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Moderate exercise and sport has been demonstrated to reduce cardiovascular morbility and mortality and to ameliorate quality of life. Indeed, to be sedentary predispose to overweight and obesity, which in turns, are related to several diseases, such as miocardial infarction, stroke, atherosclerosis, dislipidaemia, diabetes, cancer etc. However, exaggerate exercise also can lead to the same unpleasant above mentioned "side effects" on health. Indeed, inadeguate exercise was demonstrated to be related to reactive oxygen specie production and/or impairment of antioxidant defenses. Therefore, exercising people and athletes should be undergone a laboratory assessment in order to prevent oxidative stress and to monitor the effectivenes of antioxidant therapy. In this context, the aim of "d-ROMs test and sport medicine" is to provide a picture about the usefulness of d-ROMs test in sport medicine. We acknowledge the help of all Authors of clinical and experimental studies reported in this paper and, particularly, Mauro Carratelli, the "inventor of d-ROMs test".

September 2005 *Dr Eugenio Luigi Iorio, MD, PhD*

The molecular structure reported on the cover is a computer-elaborated tridimensional formula of N,N-diethylparaphenylendiamine, the chromogenic substrate of d-ROMs test.

Chapter 1. Oxidative stress and exercise

Inadequate exercise has been frequently associated to oxidative stress, a particular kind of chemical stress induced by the presence of exaggerate amounts of reactive oxygen species (ROS), which results from an increased production of reactive species and/or from a reduced ability of a living organism "to swallow" the reactive species anyhow produced.

Indeed, an exaggerate or insufficient physical activity can be responsible for an unbalance between pro-oxidant and anti-oxidant systems thus leading to oxidative stress muscle injuries in which hydroperoxides play a major role (figure 1. 1).

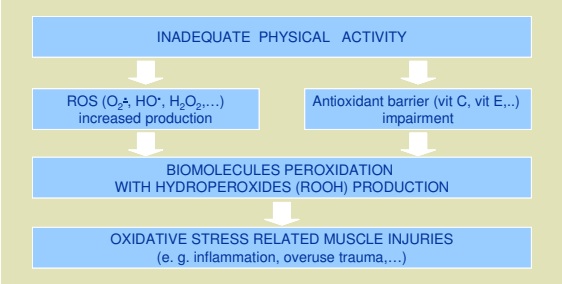

Figure 1. 1 Pathogenesis of oxidative stress-related muscle injuries

An exaggerate physical activity can induce oxidative stress either by increasing ROS production or reducing antioxidant defenses.

The primary mechanism of ROS overproduction associated to strenuous/prolonged exercise is the metabolic rate increase. Such mechanism involves the mitochondria, because these organelles are the primary metabolic source of ROS. Indeed, in their "cristae" are located the respiratory chain enzymes responsible for oxidative phosphorylation.

Several studies have demonstrated that the transfer of electrons ideally only results in the production of a molecule of water by means of the tetravalent reduction of molecular oxygen. Indeed, this process is not perfect and small but significant amounts (1-2%) of electrons are "normally" shifted from respiratory chain transporters directly to molecular oxygen, thus generating superoxide anion and/or hydroxyl radical (univalent reduction) and/or hydrogen peroxide (bivalent reduction) (figure 1. 2).

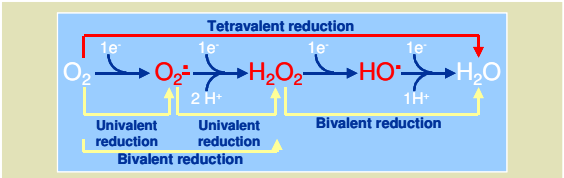

Figure 1. 2 Pathways leading to oxygen reduction

Obviously, the amount of above mentioned shifted" electrons will be very high in all the organs with a sustained metabolic activity, such as skeletal muscles, especially during intense and/or prolonged exercise. Indeed, in strenuous exercise, it was calculated that up to the 15% of the molecular oxygen of skeletal muscles can be directly reduced to ROS by this way in mitochondria.

Therefore, the overproduction of ROS associated with exaggerate physical activity is primarily due to an increased metabolic rate that results in an abnormal "shift" of respiratory chain electrons to generate $O_2^{\cdot-}$ and/or HO* and/or H_2O_2 from O_2.

