

It is where all our sores begin....

go to the origin....

at the heart of our life....

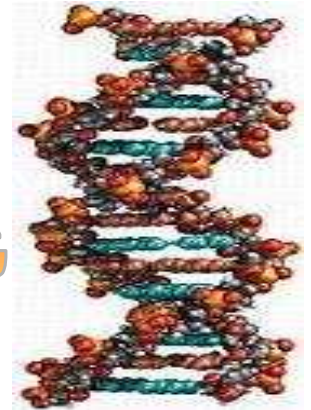


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New concept



Genetic Damage



So far we are familiar with the genetic diseases which are often beginning at birth. They are resulting from a constitutional mutation which often can lead to functional losses often mortal.

“**Genetic damage**” should be our new concept . It accumulates slowly with years under the environmental mutagen especially endogenous cancer, aging and of many degenerative diseases. This residual genetic damage develops with age through deregulation, cellular function loss or death, is the source of our sore. It is in fact the result of :

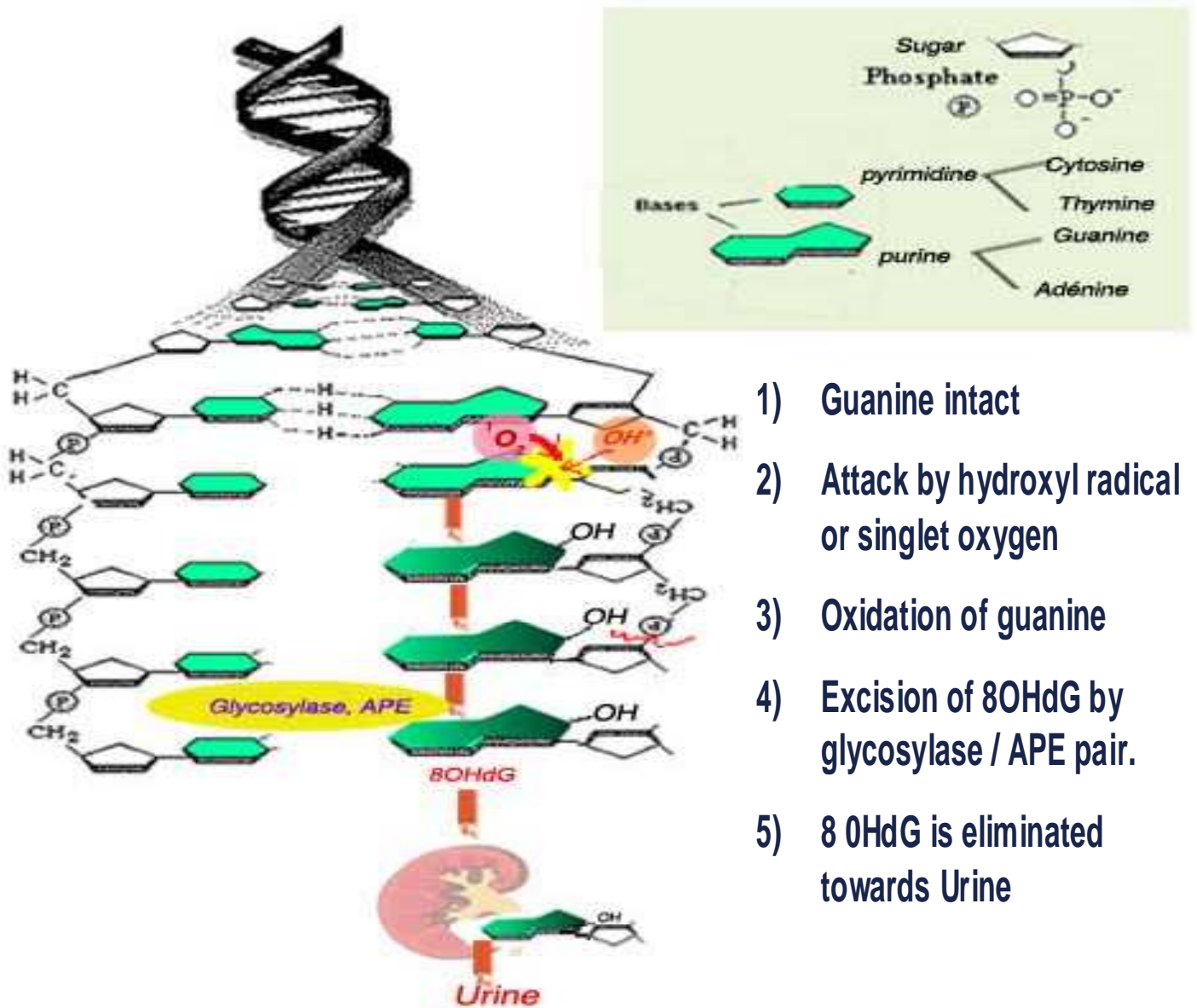


➡ **Urinary 8OHdG** is the most representative indicator of this **mutagen background**. It is resulting from the most abundant and active mutagen agents, those which producing from oxygen and nitrogen that we consume.

8OHdG Urinary

Genetic oxidative damage marker

BIOGRAPHY OF URINARY 8OHdG
from chromosome to urine.....



Urinary 8OHdG

Unification of several theories

Fifty years ago, the first theories of aging which proposed the process of genomic damage and disorder, showed many catastrophic errors. In the same time the other theories "radical theories" insisted on the effect of free radicals resulting from our cell metabolism to genome damage.

During these last 20 years, the highlight on DNA and its damage fragments in urine ^{1, 2}, integrated these 2 hypotheses into a free radical theory of aging imposing on the death and functional loss of cells.

Our genome is continuously attacked and repaired

