxidant properties” [4] but could be the consequence of effects on lens epithelial cells analogous to those on endothelial cells [5].

[1] Tassin J et al. (1979) Human lens cells have an in vitro proliferative capacity inversely proportional to the donor age. *Exp. Cell Res.* 123, 388-92.

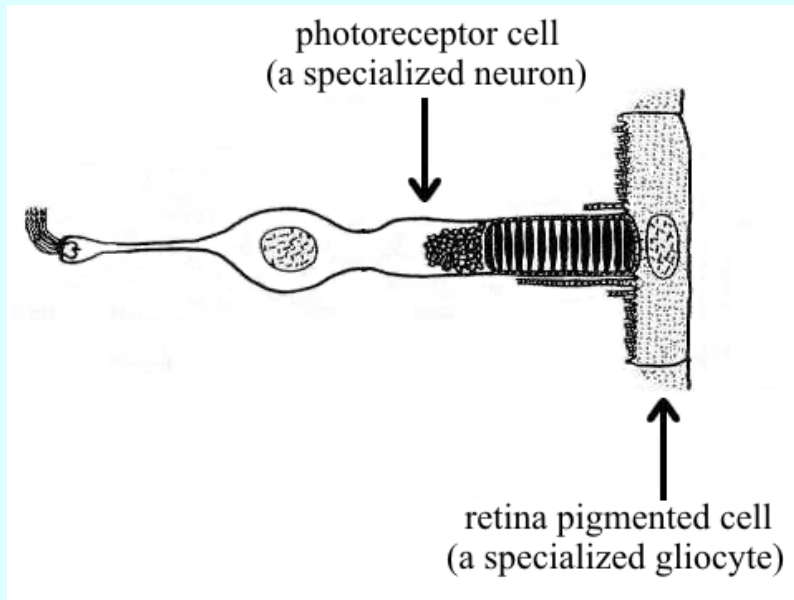
[2] Martin GM & Oshima J. (2000) Lessons from human progeroid syndromes. *Nature* 408, 263-6.

[3] Delcourt C et al. (2000) Risk factors for cortical, nuclear, and posterior subcapsular cataracts: the POLA study. *Pathologies Oculaires Liées à l'Age. Am J Epidemiol.* 151, 497-504.

[4] Klein BE et al. (2006) Statin use and incident nuclear cataract. *JAMA* 295, 2752-8.

[5] Hill JM et al. (2003) Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N. Engl. J. Med.* 348, 593-600.

4) Retinal nervous cells



Photoreceptor cells (cones and rods) are highly differentiated nervous cells with no turnover, but metabolically depending on other cells with turnover, retina pigmented cells (RPC), which are highly differentiated gliocytes.

The top of a photoreceptor cell leans on a RPC.

Each day, every RPC phagocytizes about 10% of the membranes with photopsin molecules of about 50 photoreceptor cells and, so, each day a cell of RPC metabolizes photopsin molecules of about 5 cones or rods, demonstrating a very high metabolic activity.

Without the macrophagic activity of RPC, photoreceptor cells cannot survive.

4) Retinal nervous cells - continued



Effects of ARMD on vision

With the age-related decline of RPC turnover, in RPC cells there is accumulation of damaging substances as A2E (a vitamin A-derived breakdown product) [1].

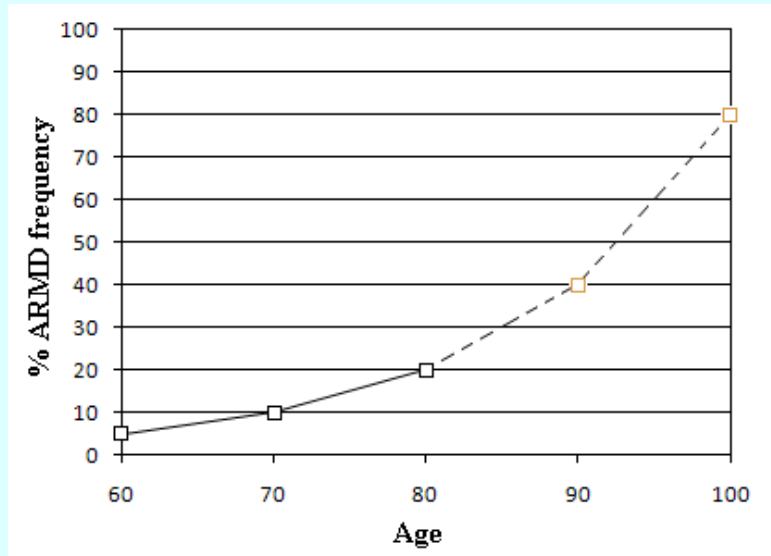
The death of RPCs by action of these substances causes holes in RPC layer and the deficiency of their function kills the photoreceptors not served.

