The fact that, in the populations of industrialised countries, disability and mortality curves are tending to come closer together would indicate that senility is a phenomenon that can be delayed. Calorific restriction and physical activity have proved effective in delaying the disability curve, whilst smoking has the opposite effect. These three elements (Fries Hypothesis) influence the extent of oxidative stress (Harman’s Hypothesis) and the latter, therefore, becomes an evaluation and systemic control parameter. Using the D-Roms test to measure the level of oxidative stress in the blood, a study was carried out on a sample of seemingly healthy individuals and on several groups of patients suffering from pathologies characterised by endothelial damage. It confirmed that the Hypotheses of Fries and Harman clearly coincide and that oxidative stress is a common component in various pathologies. It also illustrated how the administration of a balanced formulation of antioxidants (ARD Stenovit®) reduces the extent of oxidative stress.

**KEY WORDS**

SENILITY, AGING, OXIDATIVE STRESS, D-ROMS TEST, ANTIOXIDANTS

**INTRODUCTION**

The age of 65 is usually accepted (1) as the time when “aging” begins. One of the first documents written about human life expectancy and stressful conditions is Psalm 89 (known as Moses’ Psalm). Aging can be defined as a “process” comprising events in which one can intervene, within certain limits, once they are identified as being causal.

All the theories about aging (there are more than 20) are based on more or less convincing premises (2, 3), but very often the “supporting elements” of the theories cannot be tracked down at clinical level. For example, between the ’70s and ’90s one could demonstrate how cells, after about fifty replications, began apoptosis and died (4, 5) – it is a problem relating to telomeres and telomerases (6, 7) which, although extremely indicative, fails to provide a convincing clinical comparison on aging (8, 9). One of the theories (Harman) (10, 12), which is producing concrete results at epidemiological, experimental and clinical level, is linked to free radicals or, even better, to oxidative stress. This theory partially correlates with the theory on calorific restriction, as a reduced and more suitable calorific/food supply allows for an increase in the average life, simply by limiting oxidative stress (13, 14). The epidemiological bases for this theory can be traced back to research carried out in the field morbidity (disability) reduction, theorised by Fries (15). Fries points out and illustrates how, in industrialised societies, the average age of the onset of disability increases more rapidly than the average age of survival, otherwise disability and survival tend to coincide. Even though it has been subject to criticism, the theory appears to be valid.

If Fries’ hypothesis is true, it is increasingly easy to envisage the possibility of living without disability until the end of your life. One of the most valid studies on a methodological level, based on the age at which disability begins (17), illustrates that:

a. Disability can be reduced to a few years before death;

b. Being overweight and smoking are highly negative factors (increasing the period of morbidity);

c. A good level of physical activity is a positive factor (it reduces the morbidity period).
It is well-known that being overweight and smoking increase oxidative stress whilst regular physical activity (of a “salutary” nature) tends to reduce it.

The aim of this report is to show that oxidative stress is one of the components in the risk factors listed in the Fries study; that some cardiovascular diseases and lifestyles are characterised by oxidative stress; and, finally, that it is possible to control oxidative stress with an appropriate combination of low doses of antioxidants.

MATERIALS AND METHODS

EVALUATION OF OXIDATIVE STRESS

It is well-known that the alteration of molecular arrangement caused by an Xo free radical (Figure 1), when this reacts with a membrane lipid (cell or subcell), causes oxidative damage to the membrane. At that point, a gradient inversion occurs which causes cell death. The imbalance between the free radicals produced and the antioxidant power generates the “oxidative stress”.

The most valid system for identifying free radicals in the blood, is based on “spin traps” (traps for electrons) which react with the free radical, forming complexes that can be identified via ESR (Electron Spin Resonance) or via spectrophotometry (18, 19). The test used for our studies is known as the “D-Roms test” and was carried out in Italy (20) with the help of the CNR (National Research Centre). The unit used for measuring the level of oxidation is the Carratelli Unit (CARR. U.) – one CARR. U. corresponds to a Hydrogen Peroxide concentration of 0.08 mg per hundred ml. The normal values for D-Roms in serum, from between 8 and 10 in the morning, in subjects who have been fasting since the night prior to the test, fluctuate between 250 and 300 CARR. U.

For levels of over 300 CARR. U., the subject can be regarded as being “in a state of oxidative stress”. With values between 200 and 250 CARR. U., the subject can be regarded as being equipped with special antioxidant capabilities (vegetarian diets, use of antioxidants). Lower values can indicate pathological states (immuno-depression) or the result of immuno-depressive therapies. In the case of CARR. U. levels below 200, these should be studied again in detail on the basis of clinical tests on a large scale.

Two types of case underwent oxidative stress assessment:

1. Seemingly healthy subjects, with unusual or luxury lifestyles (smokers, overweight individuals with body mass indices of > 25, athletic individuals accustomed to being subjected to maximum stress on the bicycle ergometer, non-athletic individuals unaccustomed to being subjected to maximum stress on the bicycle ergometer, women undergoing contraceptive treatments). This group (with the exception of the contraceptive cases) tend to confirm the hypothesis that smoking, a sedentary lifestyle and being overweight (Fries hypothesis) have oxidative stress as a common denominator. Apart from the group being treated with contraceptives (average age of 23), all the other groups were evenly balanced (in terms of their gender) with an average age of 35. The whole sample can be seen in Table 1.