Each day, toxic substance, O₂ and N₂ reactive species, pollution, chemical garbage from our life style (tobacco, barbecues, drugs), and UV radiation, attack our genome nearly 100 000 events/cell/day (table 1). These events damage especially on bases or sugars of DNA adduct, perturbing the process of transcription and replication.

If DNA repair system do not repair damage portion quickly, the couple Polymerase / Ligase will restore the original sequence by recopy the opposit strand and connect them to free ends ^{3, 4, 5, 6}. These various mechanisms *, mutation & deletion which mean the loss of cellular controls and functions, can generate cancers, aging and death.

•Mutagen mechanisms

Transversion : change base ; ex : Nitrogen derivative transform Guanine to Thymine : G - > T

Transition : Inversion of bases couple ; ex : AT->GC : transform demethylated nitrogen derivatives Adenine to Hypoxanthine which can couple with Cytosine; the following replication will give GC which replaces by AT

Délétion : loss of a short sequence by slip of DNA Polymerase to modify base (8-OHdG) etc...

Table1. Types and Frequency of DNA damage

Damage agents	Type of damage	/cell/day
H ₂ O ₂ , UV (ROS)	Single-strand break	55,000
O ₂ and N ₂ reactive species(ROS and RNS), spontaneous	Depurination	13,000
O ₂ reactive species ·OH , ¹ O ₂ (ROS)	Oxidized DNA	10,000
PAH, alkylating agents	O(6)N(7) - Methylation	3,000
UV, Photosensibilisators	Depyrimidation	700
N ₂ O ₃ , ONOO ⁻ ,HNO ₂ (RNS)	Cytosine deamination	200
Xenobiotics, radiations, UV, Photosensibilisators	Double-strand breaks	9
Xenobiotics, radiations, UV, Photosensibilisators	Interstrand Crosslinks	8

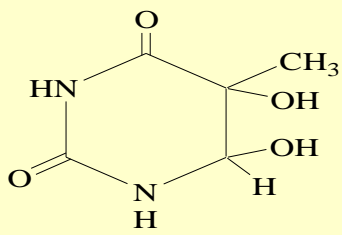
PAH = Poly Aromatic Hydrocarbon
ROS = Reactive Oxygen Species
RNS = Reactive Nitrogen Species

Each DNA attacker leaves its specific print.