This is above all manifested in the functionality of the more sensitive part of the retina, the macula - where the accumulation of A2E is more abundant [1] -, from which the name “age-related retina macular degeneration” (ARMD) [2]. ...

[1] Sparrow JR (2003) Therapy for macular degeneration: Insights from acne. *Proc Natl Acad Sci USA* 100, 4353–4.

[2] Fine SL et al. (2000) Age-related macular degeneration. *N. Engl. J. Med.* 342, 483-92.

4) Retinal nervous cells - continued



ARMD affects 5%, 10% and 20% of subjects 60, 70 and 80 years old, respectively [1], and it is likely that a large proportion of older individuals suffer from ARMD.

Smoking, diabetes, and obesity are risk factors for ARMD [2].

"The retina, with its high oxygen content and constant exposure to light, is particularly susceptible to oxidative damage" [3].

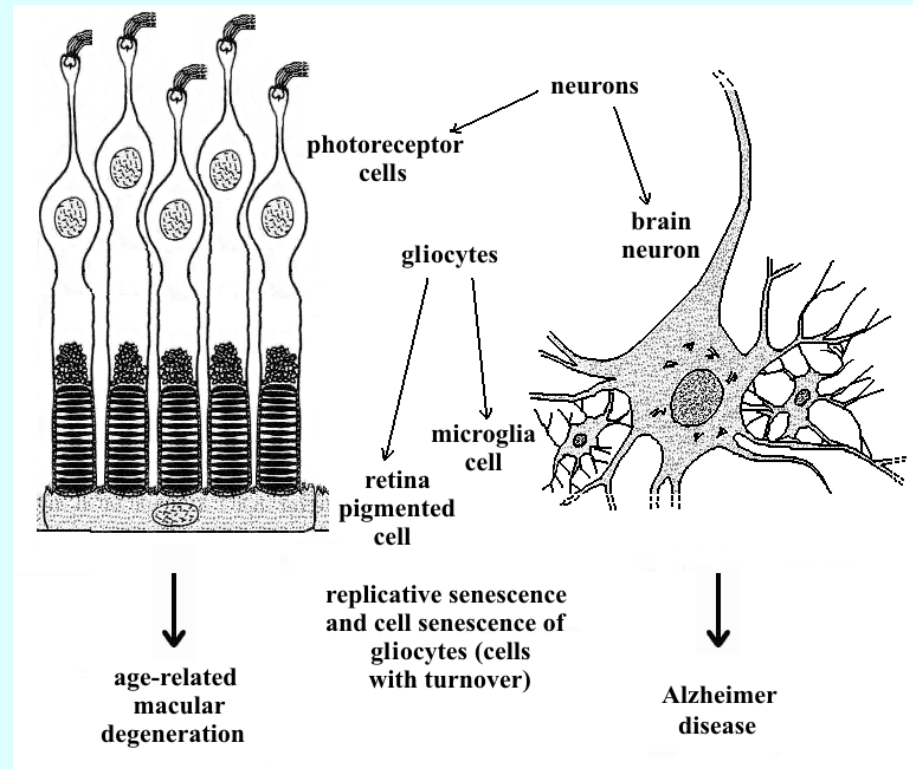
But the meta-analysis of 12 studies did not show that antioxidant supplements prevented early ARMD [3].

[1] Berger JW et al. (1999) *Age-related macular degeneration*, Mosby (USA).

[2] Klein R et al. (2007) Cardiovascular disease, its risk factors and treatment, and age-related macular degeneration: Women's Health Initiative Sight Exam ancillary study. *Am. J. Ophthalmol.* 143, 473-83.

