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Oxidative stress and aging: a clinical and biochemical study

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Abstract

The role of the free radicals in aging has been in center of research for long years. It is assumed that with advancing age, damaging effects of oxygen free radicals might be accumulated in the organisms on all components, especially on the DNA and the mitochondria. In addition, because of the decreased efficiency of the antioxidant systems, the oxidative mechanisms prevail in numerous age-dependent diseases, such as the arteriosclerosis, Parkinson and Alzheimer diseases.

The present study was aimed at revealing an eventual correlation between the oxidative balance and nutritional profile and/or psychopathological status in an ultraoctagenarian population living in a small country, Orria (SA) at home, by means of routine specific serum tests, such as d-ROMs test and BAP test.

16 on 24 ultraninethy-years-old subjects (2.36% of the country popolution), 1 of them was institutionalized and 15 living outside the institutes were studied. 9 (56%) were females and mean age was 93.4 (SD 2.44) years. Serum total oxidant capacity was determined by performing the d-ROMs test, which chemical principle is based on the ability of a biological sample to oxidize N,N-diethylparaphenylenediamine (normal range 250-300 CARR U, where 1 CARR U is equivalent to 0.8 mg/L H_2O_2), while serum total antioxidant capacity was assessed by means of anti ROMs test, which measures the ability of a serum sample to reduce iron from the ferric to the ferrous ionic form (optimal value >200 and >1000 micromol/L reduced iron for type 1 and 2 respectively).The psycho-physical state of the subjects was estimated by means of the mini mental state examination (MMSE), activities of daily living (ADL) and instrumental activities of daily living (IADL). The nutritional state and the physical activity of the subjects were evaluated through the mini nutritional assessment (MNA). All studied parameters underwent a correlation analysis of Pearson.

Statistically significant negative correlation was found between the free radical levels and the cognitive performance ($p < 0.0001$), as well as the levels of autonomy and autosufficiency, the physical activity in the total population ($p < 0.01$). These correlations were even more expressed in the institutionalized subjects. Statistically significant positive correlation seems to exist between the free radical levels and the nutritional status ($p < 0.001$).

The levels of oxygen free radicals were higher in the former group, indicating a stronger oxidative stress, influencing the psychophysical state of the elderly subjects. This may have negative consequences on the quality and duration of the life. It is difficult to define the exact role of free radicals in the determination of aging pattern, but they may be considered without any doubt as true "markers" of an enhanced oxidative stress, accompanying a non-successful aging process.

KEY WORDS: AGING, OXIDATIVE STRESS, ULTRAOCTAGENARIAN

Introduction

The aging free radical theory is based on the evidence that living organisms (aerobes) produce oxygen-centered free radicals, inducing irreversible damage to biological structures. These are formed inside cells when oxygen is used in metabolic processes to produce energy. Mitochondrial respiration produces reactive oxygen species (ROS) by leakage of intermediates from the electron transport chain [1]. These molecules are highly unstable because they have an unpaired electron; they therefore seek to achieve a stable state by appropriating electrons from nearby molecules. The latter, in turn, become unstable and so on, thus creating an instability chain reaction. Usually, the harmful activity of a small percentage of these free radicals is neutralized by cellular antioxidants. Antioxidants may be enzymatic (superoxide dismutase [SOD], catalase, glutathione peroxidase [GSHP]) and non-enzymatic (vitamins E, C, A). When, however, free radical levels increase (overeating, smoking, drug abuse, ultraviolet radiation, persistent chronic inflammation, etc.) the pool of antioxidants is saturated and the excess of free radicals damages biological structures. At first the damage is evident in mitochondria, which may affect DNA and RNA causing mutations, but may also act on proteins and lipids. Endothelial cells, fibroblasts, and tissue cells are the most affected by oxidative stress.