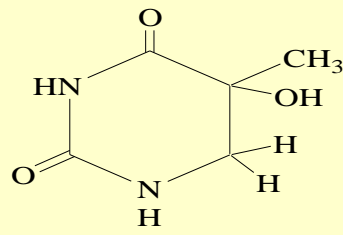
Each DNA attacker (Table 1) leaves its specific print, methyl bases for hydrocarbons and pyrolysis products, pyrimidine dimers for UV, demethylated bases for HNO₂ or ONOO⁻, oxidized bases O₂ reactive species. Of which more than a hundred of different components resulting from the attack of 4 DNA bases are currently identified ^{7,12} (fig 1).

This specific mark of oxo-nitro- radical species is currently use in pathogenic approach and later probably in degenerative diseases therapy.

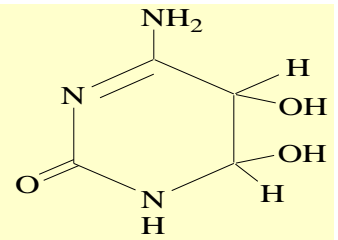
Thus « Alzheimer » DNA cortex is rich in oxidized bases like 8-hydroxyguanine which proclaims as by-product of °OH attack. Whereas Loewy body of « Senile Dementia » is rich in demethylated bases: xanthine, hypoxanthins. It shows that it has a lower part of oxygen species than nitrogen species, HNO₂ & ONOO^{-8, 9,10}.



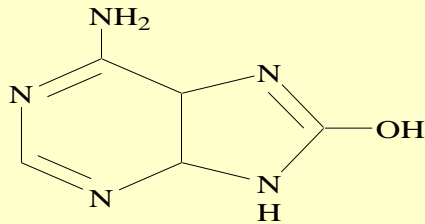
Thymine glycol



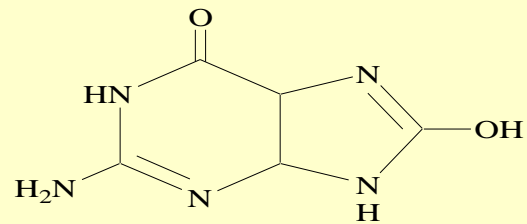
5,6-dihydrothymine



Cytosine Glycol



8-Hydroxyadenine



8-Hydroxyguanine

Fig. 1 Some oxides bases resulted from DNA attack by °OH

The principal enemies of our genome are Oxygen and Nitrogen ** that we consume

By their quantity

The radical species produced by human organism is considerable : 150 millimoles per day for O_2^{*2} & H_2O_2 , 1 millimole per day for NO_2 and NO_3 , or about 5 G and 100 Mg. They are a lot higher than toxic concentrations of environmental pollution.

by the amounts of their prints.

Moreover the inventory of various types of adducts (bases or sugars impairs) on DNA reveals that specific oxidized bases of oxidative stress are in concentration 100 to 1000 times higher than other varieties.

As an example DNA cells from a smoker lung has 50 to 500 adducts of Diol-epoxy Benzopyran, specific tobacco tar adduct for 10.8 bases whereas according to publications, oxidized bases refer only to 10.5 or 10.6 bases.

Oxidative Stress is the first responsible of DNA damage and also cancer.

It takes about 20 years or more to pass from the cancer initial stage (initiation) resulting from mutation, its clinical revelation which is shared between initiation and promotion to the third phase of progression.

The oxo-nitro-radical family activates these 3 stages cancer¹¹ (Fig.2).

• **Initiation**

By mutagen action

- radical species by generating oxidized and demethylated bases
- products of the lipidic peroxydation, MDA *, unsaturated aldehydes, mutagen by being grafted on DNA base.
- oxidation of DNA Polymerase and repair enzymes increase the mutation frequency by decreasing the reading accuracy and DNA clean up ^{7,12}.