[3] Chong EW et al. (2007). Dietary antioxidants and primary prevention of age related macular degeneration: systematic review and meta-analysis. *BMJ* 335, 755.

5) Neurons of the Central Nervous System



As photoreceptor cells (specialized types of neuron with no turnover) depend on other cells (a specialized type of gliocytes with turnover), other types of neurons - as those of the Central Nervous System - depend on other types of gliocytes.

If this is true, replicative senescence and cell senescence of these gliocytes should cause pathologies similar to ARMD. ...

5) Nervous cells of the Central Nervous System - continued

The hypothesis that Alzheimer Disease (AD) is caused by replicative senescence and cell senescence of microglia cells has been proposed [1-3].

Microglia cells degrade β -amyloid protein [4, 5] and this function is known to be altered in AD [6] with the consequent noxious accumulation of the protein. ...

- [1] Fossel MB (1996) *Reversing Human Aging*. William Morrow and Company, New York.
- [2] Fossel MB (2004) *Cells, Aging and Human Disease*. Oxford University Press, New York.
- [3] Libertini G (2009) Prospects of a Longer Life Span beyond the Beneficial Effects of a Healthy Lifestyle, Ch. 4 in *Handbook on Longevity: Genetics, Diet & Disease*, Nova Sc. Publ., New York.
- [4] Qiu WQ et al. (1998) Insulin-degrading enzyme regulates extracellular levels of amyloid beta-protein by degradation. *J Biol Chem*. 273, 32730-8.
- [5] Vekrellis K et al. (2000) Neurons regulate extracellular levels of amyloid beta-protein via proteolysis by insulin-degrading enzyme. *J. Neurosci*. 20, 1657-65.
- [6] Bertram L et al. (2000) Evidence for genetic linkage of Alzheimer's disease to chromosome 10q. *Science*, 290, 2302-3.

5) Nervous cells of the Central Nervous System - continued

Telomeres have been shown to be significantly shorter in patients with probable AD than in apparently healthy control subjects [1].

AD could have, at least partially, a vascular aetiology due to age-related endothelial dysfunction [2] but “A cell senescence model might explain Alzheimer dementia without primary vascular involvement.” [2]

An interesting comparison between AD and ARMD is possible: both are probably determined by the death of cells with no turnover as a likely consequence of the age-related decline (atrophy) of cells with turnover.

Moreover, AD frequency, as ARMD, affects 1.5% of USA and Europe population at age 65 and 30% at 80 [3] and a centenarian has a high probability of suffering from it. ...

[1] von Zglinicki T et al. (2000) Short telomeres in patients with vascular dementia: an indicator of low antioxidative capacity and a possible risk factor? *Lab. Invest.* 80, 1739-47.

[2] Fossel MB (2004) *Cells, Aging and Human Disease*. Oxford University Press, New York.

[3] Gorelick PB (2004) Risk factors for vascular dementia and Alzheimer disease. *Stroke* 35, 2620-2.

5) Nervous cells of the Central Nervous System - continued

There is association between Alzheimer disease and cardiovascular risk factors [1, 2].

"Protective drugs" as statins, ACE-inhibitors and sartans, are effective against Alzheimer disease [1, 3].

[1] Vogel T et al. (2006) [Risk factors for Alzheimer: towards prevention?] [Article in French] *Presse Med.* 35, 1309-16.

[2] Rosendorff C et al. (2007) Cardiovascular risk factors for Alzheimer's disease. *Am J Geriatr Cardiol.* 16, 143-149.

[3] Ellul J et al. (2007) The effects of commonly prescribed drugs in patients with Alzheimer's disease on the rate of deterioration. *J. Neurol. Neurosurg. Psychiatry* 78, 233-9.



Possible cures for ARMD and for AD

1) Cures that are rational but effective within obvious limits:

- Reduction or avoidance of modifiable risk factors;
- Use of "protective drugs" against the effects of modifiable risk factors.

LIMITS: ineffective against age-related increasing risk of ARMD and AD (age is a non-modifiable risk factor and is not contrasted by “protective drugs”!)