In order to understand how free radicals are involved in the ageing process it is worth reviewing the production and fate of ROS at the tissue level. Oxidases and mitochondria are the main ROS producers, which include highly reactive molecules such as superoxide anion. The fate of this molecule depends on the total oxidation-reduction equilibrium at the cellular level, which is controlled by SOD which produces H_2O_2 [1] followed by conversion into H_2O and O_2 through the action of catalase or GSHP. When the production of superoxide anion or H_2O_2 is such as to saturate the reductive capacity of the SODs or of GSHP, these molecules become substrates for the creation of highly reactive molecules such as hydroxyl (by means of a Fenton and/or Haber-Weiss reaction) which are responsible for cell and tissue damage [2]. The superoxide anion can react with nitric oxide in a diffusion limited reaction generating peroxynitrite, itself a powerful ROS. Most ROS are limited in their diffusion potential by their insolubility in lipids, and thus their effect is generally restricted to the intracellular compartment. Conversely, H_2O_2 can pass through cell membranes and thus react farther away from the production site. This theory posits that mitochondrial production of ROS regulates the ageing rate, since oxidative damage builds up over time. According to this hypothesis long-lived species produce fewer ROS by comparison to shorter-lived species, and mice subjected to calorie restriction live longer and produce fewer ROS than controls [1].

The aging process is one of the best examples of the effects of a deterioration of homeostasis, since aging is accompanied by an impairment of the physiological systems including the immune system. We propose an integrative theory of aging providing answers to the how (oxidation), where first (mitochondria of differentiated cells) and why (pleiotropic genes) this process occurs. In agreement with this oxidation-mitochondrial theory of aging, it was observed that the age-related changes of immune functions have as their basis an oxidative and inflammatory stress situation, which has among its intracellular mechanisms the activation of nuclear factor-kB (NF-kB) in immune cells [2]. Therefore, NF-kB overactivation on immune cells, by intensifying inflammatory-oxidative stress, has been hypothesized to play a major role in oxi-inflamm-aging. [2]. Moreover, it was also observed that several functions of immune cells are good markers of biological age and predictors of longevity [2]. Therefore, the theory of oxidation-inflammation was proposed as the main cause of aging [2]. Accordingly, the chronic oxidative stress that appears with age affects all cells and especially those of the regulatory systems, such as the nervous, endocrine, and immune systems and the communication between them. This prevents an

adequate homeostasis and, therefore, the preservation of health. It was also proposed that the immune system plays a key role in the aging process, specifically in the rate of aging, since there is a relationship between the redox state and functional capacity of immune cells and longevity of individuals [2]. Moreover, the role of the immune system in senescence could be of universal application. A confirmation of the central role of the immune system in oxi-inflamm-aging is that the administration of adequate amounts of antioxidants in the diet improves immune function, decreases their oxidative stress, and consequently increases longevity [2].

The present study was aimed at revealing an eventual correlation between the oxidative balance and nutritional profile and/or psychopathological status in an ultraoctagenarian population living in a small country, Orria (SA) at home, by means of routine specific serum tests, such as d-ROMs test and BAP test.

Methods and Patients

16 on 24 ultraninethy-years-old subjects living in a small country, Orria (SA) (2.36% of the country population), 1 of them was institutionalized and 15 living outside the institutes were studied. 9 (56%) were females and mean age was 93.4 (SD 2.44) years.

In tutti è stato valutato lo stress ossidativo attraverso un prelievo ematico. Tutti i soggetti sono stati sottoposti ad una dettagliata valutazione cognitiva, clinica generale e nutrizionale attraverso scale cliniche dedicate.

Oxidative status measurements

Blood samples taken after a minimum of 12 hours fasting were centrifuged and serums were divided into aliquots and stored at –80°C until used. Defrozen samples were used to assess serum total oxidant capacity (S-TOC) and serum total antioxidant capacity (S-TAC), by using a dedicated photometer (FREE, Diacron International, Grosseto, Italy).

Serum total oxidant capacity was determined by performing the d-ROMs test (Diacron International, Grosseto, Italy), whose chemical principle is based on the ability of a biological sample to oxidize N,N-diethylparaphenylenediamine (DPPD), as described elsewhere [3]. Briefly, 20 µL of serum were added to 1 mL acetate buffer pH 4.8 containing 20 µL aqueous solution of DPPD $3.7x10^{-3}$ M. The change in absorbance was photometrically monitored at 505 nm (optical path 1 cm), using the kinetic procedure, for 5 min., at 37°C [16, 17]. The results were expressed in Carratelli Units (CARR U), where 1 CARR U is equivalent to 0.08 mg/dL hydrogen peroxide [4]. This "equivalence" does not mean that a normal serum (300 CARR U) really contains 7054 µmol/L or 24 mg/dL of hydrogen peroxides or hydroperoxides, as erroneously reported by some authors [5], but only that, in calibration experiments, 1 CARR U shows the same absorbance change, and therefore is equivalent to 0.08 mg/dL hydrogen peroxide [6]. The normal range is between 250 CARR U (equivalent to 20.08 mg/dL hydrogen peroxide) and 300 CARR U (equivalent to 24.00 mg/dL hydrogen peroxide). The d-ROMs test proves linear up to 500 CARR U and in our study showed a 2.4% within-run variation coefficient and a 3.5% between-run coefficient, both assessed on 20 aliquots of frozen serum [7].