Promotion

By the proliferative action

Extracellular H₂O₂, O₂ start mitogenic signal by activating membranes receivers (PDGF, EGF etc.), then intracellular messengers relay the signal and activating mitogenic factors of transcription NF-kB & AP-1 (redox mechanisms?)¹³.

Progression

By amplifying short cut activity

implied in the extension and invasion processes.

****Nitrogen derivative species**

An non-reactive NO₂ & O₂ overproduction, can generate aggressive molecules such as HNO₂, N₂O₃, ONOO⁻. Which can cause nitration and oxidation reaction with many target molecules specially DNA. Activated O₂ form are however pathologic responsible in specific etiopathogenic and inflammatory (rheumatism & regional enteritis inflammatory, hepatitises, parasitoses, athrome etc.)

Uric acid and ascorbic acid are excellent quencher of these nitrogen active derivatives.



Fig.2. 3 stages of impact Stress Oxidative on cancer

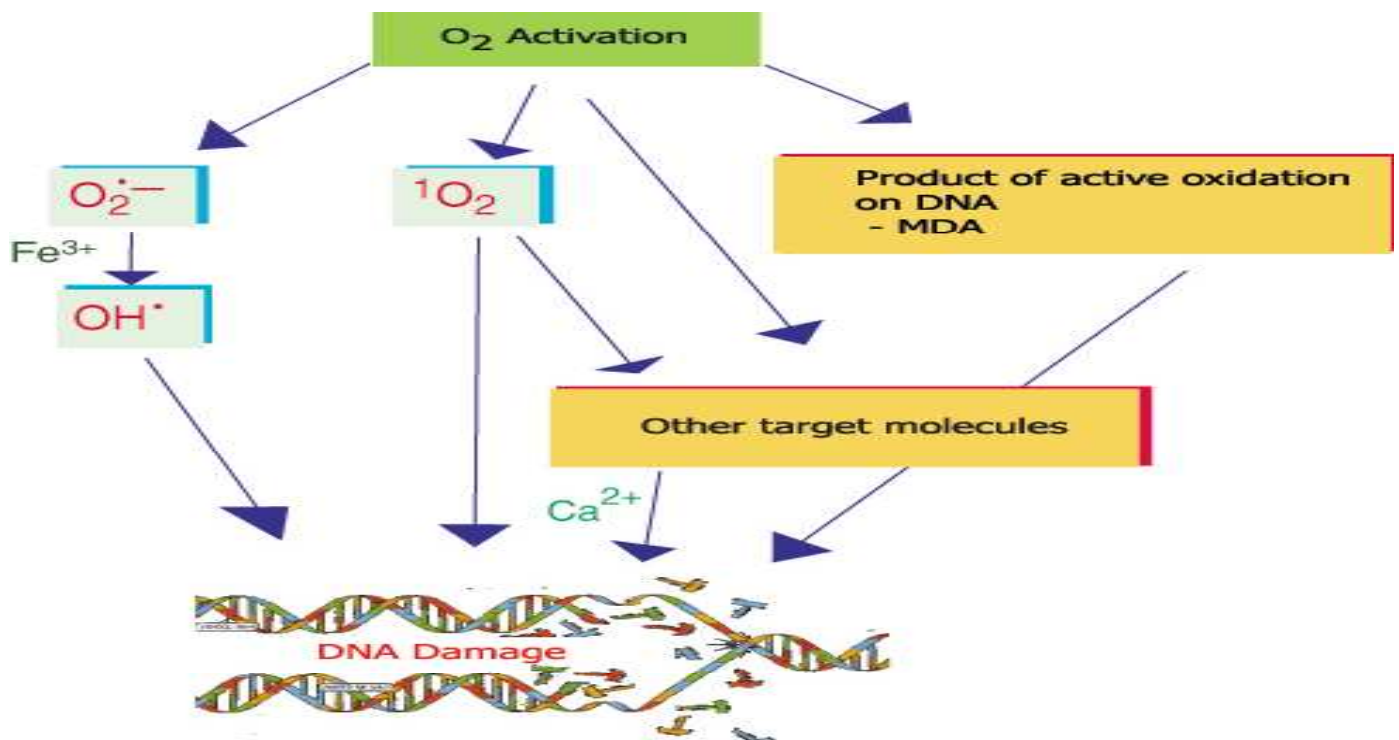


Fig.3 Direct and indirect reactive forms of oxygen.