ROS (e. g. HO*), now available in great amounts, are able to attack any substrate (R– H) by extracting electrons to reach their stability. This process generally trigs a radical chain reaction and if this process is not opportunely blocked a functional and therefore structural cell damage can results (figure 1. 3).

$$R–H + HO^* \rightarrow R^* + H_2O$$

$$R^* + O_2 \rightarrow ROO^*$$

$$ROO^* \rightarrow ROOH + R_1^*$$

$$R_1^* + R_1^* \rightarrow R_1^*–R_1^*$$

Figure 1. 3 Radical chains reactions

In this context, the primary mechanism involved in cell and tissue damage is the production of hydroperoxides (R-OOH) a class of compounds which belong to the reactive oxygen metabolites (ROMs).

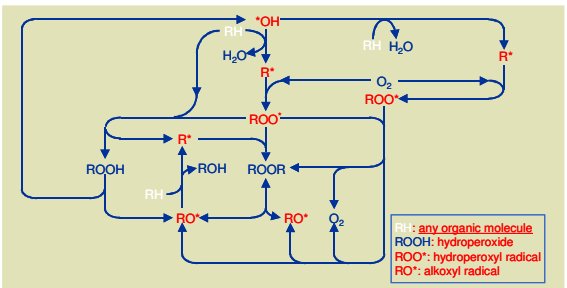

Figure 1. 4 Peroxidation pathways

Normally, muscular cells have an efficacious defense system to control the overproduction of

these reactive species. Indeed, endogenous or dietary antioxidants play a protective role, being capable of scavenging free radicals, and thereforethey may prevent muscle damage. Moreover, several studies have shown that training results in increased activity of antioxidant defence and also that dietary supplementation with antioxidants has favourable effects on peroxidation processes after exercise.

However, if ROS production is exaggerate and/or muscle cell ability to inactivate such reactive species is reduced, cell undergoes free radicals damage, despite antioxidant system.

Indeed, ROS can attack any organic substrates thus producing hydroperoxides.

Hydroperoxides, the end products of peroxidation process, in turn, if transition metals and/or specific enzymes are available, can generate alkoxyl and peroxyl radicals, finally responsible for muscle oxidative damage (figure 1. 5).

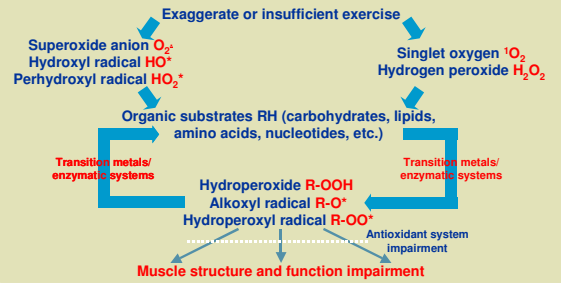

Figure 1. 5 Mechanisms of muscle impairment induced by ROS

It is likely also that muscular oxidative damage can be amplified during intense and/or prolonged exercise by the collapse of mitochondrial membrane potential, secondary to lipid peroxidation, that further reduces ATP production and increases ROS generation.

Moreover, it was postulated that strenuous exercise in skeletal muscle reproduces a condition very similar to heart muscle ischemia. In other words, oxidative damage after intense and/or prolonged physical activity should follow the model of ischemia-reperfusion damage.

Indeed, during muscle contraction, ATP is converted to ADP + P_i to allows mechanical work. ADP is recharged to ATP from the local creatine phosphate stores and from respiratory chain activity of mitochondria.

In skeletal muscle under strenuous exercise, such as in heart muscle during severe ischemia (e. g. myocardial infarction), AMP is accumulated and deaminated to IMP. This latter is converted to inosine by 5'-nucleotidase and, finally, to hypoxanthine, by purine-nucleoside phosphorylase. Provided that xanthine dehydrogenase is converted to xanthine oxidase, hydrogen peroxide and superoxide radical are formed from hypoxantine and xantine, respectively, during uric acid generation. Such ROS can noticeably amplify oxidative damage primary induced by mitochondrial activation (figure 1. 6).