Serum total antioxidant capacity was assessed by means of two assays, the OXY-Adsorbent and the BAP tests (Diacron International, Grosseto, Italy). The OXY-adsorbent test provides a reliable measure of serum's ability to oppose the massive oxidant action of a hypochlorous acid solution, as described elsewhere [8]. The unreacted acid is then photometrically measured at 505 nm by using DPPD, a chromogenic oxidizable substrate. Briefly, 10 µL of serum, previously diluted with distilled water 1:100, was added and mixed with 1 mL of oxidant solution (R_1), containing highly concentrated HClO. After 10 min. incubation at 37°C, 10 µL of chromogenic mixture (R_2) -- an aqueous solution of DPPD $3.7x10^{-3}$ M --, was added, for each series of assays, a standard serum with assigned value, previously diluted with distilled water 1:100, as is done for the samples,

and a blank reagent, obtained by replacing serum with distilled water, were included. Absorbance was measured immediately at 505 nm (optical path 1 cm); the absorbance value of the reagent blank was subtracted from those of the standard and the samples. Antioxidant capacity, expressed as µmoles HClO/mL of sample, were calculated with the following formula: (Abs blank-Abs sample)/(Abs blank-Abs standard) x [standard], where Abs are the absorbances and is the standard concentration [8]. Serum levels higher than 350 ìmoles HClO/mL are considered normal; lower levels correlate directly with the severity of plasma antioxidant reef impairment [8]. In our laboratory, the intra-assay variation coefficient assessed on 20 aliquots of fresh serum was 2.5%, while the inter-assay one was 6.0%. The BAP (Biological Antioxidant Potential) test measures the ability of a serum sample to reduce iron from the ferric to the ferrous ionic form, as described elsewhere [9]. The reduction in iron causes loss of colour in the initially coloured Fe^{3+}-thiocyanate derivative solution. The colour change is then photometrically monitored at 505 nm and measured according to a fixed-time procedure. Briefly, 10 µL serum were dissolved in a coloured solution, which was previously obtained by mixing a ferric chloride ($FeCl_3$) solution (R_2) with a thiocyanate derivative solution (R_1). After 5 min of incubation at 37 °C, the intensity of decolouration was photometrically evaluated by monitoring the absorbance change (505 nm, 1 cm optical path) using as a standard a solution of ascorbic acid [9]. Levels above 2200 µEq/L of reducing agents (as measured by using ascorbic acid as a standard) are considered optimal in humans [9].

The anti-ROMs Test - last in terms of development compared to the BAP test and to the OXY-Adsorbent Test - aims in some ways like an evolution of the BAP test that retains, however, its validity and its specific indications. The principle, in fact, is substantially identical, both the tests measuring the antioxidant capacity of the plasma in terms of iron-reducing capacity.
The innovative aspect that distinguishes the anti-ROMs test is that it relies on the development of a reaction in two sequential stages. In the first is measured the iron-reducing ability of antioxidants "Fast", such as vitamins C and E, which intervene immediately to eliminate any oxidizing species, in the second one of the antioxidants "lenses", such as uric acid and certain thiols (es. cysteine), which come into play later in life in the defense against free radicals. On this basis, the anti-ROMs Test provides two different results. In normal conditions, are considered optimal values greater than 200 micro-equivalents / L for the first, and higher than 1000 micro-equivalents / L for the second. Values below these limits are indicative of a condition of oxidative stress.

Mini mental state examination (MMSE)

The mini mental state examination (MMSE)[5] is the most commonly used instrument for screening cognitive function. This examination is not suitable for making a diagnosis but can be used to indicate the presence of cognitive impairment, such as in a person with suspected dementia or following a head injury[6]. The MMSE is far more sensitive in detecting cognitive impairment than the use of informal questioning or overall impression of a patient's orientation.