8-hydroxydesoxyguanosine is the most representative of DNA oxidative damage.

Among forty oxidized bases resulted from DNA oxidative background noise, 8-OHdG was retained by specialists because :

Produced in significant quantity

Eventhough it is not the most abundant adduct*

DNA specific

Desoxyribose exists only in DNA.

Exclusively endogenous

8-OHdG is hydrolized into 8-oxoguanine and desoxyribose by intestinal mucous membrane before absorption.

Accessible technic

Eventhough 8-OHdG is already oxidized product, it can still easily oxidize with low potentials which is the property for liquid chromatography with electrochemical detection ⁷.

Its evaluation :

- in cellular genome (lymphocyte) where it is shown in a number of 8OHdG for 10.6 bases
- in urine where it constitutes the total oxidative damage index of organism, and shown by 24 hours, mmol of creatinine and / or kg of weight.

pmole creatinine / weight (kg)	nmole / mmole creatinine	nmole/ 24 h.
131-173	1.09-1.43	10-13.2 (men) 7.9-1.07 (women)

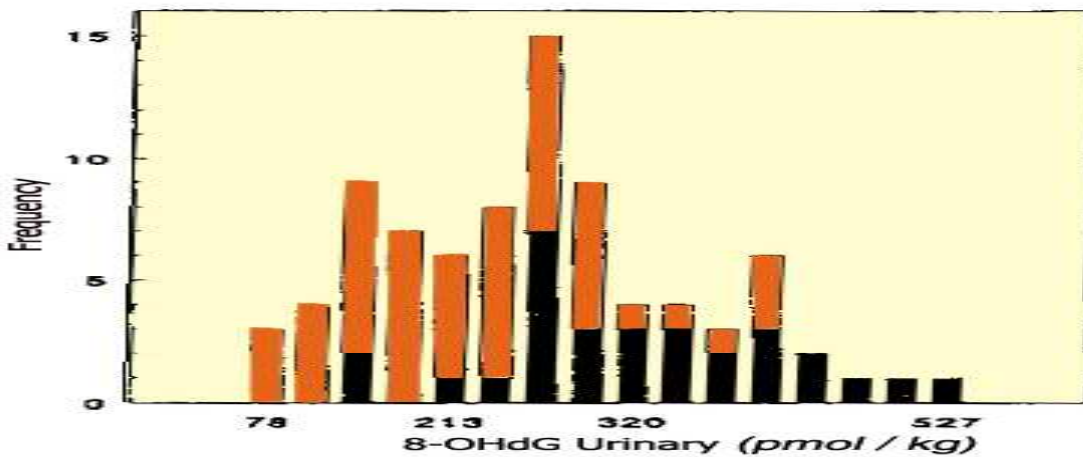


Fig.4

- average values distribution in a population of 84 specimen in good health and smokers (black) (Fig 4).
- its interindividual principal variation factors are tobacco, sex, weight : for example a thin smoker man eliminates 2 times more 8OHdG than non-smoker fat woman.

Extraordinary significant density of 8OHdG.

Oxidative stress is being implied in the majority of human pathologies, cardiovascular, degenerative, inflammatory, cancer, aging. Its evaluation indicates like the risk parameter, evolutionarity and gravity of all these affections.

Cardiovascular marker

8-OHdG is a predictive marker of morbidity cardiovascular especially of young subject. A recent European study shows a powerful correlation ($r=0.95$) between lymphocytary 8OHdG rate and the male cardiovascular mortality according to a gradient decreasing of North, West and South of Europe ¹⁴ (fig.5).