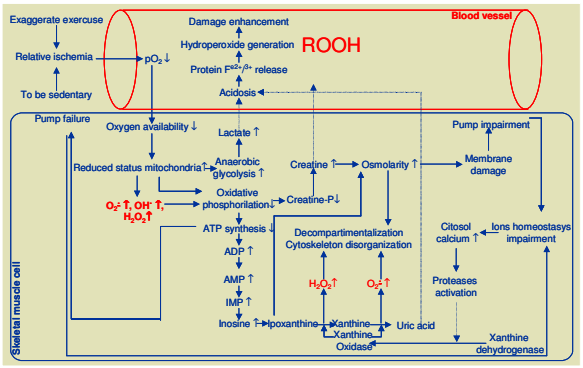

Figure 1. 6 Ischemia-reperfusion damage in skeletal muscle cell

On the basis of these observation oxidative stress during exercise can be related to two mechanisms: a reduced efficacy of cellular respiration (oxidative stress type II) and a change in the oxygen pressure (oxidative stress type IV), according to the general classification of oxidative stress mechanisms (table 1. 1).

Table 1. 1 Primary mechanisms of oxidative stress (OS) and their relations with some clinical situations

SO*	Cell site†	Mechanisms†	ROS/ROM	Relations
I	Plasmamembrane	Arachidonic acid generation	Hydroperoxides, superoxide anion	Reactive proc. (inflammation)
		NADPH oxidase activation	Superoxide anion	Reactive proc. (inflammation)
II	Mitochondria	Metabolic activation	Superoxide anion Hydr. peroxyde	Ipernutrition, inadequate ex.
		Mitochondrial dysfunction	Superoxide anion Hydr. peroxyde	Mitochondrial diseases
III	Microsomes	Cytochromes P_{450}/b_5 activation	Various	Alcohol, drugs, xenobiotics
IV	Citosol	Xanthine oxidase activation	Superoxide anion Hydr. peroxyde	Ischemia-reperfusion dis.
V	Two at least	Multiple	Variably centered‡	Cigar. smoking, polluttants, radiations

I: OS by reactive changes of cell surface; II: OS by cell respiration reduced effectiveness; III: OS by pharmaco-metabolic induction; IV: OS by intracellular pO_2 changes; V: OS by mulltiple mechanisms. † Primarily. ‡Carbon, nitrogen, chlorine etc

However, ROS overproduction after strenuous exercise make prone skeletal muscle, tendon and joints to trauma and overuse lesions, all conditions characterized by activation of inflammatory system.

In particular, monocyte-macrophage and polimorphonuclear leukocytes (PMN) activation, can lead to production of superoxide anion and hydrogen peroxide, according to the model of oxidative stress by activation of plasmamembrane (oxidative stress type I).

Indeed, byproducts od tissues damage derived by muscle trauma can activate NADPH oxidase and lipoxygenase systems located in PMN plasmamebrane, finally responsible for ROS/ROMs production.

Thus, it was postulated that a third cellular source of ROS, other than mitochondria and citosol, can be involved in oxidative stress in exercising people (figure 1. 7).

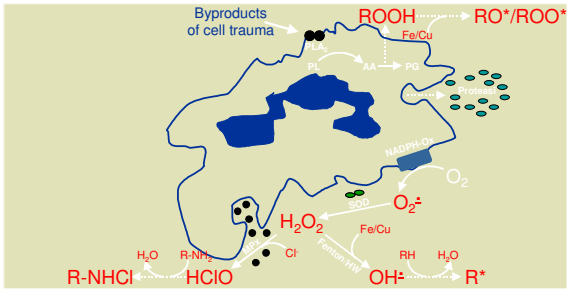

Figure 1. 7 Inflammatory damage in skeletal muscle cell

Moreover, the pathological role of hydroperoxides, which are considered not only the “amplifiers” but also “the witnesses” and the “markers” of the tissue damage, must be further outlined.

Indeed, hydroperoxides, which are generated in the cell, was postulated to be ejected in extracellular fluids where their still maintain a good oxidant capacity. Because this property, they are able to promote also in biological fluids (e. g. plasma, synovial fluid etc.) a “branch reaction” if a transition metals such as iron is available as catalyst (figure 1. 8).