· The test takes only about 10 minutes, but is limited because it will not detect subtle memory losses, particularly in well-educated patients [7].

· People from different cultural groups, or of low intelligence or education, may score poorly on this examination in the absence of cognitive impairment[8] and well-educated people may score well despite having cognitive impairment [9].

· The MMSE provides measures of orientation, registration (immediate memory), short-term memory (but not long-term memory) as well as language functioning.

· The examination has been validated in a number of populations. Scores of 25-30 out of 30 are considered normal; NICE classify 21-24 as mild, 10-20 as moderate and <10 as severe impairment. The MMSE may not be an appropriate assessment if the patient has learning, linguistic/communication or other

disabilities (eg sensory impairments) [10-11].

Before administering the MMSE it is important to make the patient comfortable and to establish a rapport with the patient. Praising success may help to maintain the rapport and is acceptable, but persisting on items the patient finds difficult should be avoided.

Activities of daily living (ADL)

Activities of daily living (ADLs) is a term used in healthcare to refer to daily self-care activities within an individual's place of residence, in outdoor environments, or both. Health professionals routinely refer to the ability or inability to perform ADLs as a measurement of the functional status of a person, particularly in regards to people with disabilities and the elderly [12].

Basic ADLs (BADLs) consist of self-care tasks, including:[13]

· Bathing and showering (washing the body)

· Bowel and bladder management (recognizing the need to relieve oneself)

· Dressing

· Eating (including chewing and swallowing)

· Feeding (setting up food and bringing it to the mouth)

· Functional mobility (moving from one place to another while performing activities)

· Personal device care

· Personal hygiene and grooming (including washing hair)

· Sexual activity

· Toilet hygiene (completing the act of relieving oneself)

A useful mnemonic is DEATH: dressing, eating, ambulating, toileting, hygiene.

In our study the levels of autosufficiency were evaluated by means of the scale for activity of daily living (ADL) according to Katz et al. (1970) [14]. Autonomy was evaluated by using the instrumental activity of daily living (IADL) scale (Lawton and Brody, 1969) [15].

Most models of health care service use ADL evaluations in their practice, including the medical (or institutional) models, such as the Roper-Logan-Tierney model of nursing, and the resident-centered models, such as the Program of All-Inclusive Care for the Elderly (PACE).

Mini nutritional assessment (MNA)

Nutritional status was assessed by anthropometric measurements recorded by a single, trained observer and by measurements of biochemical markers. Height (defined as the patient's height before the age of 50 y, before the possible onset of osteoporosis or kyphosis) was recorded. Weight was measured by using a standing scale with the patient in nightclothes and hospital slippers. If the patient was wheelchair bound, weight was measured with the patient in the wheelchair. Then, the wheelchair was weighed alone and the latter weight subtracted from the former. If the patient was bed bound, a bed scale was used for weighing.

Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Subjects were asked about their history of weight loss in the past 6 mo. Biochemical data, when present, were collected for the following: albumin, cholesterol, sodium, serum urea nitrogen, and creatinine. The laboratory data were collected within 10 d of, or on, the admission day. The ratio of serum urea nitrogen to creatinine was calculated as an index of dehydration at the time of admission.

A Mini Nutritional Assessment (MNA; 16) score was obtained at the time of admission in patients admitted over 2 nonconsecutive, 1-mo periods during the study (n = 104). The MNA is a rapid and simple assessment tool and has been cross-validated in 3 studies involving >600 elderly persons in Europe and the United States. About 75% of elderly subjects can be correctly classified with the use of the MNA [17] About 25–30% of subjects fall

into an intermediate zone between well-nourished and undernourished. These subjects are classified as borderline or at risk of malnutrition and require further assessment through measurement of biochemical markers or additional clinical evaluation. With the use of the validated cutoffs of adequate nutritional status (MNA score ≥ 23.5), at risk of malnutrition (MNA score between 17 and 23.5), and malnutrition (MNA score < 17), the sensitivity of the MNA score is 96%, the specificity is 98%, and the positive predictive value is 97% for malnutrition [18, 19].

The values obtained in the tests, divided into 2 groups (A and B) were compared by statistical analysis with the t-test. All studied parameters underwent a correlation analysis of Pearson.