Country	8OHdG lymphocyte rate for 10^5 bases	Coronary mortality for 10^5 people
Ireland	1.2	120
Netherland	0.8	80
France	0.4	30

This epidemiologic study which focus on the oxidative stress in premature atherogenase, is very close to Halliwell study of atheroma. Which described that atheroma body is like a prooxidant pool, rich in catalytic iron and copper that generates hydroxyl radicals and peroxides intensely in vitro¹⁵ of lipids.

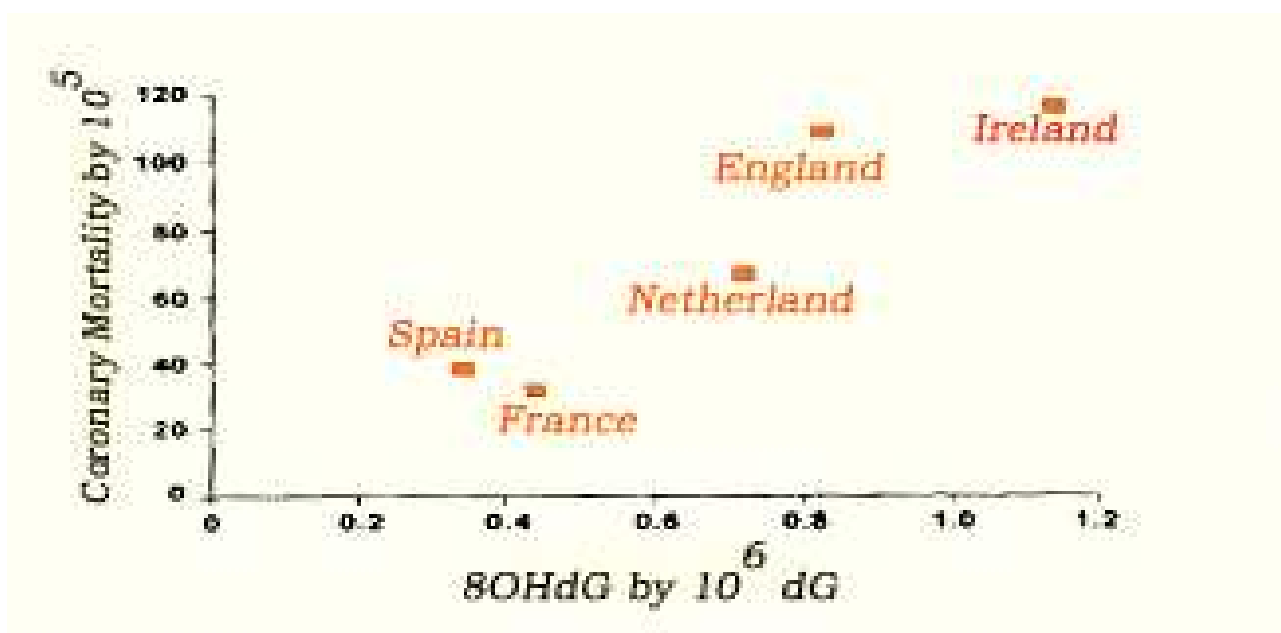


Fig.5 Average Relationship between 8OHdG in lymphocyte DNA and Human coronary mortality.

Toxic display marker

Environmental toxics (urban or industrial) rich in transition metals, nitrogen oxide derivatives, or O₂ reactive species, increase an oxidative damage. This effect can be translated by an increase of 8OHdG. It has already been used in many studies like surveillance examination of exposure professional depending on their lifestyle^{16,17}. This urinary 8OHdG marker is much more sensitive than urinary toxic thresholds which seldom reach.

Marker of cancer risk

The oxidation stress action, is shown on the first phases of cancer (initiation and promotion). Urinary 8OHdG is the prediction of cancer risk.

High rates of 8OHdG were found in the majority of cancer patients related to how far and how serious of the affection and with therapeutic associated (genotoxic)*^{17,18,19}.