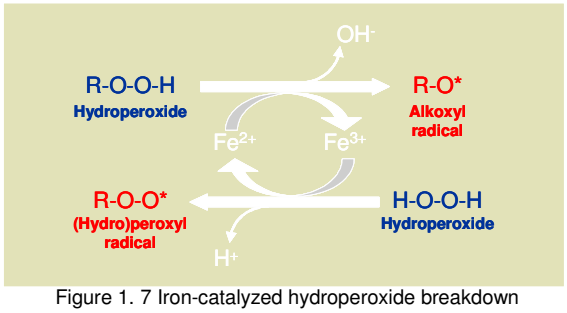

Figure 1. 7 Iron-catalyzed hydroperoxide breakdown

Iron is normally bound in a chelated, harmless form, to specific proteins (i. e. transferrin). However, in some situations (e. g. acidosis after muscular effort) this metal can be released as free ion. Such free iron (or other transition metal) is then able to catalyze a Fenton-like reaction to produce alkoxyl and hydroperoxyl radicals. In turn, such radicals can attack LDL and endothelial cell thus amplifying the oxidative stress damage (figure 1.8).

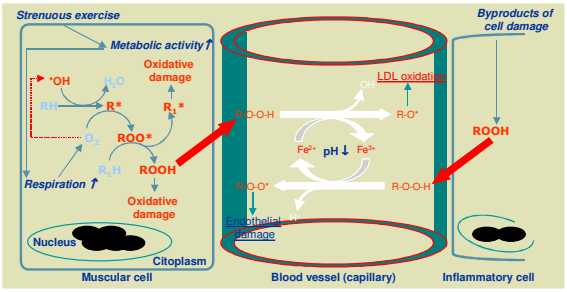

Figure 1. 8 Cell amplification damage hydroperoxide-mediated

The combination of branch and chain reactions are the principles of the cascade reaction and the production of hydroperoxyl radicals and hydroperoxides will continue as long as molecular oxygen is present and no antioxidants are present to break the “production line” and terminate the chain reactions.

Because their relative stability and their good oxidant capacity, hydroperoxides are detectable in biological fluids by means the d-ROMs test, a clinical tool to diagnose the presence of radical formation and estimate its magnitude. The d-ROMs test take advantage by the property of hydroperoxides to generate hydroperoxil and alkoxyl radical in the presence of iron ions (*see below*). In such sense hydroperoxides of biological fluids are considered as “witnesses” and “markers” of the tissue oxidative damage. Plasma levels of malondialdehyde (MDA) or expired pentane and ethane are also measures of radical formation but only if the antioxidant system in the biological milieu is exhausted (*see below*).

Chapter 2. Oxidative stress assessment in exercising people

To study radical metabolism "in vivo", electron spin resonance (ESR) or nuclear magnetic resonance spectroscopy (NMRS) has bee used. However, in humans in general and in athletes in particular, neither of these methods is applicable to any major extent.

Consequently, different methods, referred to as "fingerprinting", must be applied. According to this approach, a radical is inferred from the molecular nature of the damage it causes to biological molecules. Indeed, it is now clear that if oxidative stress is great enough to overcome the antioxidant defence, the reactive radical species can damage practically every component of the cell, including amino acids and proteins, nucleic acids in DNA and RNA, and lipids. A peroxidation process occur and hydroperoxides are formed. These damaged molecules or products are the "fingerprints" of oxidative stress.

In this context, the d-ROMs test is a spectrophotometric test that allows to assess, in a biological sample, the concentration of hydroperoxides (ROOH).

Such compounds are generated into the cells by the oxidative attack of ROS on a number of organic substrates (e. g. carbohydrates, lipids, amino acids, proteins, nucleotides etc.).

The initials "ROMs" underlines that the analites measured by this test, i. e. hydroperoxides, belong to the reactive oxygen metabolites (ROMs).

The d-ROMs test is based on the interaction with transition metals, a mechanism involved in the initiation of radical chain reactions.

The principle is those of Fenton's reaction, verified the first time for hydrogen peroxide and after amplified by Haber and Weiss. According to these reactions, a transition metal ion (e. g. iron or copper) catalyze hydroperoxide breakdown, thus generating new radical species, such as hydroperoxyl (ROO*) and alkoxyl (RO*) radicals, concomitantly to the oxidation ($Fe^{2+} \rightarrow Fe^{3+}$ or $Cu^{+} \rightarrow Cu^{2+}$) or the reduction ($Fe^{3+} \rightarrow Fe^{2+}$ or $Cu^{2+} \rightarrow Cu^{+}$), respectively, of the catalyzing ion.

If to a solution containing this system (hydroperoxides and catalyst) is added a compound having a reduction potential that allows to extract the needed electron to reach its stability, such compound will become a radical, according to second step of radical chain reactions.

It is obvious that if such compound have the optical property to change its color when oxidized and if this compound is sufficiently stable in such form, it is possible, with adequate spectrophotometric techniques, assess its concentration.