Results

The study sample refers to the population of the City of ninety Orria that compared to the corresponding Italian population is markedly higher than about 3 times (ISTAT data) (Table I). Furthermore, the sample was divided into 2 groups according to the score obtained through the MMSE cognitive assessment: in particular in the group A (6 M) were included subjects with values > 20 (normal or slight deterioration) and in the group B (9 W, 1 M) subjects with values <20 (moderate to severe impairment) (Table III and IV).

Of the 24 subjects nineties the population Orriese 16 (66.6%), mean age 93.4 years (SD 2.4) represent the sample studied, 9 females (56.25%) and 7 males (43.75%) (Table II).

The calculated values for Body Mass Index were: thinness (<18.5) in 3 (19%) subjects; regular (18.5 <> 24.9) in 8 (50%) subjects, overweight (25 <> 29) in 4 (25%) subjects, obese (> 30) in 1 (6%) subject.

Pathologies associated detected in the studied subjects are represented by diabetes in 3 (19%) subjects and major vascular pathologies (cardiocerebral) in 8 (50%), and finally no significant pathology was observed in 5 (31%) subjects.

Familiarity is noted for diabetes in 3 (19%) subjects, cardio-cerebrovascular disease in 10 (56%), and finally no significant pathology was found in 5 (31%) subjects.

The administration of the Mini-Mental State Examination (MMSE) showed the following scores: 0-9 (severe decline) in 7 (44%) subjects, 10-19 (moderate decline) in 3 (19%) subjects, 20-24 (slight decline) in 4 (25%) subjects and> 25 (normal) in 2 (12%) subjects (Fig. 1). In 6 subjects was not possible to administer the test for cognitive decline too advanced. The total average was 20.4 (SD 4.1) in the group A 23.2 (SD 2.1) in group B and 16.3 (SD 2.8).

Fig. 1 MMSE

The nutritional study by administering the Mini Nutritional Assessment (MNA) showed the following values: 0-17 (poor nutritional status) in 4 (25%) subjects, 17.5 <> 23.5 (risk of malnutrition) in 7 (44%) subjects and > 24 (normal nutritional status) in 5 patients (31%). The total average value is the result of 20.7 (SD5.8) that differentiated and compared for the group A, 26 (SD0.7), and for group B, 18.1 (SD5.4) shows a statistically significant difference (P = 0.003) in favor of those with better cognitive mindset. Also in group A the average has been calculated only on 4 subjects, because the remaining 6 were not testable (Fig. 2).

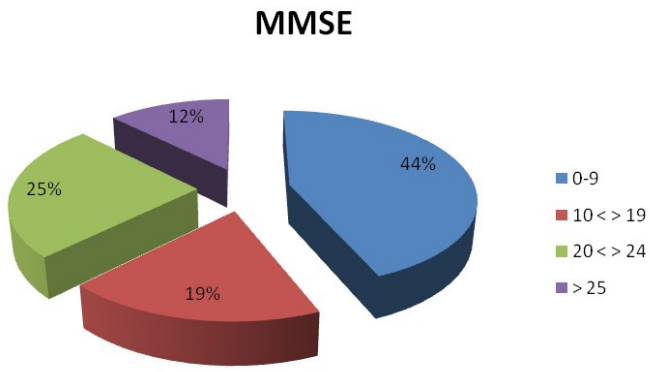
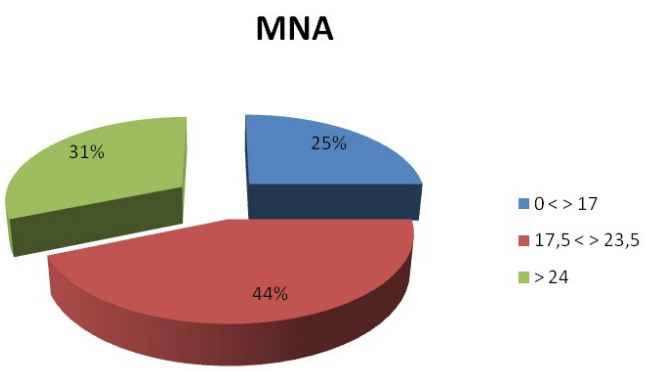