Kinetic aging marker

Organism DNA oxidative damage is principal actor of aging. Urinary 8OHdG is the essential biological reference which is the strategy of fighting against aging²⁰.

Life expectancy Marker

The rat consumes 5 times more oxygen per kg of weight, eliminates 15 times more Thymine glycol, oxidative waste of DNA which is the same significance as 8 OHdG, and lives 30 times less longer than human^{21,22}.

The species lifespan of is inversely proportional to the DNA oxidative damage, and proportional to metabolic activity evaluation (O₂ consumption) per kg of weight (fig 6)

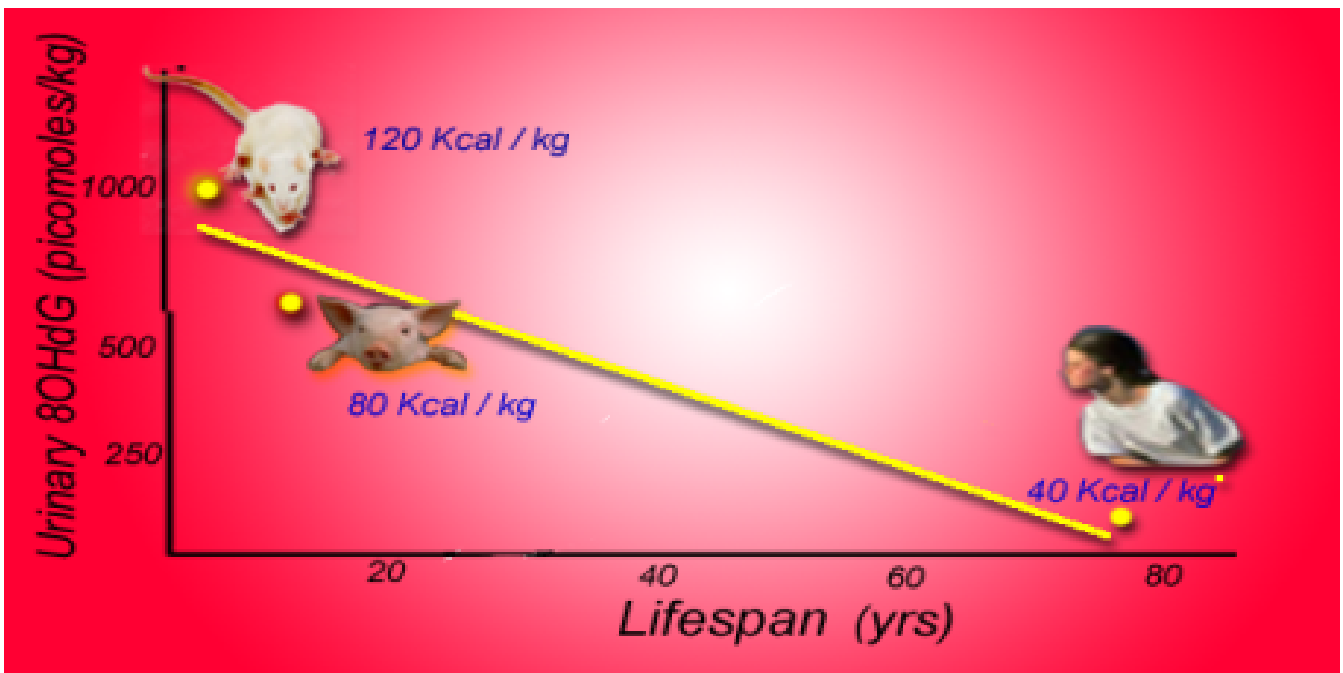


Fig 6. The species lifespan is inversely proportional to 8-OHdG elimination and O₂ consumption rate.