Such concentration will be directly proportional to those of radical species generated in vitro and, in summary, to those of hydroperoxides initially present in the tested sample.

In the d-ROMs test, therefore, hydroperoxides of a biological sample, e. g. blood serum, are posed in the same conditions of the Fenton's reaction to generate *in vitro* alkoxyl and peroxyl radicals. Practically, a small amount of serum is diluted in a acidic buffered solution (pH 4.8). In these conditions, iron ions before bonded to the serum proteins become available to catalyze *in vitro* the breakdown of blood hydroperoxides to alkoxyl and peroxyl radicals (figure 2. 1).

The reactions of d-ROMs test

1A) $R\text{-}OOH + Fe^{2+} \rightarrow R\text{-}O^{*} + Fe^{3+} + OH^{-}$
1B) $R\text{-}O^{*} + A\text{-}NH_2 \rightarrow R\text{-}O^{-} + [A\text{-}NH_2{}^{*}]^{+}$

2A) $R\text{-}OOH + Fe^{3+} \rightarrow R\text{-}OO^{*} + Fe^{2+} + H^{+}$
2B) $R\text{-}OO^{*} + A\text{-}NH_2 \rightarrow R\text{-}OO^{-} + [A\text{-}NH_2{}^{*}]^{+}$

where:
– R-OOH is a generic hydroperoxide
– R-O is the alkoxyl radical of a generic hydroperoxide*
– R-OO s the hydroperoxyl radical of a generic hydroperoxide*
– A-NH_2 is N, N-diethyl-paraphenylendiamine, i. e. the chromogenic substrate of d-ROMs test
– $[A\text{-}NH_2{}^{*}]^{+}$ is the coloured radical cation of the chromogenic substrate

Figure 2. 1 The principle of d-ROMs test

A compound (chromogen) that have the ability to change its color when is oxidized by hydroperoxyl and alkoxyl radicals is then added to this solution. The chromogenic substrate used in the d-ROMs test is N,N,-diethylparaphenylen-diamine, that possess the feature of being oxidized by hydroperoxyl and alkoxyl radicals, thus transforming itself in a pink to red colored cation.

Such cation is a radical but it is sufficiently stable so that it is possible to assess its quantity by means of a photometer (work conditions: wavelength 505 or 546 nm, optical path 1 cm, temperature 37 °C, kinetic or endpoint mode). The concentration of colored complex will be directly related to the hydroperoxides levels of the tested biological sample.

Because the chemical heterogeneity of alkoxyl and peroxyl radicals generated by hydroperoxide breakdown, the results of d-ROMs test are expressed as arbitrary units, the CARRATELLI UNITS (CARR U), where 1 CARR U correspond to 0.08 mg/100 mL H_2O_2.

Normal range on d-ROMs test in healthy peoples was shown to be 250 – 300 CARR U.

It is very important to remark the substantial differences existing between d-ROMs test and TBARs-test.

The T-BARs ia commonly used test to assess oxidative stress in athletes based on the dosing of all the substances which react with thiobarbituric acid, suc as the well known malonyldialdehyde (MDA, CHO–CH_2–CHO).

In peroxidation process, MDArepresents one of the final products in the chain of reactions set off in the cellular membranes by the oxidative attack, on behalf of some free oxygen radicals (like the hydrossil radical), on the polinsatured fatty acids (like arachidonic acid, an inportant costituent of the membrane phospholipids) (figure 2. 1).

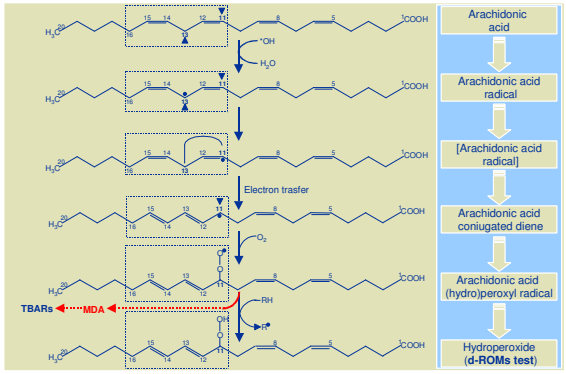

Figure 2. 1 Peroxidation of arachidonic acid

This chain of reaction in the specific case of arachidonic acid, has the peroxide radical as the "key" of the chemical species of the entire process. The last, in fact, is at a "fork" of two possible metabolics, as it can be converted into hydroperoxide (by acquisition of an H) or "take" the road leading to cyclic peroxides which, after other oxidative attacks gives a series of terminal products, one of which is the MDA. These further "oxidative attacks" and therefore, the formation of MDA, are possible thanks to the passing of the antioxidant "defences" of the medium in which the same oxidative process takes place.