2. Patients suffering from cardiovascular diseases included in an epidemiological study carried out in Italy (PAP/PEA study, San Valentino – Pescara) and analysed before and after treatment of the basic illness (one month of treatment prescribed by the attendant doctor). All the pathologies examined compromised the endothelium. In addition, these patients, with the exception of Raynaud’s disease (all females), were evenly balanced (in terms of gender) with an average age of 55. The full sample can be seen in Table 2.

ANTIOXIDANTS

Antioxidants can be divided into six categories (21, 22) that can be subdivided according to their potency level as follows:

1. Antioxidant systems (e.g. GSH (Glu-
tathione), Catalase, super oxide dismutase, peroxidase);  
2. Antioxidants with “shock absorber” function (e.g. albumin, uric acid, bilirubin);  
3. Essential vitamin antioxidants (e.g. Vitamin E, Vitamin C);  
4. Antioxidants of mixed endogenous/food type (e.g. Coenzyme Q10, polyunsaturated fatty acids);  
5. Carotenoid antioxidants;  
6. Flavonoid antioxidants.

We have used a combination of antioxidants (20) (ARD Stenovit®) comprising Vitamin C, Vitamin E, Vitamin A, Vitamin B6, L-Cysteine, Coenzyme Q10, Bioflavanoids extracted from citrus fruits (orange, grapefruit, citron), Selenium and Zinc. Within the limits of the RDA (Recommended Daily Allowance), the single doses were administered in a two-stage formulation (powder in a plunger cap and liquid in a 10 ml phial) which was to be mixed together just before administration. The product was administered in the morning, for a period of 7-15 days to three groups of subjects: a) seemingly healthy subjects (6 males and 6 females with an average age of 56, treated for 7 days); b) subjects suffering from Intermittens Claudicatio (cramp-like pain and motorial stop of between 200 and 500 mt) (9 Males and 6 females with an average age of 65, treated for 10 days); c) subjects suffering from Asymptomatic stenosis of the carotid artery (27 Males with an average age of 65, treated for 15 days).

**RESULTS**

In seemingly healthy individuals who were overweight (Body Mass Index > 25), we were able to identify high levels of oxidative stress (TABLE 1).

Physical stress (TABLE 1) produces a temporary increase in oxidative stress corresponding to the moment when O2 consumption increased under stress; In addition, when they entered into the anaerobic phase, the accumulation of Hypoxanthine began which, when re-oxygenation (reperfusion) occurred, turned into massive amounts of Xanthine with the corresponding production of OH- radicals.

After maximum stress on the bicycle ergometer, you can see a high level of oxidative stress which, in the athletic individual, is recovered in a short period of time (within 1 hour); in the non-athletic subject, however, the oxidative stress persists for a much longer period of time.

The athletic individual, accustomed to stress, triggers a series of antioxidant systems (in addition to blocking acidosis), resulting in the increase in oxidative stress being only temporary and more than adequately compensated by the efficacy of the antioxidant systems (which can be defined as shock absorbers). Smoking and the use of the contraceptive pill cause a significant increase in the CARR. U. values.

Smoking causes an increase in free radicals in around 70% of smokers (within the limits of 20 cigarettes a day) with values ranging between 350 and 480 CARR. U. These subjects would have been tested not only in the morning but in the evening as well (at the moment of maximum manifestation). The use of contraceptives almost always leads to a significant increase in levels that exceed 400 CARR. U., probably as a result of a change in the bioavailability of the Vitamin E.

High levels of oxidative stress have also been found in pathologies that have an endothelial change in common (Table 2), both before and after the attempted therapeutic compensation (one month of treatment with a therapy specifically for the pathology). The treatment of these patients was carried out by the doctor in charge.

These pathologies were put under observation because the endothelium undergoes a double aggression by the OH- radical. The first is due to the compression caused by the oxidized lipids [Oxidation and inflammation theory of Atherosclerosis (24)], and the second, to the antiproteases’ greater sensitivity to oxidation (25). Their oxidation can allow the proteases a great deal of freedom of action, and they tend to attack the intercellular junctions of the endothelium, opening access to the oxidized lipids by the aforementioned free radicals, thus enabling the macrophages to penetrate more easily into the subendothelium (24).

All the pathologies analysed have been evaluated before any specific therapy,

<table>
<thead>
<tr>
<th>SITUATION</th>
<th>NUMBER OF CASES</th>
<th>AVERAGE CARR. U. VALUES ± SD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMOKING</td>
<td>10</td>
<td>405 ± 62.2</td>
</tr>
<tr>
<td>USE OF CONTRACEPTIVE PILLS</td>
<td>28</td>
<td>440 ± 34.0</td>
</tr>
<tr>
<td>EXCESS WEIGHT</td>
<td>12</td>
<td>&gt; 350 °</td>
</tr>
<tr>
<td>BYCYCLE ERGOMETER</td>
<td>20</td>
<td>&gt; 350 **</td>
</tr>
<tr>
<td>1 H. AFTER USING BY CYCLE ERGOMETER (non-athletic individuals)</td>
<td>10</td>
<td>&gt; 350 °</td>
</tr>
<tr>
<td>1 H. AFTER USING BYCYCLE ERGOMETER (athletic individuals)</td>
<td>10</td>
<td>&lt; 300 °</td>
</tr>
</tbody>
</table>

* Standard Deviation  
** No subject showed levels lower than those indicated  
*** No subject showed levels lower than those indicated

Table 1  
D-Roms test values in various situations.
immediately after confirmation of the clinical diagnosis.