Of biological identical to aging, cancer related to age behaves in frequency in an exponential way like power 4 of age (fig 7)

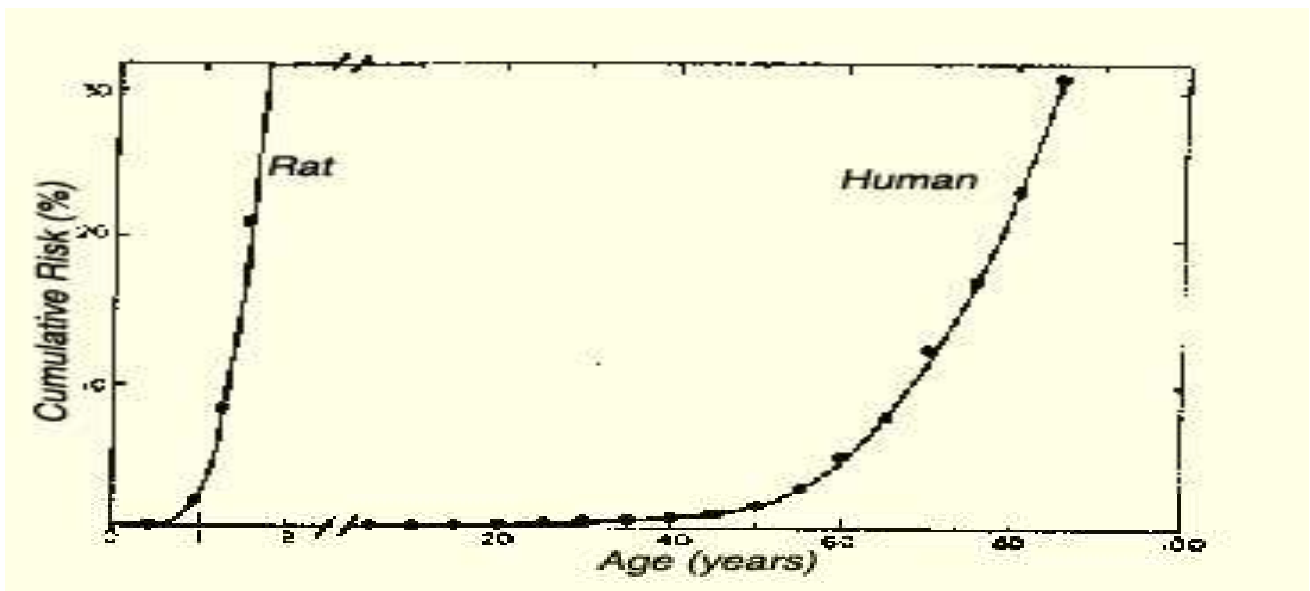


Fig 7. The rat presents a stronger exponential growth of cancer impact related to age than human.

Observation conforms to aging radical hypothesis : an increased O₂ consumption generates an increased production of radical species which causes an increased genetic damage.

Detrimental threshold marker of physical exercise

During physical effort, muscular mitochondria treats 200 times more O₂ than at rest , producing inevitable radical explosion (table 2) specially DNA ²³.

Generally an intense and prolonged effort is accompanied by an increase in carbonyl markers and malonyldialdehyde controlled by the antioxidants and training ²⁴.

The increase in 8OHdG marker of " DNA breakage ", can constitute the detrimental threshold of sport activity.

Table 2 Physical exercise potential Sources of radical explosion

- O₂ overconsumption
- anoxia-reoxygenation resulting from alternation effort-rest
- muscular damage
- increasing pollution inhalation increases catecholamine oxidation

Effect of nutritional supplementation and intervention marker.

The development of antioxidant activity markers to brief and medium term (month) allows the reduction of study time and evaluation objective of the different natural antioxidants²⁵ effectiveness.

Several studies using the urinary 8OHdG showed that the majority of antioxidants except Brussels sprout (phytonutriments) have no effect on oxidative genetic damage ^{26,27} .

*" Adduct " design as damaged molecule by toxic, oxidizing species or radicals etc...).

* Carbonyl proteins and malonyldialdehyde (MDA) are witnesses of the oxidative damage undergone by muscular cell first on structure and enzymatic proteins level and second on phospholipid membrane.

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