Fig. 2 MNA

Oxidative status measurements

The average values determined for Droms, anti



ROMs 1 and 2 were respectively 324.1 (SD 79.4), 234.4 (SD 99.7) and 1188.8 (SD 433.3); in group A 243.3 (SD 33.5), 203.2 (SD 37.4) and 1148.2 (SD 317.1); in group B 347.8 (68.7) 233.5 (SD 116.4) and 1182.7 (SD 453.0) (Tab II, III, IV). In particular, values are recorded in the normal range in the various groups (total, A and B) and for the data of Droms, anti ROMs 1 and 2 respectively in 5 (31.2%), 4 (66.6%) and 1 (10%) subjects; 9 (56.2%), 3 (50%) and 6 (60%) subjects, 11 (68.7%), 4 (66.6%) and 7 (70%) subjects. In the comparison between groups A and B were statistically significant differences for the values of Droms (P = 0.004) and not significant for antiROMS 1 and 2 (P = 0551 and P = 0.873 respectively).

Discussion

The sample studied represents a population of particular interest because it consists of all nineties and is the first series in the world of such an advanced age. This segment of the population in the municipality of Orria is greater than about three times the national average (2.36% vs. 0.74%). Therefore, the study of one of the recognized factors (oxidative stress) related to aging constitutes a particular item of interest. In fact the values of the test related to oxidative stress showed a slight increase for the dROMS (324.1, SD 79.4) and values within the limits for antiROMs 1 and 2 (234.4, SD 99.7 and 1188.8, SD 433.3) to mean an overall good control of oxidative balance. The data were then divided into 2 subgroups according to cognitive status detected through a test widely used as the MMSE, to assess the impact of the oxidative balance on cognitive functions in a sample selected for advanced age. We have already mentioned that high values of dROMS were recorded in the total sample of subjects evaluated, but significantly elevated values were recorded especially in group B that is, the subjects with greater impairment of cognitive functions. In group A with better cognitive performance mean values are within normal range.

In fact, high values of d-ROMs test are observed in subjects exposed to risk factors for oxidative stress (eg, cigarette smoking, alcohol abuse, inadequate exercise, how many diets / quality unbalanced etc..) Or diseases associated with alterations of oxidative balance (eg. cardiovascular diseases, neurodegenerative disorders, metabolic syndrome, tumors, etc..) or treatments capable of increasing the level of oxidizing species (eg, oral contraceptives, radio / chemotherapy, dialysis interventions bypass, etc..). The recorded values for anti ROMs 1 and 2 test based on the development of a reaction in two sequential stages in which the first is measured by the ability of iron-reducing antioxidants "fast", such as vitamins C and E, which are involved in immediately remove any oxidizing species, and the second that of the antioxidant "slow", such as uric acid and some thiols (eg, cysteine), which come into play later in life in the defense against free radicals highlight values substantially limits for all groups considered , total, A and B. Therefore there is a good supply of antioxidants in the diet. The group A, he is also the one with best results in the test of nutritional assessment (MNA) also significantly greater in the group with less good cognitive impairment.

The results of the present study indicate that the levels of oxygen free radicals were higher in the former group, indicating a stronger oxidative stress, influencing the psychophysical state of the elderly subjects. This may have negative consequences on the quality and duration of the life. It is difficult to define the exact role of free radicals in the determination of aging pattern, but they may be considered without any doubt as true “markers” of an enhanced oxidative stress, accompanying a non-

successful aging process.

Our study confirms and extends previous studies indicating that oxidative stress may induce both the initiation and the progression of PD [20-22].

Mitochondrial respiration produces reactive oxygen species (ROS) by leakage of intermediates from the electron transport chain [23]. These molecules are highly unstable because they have an unpaired electron; they therefore seek to achieve a stable state by appropriating electrons from nearby molecules. The latter, in turn, become unstable and so on, thus creating an instability chain reaction. Usually, the harmful activity of a small percentage of these free radicals is neutralized by cellular antioxidants. Antioxidants may be enzymatic (superoxide dismutase [SOD], catalase, glutathione peroxidase [GSHP]) and non-enzymatic (vitamins E, C, A). When, however, free radical levels increase (overeating, smoking, drug abuse, ultraviolet radiation, persistent chronic inflammation, etc.) the pool of antioxidants is saturated and the excess of free radicals damages biological structures. At first the damage is evident in mitochondria, which may affect DNA and RNA causing mutations, but may also act on proteins and lipids. Endothelial cells, fibroblasts, and tissue cells are the most affected by oxidative stress.