Therefore, the presence of MDA in the biological liquids will only be seen when the whole endogene antioxidant system of the medium where the oxidative attack has taken place has finished. Taking these considerations into account, we realize that the MDA, a nearly "terminal" product of oxidation of various biological sublayers, such as membrane polinsature fatty acids, is a tardy indicator of oxidative stress.

Thus, one of the greatest disadvantages of the test based on the determination of the MDA is related to the fact that it is not always able to show a precocious altered oxidative state.

Instead, the d-ROMs test is based on the determination of the level of hydroperoxides, the other class of composts that can develop starting from peroxide radicals (chemical species "key" of the chain of reaction that takes to oxidation of the polyunsatured fatty acids of the membrane). Unlike MDA, the hydroperoxides are compounds which are formed precociously in the sequence of oxidative reactions of the membrane lipids, they are relatively stable and, still conserving a discreet oxidant capacity, can be revealed thanks to an adequate redox system (like the N,N-diethylparaphenylendiamine of the d-ROMs test).

Therefore, regarding tests that give MDA values, the d-ROMs test is able to show altered oxidative states much more quickly, with enormous advantages for the clinical side regarding prevention and therapeutic recording. As already seen many times in literature, MDA run into many secondary reactions that reduce the accuracy of the obtained results; in fact, as a bifunctional reaction (double CHO aldehydic group) the MDA can form crossed relationships with proteins or nucleotidis (giving formation of Shiff bases) and it can be degraded by hydrogen peroxide or oxidated by peroxidase and xantinaoxidase. Even as a marker of lipidic peroxidation the MDA results are specificly scarse; in fact, it has been identified amongst the products of oxidative decomposition of amminoacids, carbohydrates and prostaglandins. Lastly, the MDA can also be an ascorbic acid oxidation product, and this makes its dosage useless regarding an eventual therapeutic recording during antioxidant treatment (figure 2. 2).

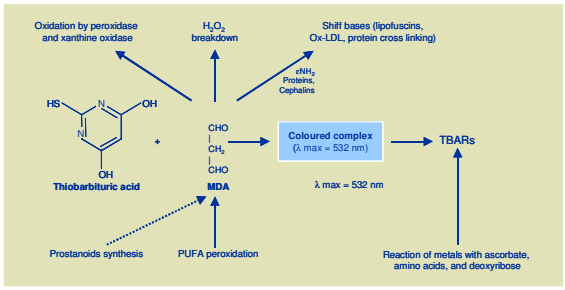

Figure 2. 2 Principles and disadvantages of T-BARs Test

Vice versa, the d-ROMs test has been successfully used in monitoring of antioxidant treatment (e. g. A_{RD} Stenovit) and also during specific pharmacological treatment (dipyridamole, rutosides, D-penicillamine, etc).

Chapter 3. d-ROMs test and oxidative stress assessment in athletes

3. 1 Ergometer bicycle test

In healthy subjects at rest, the level of hydroperoxides are lower than those after physical activity. Usually, values tend to increase following activity but do not exceed the threshold of 350 CARR U or 28.00 mg H_2O_2/dL of serum (1). If the activity of a subject involves a muscular effort the values of d-ROMs test temporally may exceed this threshold (e. g. exercise with ergometer bicycle). This it true obviously for not trained individuals, as shown in table 3. 1 (2).

After maximum effort on ergometer bicycle, the high level of oxidative stress rapidly decreased only if the subject is trained (1, 2).

Table 3. 1 d-ROMs test mean values in healthy peoples (ergometer bicycle test)

Timing	n	CARR U	Mg H_2O_2/dL
Immediately after maximum effort	20	> 350*	>28.00*
One hour after maximum effort (not trained subjects)	10	> 350*	> 28.00*
One hour after maximum effort (trained subjects)	10	< 300**	< 24**
*None of subjects had levels inferior to 350 CARR U (i. e. 28.00 H_2O_2/dL). ** None of subjects had levels superior to 300 CARR U (i. e. 24.00 H_2O_2/dL).			