Table 2 shows how the average CARR. U. levels in all the pathologies examined are clearly higher than normal levels. If you exclude any case of non-insulin-dependent diabetes, all the individual cases analyzed presented levels of over 300 CARR.U., proving that the patients were in conditions of oxidative stress. It is important to point out that despite the relevant therapeutic compensation, the CARR.U. levels did not return to normal, even if a significant drop was observed in the patients who were hypertensive and suffering from hypertriglyceridemia. What emerged is that oxidative stress appears to precede clinical manifestation and, as a result, the determination of its quantity assumes an important diagnostic role. Within the various groups, we observed cases where basic therapy had succeeded in compensating the oxidized state, although in the majority of cases this effect was not produced, indicating that the state of oxidation must be regarded as an independent parameter.

**CONTROL USING ANTIOXIDANTS**

With these patients, there was the problem of whether to control the state of oxidation by means of food or by using integrators with antioxidant action.

This therapeutic approach was used in three groups of subjects as shown in Table 3.

The administration of a balanced pool of antioxidants proved to be capable of reducing oxidative stress both in normal individuals and in patients suffering from intermittent claudication and asymptomatic stenosis of the carotid artery.

From the data analysis, it is clear that the therapeutic administration of antioxidants may be capable of reducing oxidative stress. This does not mean that by using antioxidants one can cure the basic disease, but it definitely indicates that one can remove an important risk factor.

**DISCUSSION**

Fries’ Hypothesis suggests that there are three “large controllers” - smoking, calories and physical inactivity - that are responsible for an increase in morbidity and, therefore, the progression of senescence towards senility. Controlling the oxidation state of these “large controllers” clearly indicates that they share oxidative stress as a common element and, therefore, they are getting close to the hypotheses of Fries and Harman. Even if the fundamental causes of morbidity are the various cardiovascular diseases, it seems that these also have in common the abnormal production of free radicals. The chapter relating to the various ways in which free radicals form is currently in preparation and is not the subject of this article. However, we can glean from the studies we are currently carrying out (data not yet published) that these causes can be confined using reactive, caloric and metabolic methods.

Treatment with antioxidants is effective – however, we cannot take for granted that any pool of antioxidants would be effective; instead, it appears that any pathology can benefit from specific formulations. We are certain that the type
of formulation should include low concentrations of fluid forms in order to prevent any delay in the contrasting effect, or in pro-oxidation. Restriction of calories and physical exercise are other factors that positively influence the disability curve and which can have a reducing effect on oxidative stress and they should, therefore, also be systematically evaluated.

The occurrence of oxidative stress should therefore be regarded as an alarm bell prompting further diagnostic investigations. However, there are therapeutic resources (dietetic, pharmacological and salutary) enabling you to slow down the dual concept of oxidative stress and senility. An extremely simple regime, to reduce the calorific intake of fluid forms in order to achieve longevity in relation to genetic and salutary) enabling you to play a role in the treatment of senility as well as in dietetic integrators (30, 31). There are clearly other factors that reduce mortality and morbidity rates – high socioeconomic levels (32) and a high level of education (33).

The quality and duration of life are much more closely related concepts than you would imagine – they can actually be defined as “heterozygotic twins” that are born out of the same matrix. They both emerge from a unitary context in which food, education and relationship with the environment are in harmony with each other in a holistic way.

This test uses the same unit as that used to measure body temperature in the case of infectious states or for measuring arterial pressure in the case of cardiovascular diseases, both of which give a precise indication of a diagnosable pathological condition.

The use of antioxidants has proved useful in reducing morbidity and mortality rates (26, 29) – they are included in intervention methods in the treatment of senility as well as in dietetic integrators (30, 31). There are clearly other factors that reduce mortality and morbidity rates – high socioeconomic levels (32) and a high level of education (33).

In short, we cannot overlook subjects’ “lifestyles” in our overall assessment.

Psalm 89, which posed the problem, pointed out the solution “…teach us to number our days, so that we may apply our hearts unto wisdom”. In exegetic terms, “number” the days means living them, taking care of yourself and others.

We take care of ourselves using the means Science puts at our disposal. For some time, there has been a diagnostic view of the state of oxidation that tends to see it as going beyond the task of defending the body and becoming an uncontrolled cause of damage that precipitates the onset of senility.

Oxidative stress can be evaluated using many methods of varying levels of effectiveness that will certainly improve with time. It is currently thought that the D-Roms test is a valid method and achieves the aim of triggering alarm bells and prompting diagnostic investigation into senility.

References