In order to understand how free radicals are involved in the neurodegenerative process it is worth reviewing the production and fate of ROS at the tissue level. Oxidases and mitochondria are the main ROS producers, which include highly reactive molecules such as superoxide anion. The fate of this molecule depends on the total oxidation-reduction equilibrium at the cellular level, which is controlled by SOD which produces H_2O_2 [23] followed by conversion into H_2O and O_2 through the action of catalase or GSHP. When the production of superoxide anion or H_2O_2 is such as to saturate the reductive capacity of the SODs or of GSHP, these molecules become substrates for the creation of highly reactive molecules such as hydroxyl (by means of a Fenton and/or Haber-Weiss reaction) which are responsible for cell and tissue damage [24]. The superoxide anion can react with nitric oxide in a diffusion limited reaction generating peroxynitrite, itself a powerful ROS. Most ROS are limited in their diffusion potential by their insolubility in lipids, and thus their effect is generally restricted to the intracellular compartment. Conversely, H_2O_2 can pass through cell membranes and thus react farther away from the production site. This theory posits that mitochondrial production of ROS regulates the ageing rate, since oxidative damage builds up over time. According to this hypothesis long-lived species produce fewer ROS by comparison to shorter-lived species, and mice subjected to calorie restriction live longer and produce fewer ROS than controls [23].

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Total Population		Ultraninethy-Years-Old Subjects	
ITALY	ORRIA	ITALY	ORRIA
60.340.328	1225	447.063 (0.74%)	29 (2.36%)
31.052.925 W	637 W	334.957 W (1.07%)	20 W (3.13%)
29.287.403 M	588 M	112.106 M (0.38%)	9 M (1.53%)

Tab I. Italian population vs. Orriese population (1.1.2010)

	Sex	Age	Height	Weight	MMSE	MNA	dROMS	antiROMs	antiROMs
1	m	91	168	65	23	26,5	188	230	1241
2	m	94	165	50	24	17,5	246	211	927
3	m	91	170	80	21	27,5	293	169	1265
4	f	99	150	50	n.t.	11,5	319	131	1017
5	f	92	150	50	n.t.	20,5	387	115	688
6	f	92	155	43	n.t.	12,5	280	293	1201
7	f	95	150	43	n.t.	17	278	379	1274
8	m	91	170	85	13,2	22,5	242	179	1044
9	f	97	155	80	14	22,5	330	224	993
10	m	91	155	60	26	27	244	261	1612
11	f	92	145	50	n.t.	13	483	414	2243
12	m	92	170	75	25	25,5	284	172	683
13	m	95	150	40	20,3	25,5	202	176	1161
14	f	94	160	50	n.t.	13	412	58	627
15	f	92	155	55	19	29	380	313	1308
16	f	97	155	59	19	20	367	229	1432

Tab. II: General Sample

Pz	Sex	Age	Height	Weight	MMSE	IADL	ADL	MNA	dROMS	Anti ROMs	Anti ROMs
1	m	91	168	65	23	7	6	26,5	188	230	1241
2	m	94	165	50	24	7	6	17,5	246	211	927
3	m	91	170	80	21	5	6	27,5	293	169	1265
10	m	91	155	60	26	7	6	27	244	261	1612
12	m	92	170	75	25	7	6	25,5	284	172	683
13	m	95	150	40	20,3	5	6	25,5	202	176	1161

Tab. III Sample with MMSE >20 (gruppo A)

Pz	Sex	Age	Height	Weight	MMSE	IADL	ADL	MNA	dROMS	Anti ROMs	Anti ROMs
4	f	99	150	50	n.t.	1	1	11,5	319	131	1017
5	f	92	150	50	n.t.	1	1	20,5	387	115	688
6	f	92	155	43	n.t.	0	0	12,5	280	293	1201
7	f	95	150	43	n.t.	0	0	17	278	379	1274
8	m	91	170	85	13,2	3	4	22,5	242	179	1044
9	f	97	155	80	14	3	3	22,5	330	224	993
11	f	92	145	50	n.t.	1	1	13	483	414	2243
14	f	94	160	50	n.t.	0	0	13	412	58	627
15	f	92	155	55	19	3	3	29	380	313	1308
16	f	97	155	59	19	4	3	20	367	229	1432

Tab. IV Sample with MMSE <20 (gruppo B)