2) Cures that are in accordance with the view that ARMD and AD are caused by the accumulation of damaging substances:

- For ARMD, dietary antioxidants: FAILURE shown in the meta-analysis of 12 studies [1];
- For AD, drugs against the formation of β -amyloid peptide: FAILURE [2];
- For AD, vaccine against β -amyloid peptide: "Post-mortem analyses showed that almost all the patients had stripped-down amyloid plaques, despite most of them having progressed to severe dementia before they died" [2] ...

[1] Chong EW et al. (2007). Dietary antioxidants and primary prevention of age related macular degeneration: systematic review and meta-analysis. *BMJ* 335, 755.

[2] Abbott A (2008) The plaque plan. *Nature* 456, 161-4.

Possible cures for ARMD and for AD - continued

3) Cures that treat cognitive alterations:

- For AD, cholinesterase inhibitors (donepezil, galantamine, rivastigmine) and NMDA receptor antagonist (memantine): "They are marginally effective at best" [1]
- For AD, antipsychotic drugs: Increase of long-term risk of mortality [2]

4) Cures that treat the key mechanism of ARMD and, likely, of AD, that is the turnover progressive failure of EPC and neuron-satellite microglia, respectively:

It is well known from 1998 that with the activation of telomerase, telomeres result elongated and cells acquire unlimited duplication capacities [3-6]. ...

[1] Abbott A (2008) The plaque plan. *Nature* 456, 161-4.

[2] Ballard C et al. (2009). "The dementia antipsychotic withdrawal trial (DART-AD): long-term follow-up of a randomised placebo-controlled trial". *Lancet Neurology* 8, 151-7.

[3] Bodnar AG et al. (1998) Extension of life-span by introduction of telomerase into normal human cells. *Science* 279, 349-52.

[4] Counter CM et al. (1998) Dissociation among *in vitro* telomerase activity, telomere maintenance, and cellular immortalization. *Proc. Natl. Acad. Sci. USA* 95, 14723-8.

[5] Vaziri H (1998) Extension of life span in normal human cells by telomerase activation: a revolution in cultural senescence. *J. Anti-Aging Med.* 1, 125-30.

[6] Vaziri H & Benchimol S (1998) Reconstitution of telomerase activity in normal cells leads to elongation of telomeres and extended replicative life span. *Cur. Biol.* 8, 279-82.

Possible cures for ARMD and for AD - continued

Moreover, in the first experiment, a very important study by Bodnar et al. (which Google Scholar reports has been cited 2,771 times):

"two telomerase-negative normal human cell types, retinal pigment epithelial cells and foreskin fibroblasts, were transfected with vectors encoding the human telomerase catalytic subunit. In contrast to telomerase-negative control clones, which exhibited telomere shortening and senescence, telomerase-expressing clones had elongated telomeres, divided vigorously, and showed reduced staining for -galactosidase, a biomarker for senescence. ...

The ability to maintain normal human cells in a phenotypically youthful state could have important applications in research and medicine." [1]

[1] Bodnar AG et al. (1998) Extension of life-span by introduction of telomerase into normal human cells. *Science* 279, 349-52.

Conclusion

Well, this is an extraordinary coincidence: it is not necessary to demonstrate that RPC can be rejuvenated by action of telomerase.

It is rational to hint that by action of telomerase it could be possible to reactivate the turnover of RPC and, so, to cure the key mechanism of ARMD.

Furthermore, it is rational to hint that by action of telomerase it could be possible to reactivate the turnover of neuron-satellite microglia and, so, to cure the key mechanism of AD.

Conclusion - continued

ARMD and AD are terrible diseases and the cure of them by the correction of their key mechanism is very important *per se*.

**But this type of cure is important
in a more general perspective.**

**ARMD and AD are the pivotal expression
of aging for the nervous system.**

This type of cures could be:

- the first step in the control of aging,
- the demonstration that aging is a tameable process,
- the proof that the ambitious goal of an *Homo sapiens liberatus* is a real aim and not utopia.

This presentation is on my personal pages too:

www.r-site.org/ageing.

Please, write your possible questions (now or when you will prefer).

Any question will have a written and public answer

(e-mail: giacinto.libertini@tin.it)

**Thanks
for your attention**