Similar results were found in another study on 10 volunteers subjected to the same test until appearance of physical stress and/or cramps (3). Most individuals were shown to have a higher level of hydroperoxides circulating after super muscular strain on ergometer bicycle than at rest.

These findings suggest that the dosage of serum hydroperoxide by the d-ROMs test is useful to guarantee the possibility of improving training, therefore assuring longer physical strains without damage that could be induced by free radicals.

The d-ROMs test was shown also to be very useful to monitor oxidative stress in athletes, according to the results of several studies reported below.

3. 2 Football players

The serum level of hydroperoxides during a whole football season was evaluated in 26 professional football players of the Bologna Football Club, a team participating to Italian Premier League (4). Five serum samples for each athlete – typically performing regular heavy exercise – were done, every two weeks during the period of training, then every two months.

The mean values of d-ROMs test are reported in Table 3. 2.

Table 3. 2 d-ROMs test mean values in football players during a whole football season

Athletes	Assays	CARR U (mean value ± SD)	Range
26	120	258 ± 37.9	160-376

The level of hydroperoxides was ≥ 300 CARR U in 26/120 (22%) assays. A level of hydroperoxides ≥ 300 CARR U, at least once, was observed in 10/27 (37%) athletes (2/10 on 4 samples, 2/10 on 3 samples, 4/10 twice; 2/10 once; none persistently). During the season, the trend was stable on high levels in 3/20, stable on border-line levels in 3/20, stable within reference range in 9/20, in decrease in 4/20 (for 3 is a big decrease due to supplementation). The trend was rising only in 1/20.

Total antioxidant status (TAS, Randox, Crumlin, UK) was also evaluated. Exactly as for d-ROMs, 10/27 (37%) players showed significant modifications of the test (TAS ≥1.30 mmol/L) at least once, but only for 3/10 TAS was ≥1.30 mmol/L twice on 2 sampes, for remaining 7/10 was significant only once. The average (n=85 tests) was 1.40±0.05 mmol/L (range 1.20-1.56); 13/85 tests were ≥1.30 mmol/L and 8/13 also shown a hydroperoxides increased value.

Correlation between d-ROMs and TAS levels shown a slight, inverse trend (y=0.0029x + 2.16; R2=0.52), as expected. In 8/85 tests the Authors found TAS ≥1.30 and hydroperoxides level ≥300 CARR U, in 11/85 TAS >1.30 mmol/L and hydroperoxides levels <300 U CARR, in 5/85 TAS ≥1.30 mmol/L and hydroperoxides levels ≥ 300 CARR U and in the remaining 61/85 TAS >1.30 mmol/L and hydroperoxides levels <300 CARR U, thus confirming the capability of antioxidants system to limit the free radicals generation.

Finally, the proportion of players with at least one episode of exercise induced muscular damage was evaluated. On 27 elite players, 6/10 (60%) athletes with a level of hydroperoxides ≥300 and 7/17 (41%) shown at least one event of muscular injury. Although the frequency of muscular damage was not statistically correlated to any test evaluated, athletes with highest hydroperoxides levels largely supplemented reduced however the incidence of their accidents compared to previous football season.

These results globally suggest that d-ROMs test is useful to monitoring professional players during a football season. Particularly, concentrations of hydroperoxides above the upper

reference limit were more frequently found, but following values were generally stabilized around a specific level, probably reflecting the individual response to the oxidative stress. In this way, dietary supplementation of players with high persistent values led to hydroperoxides reduction on following samples.

3. 3 Softball players

Eight elite athletes (all females and members of the Italian National Team) were tested (5).

The d-ROMs test was performed in basal conditions, after a training session and after a 3,000 meter race.

The level of hydroperoxides was shown to increase significantly after aerobic activity related to effort (figure 3. 1).

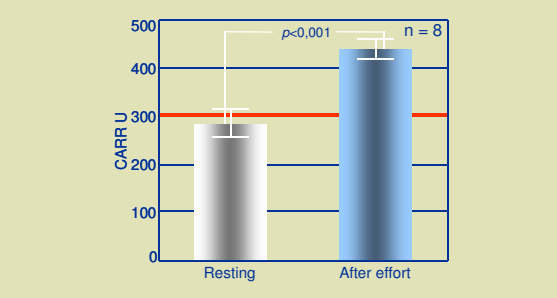

Figure 3. 1 The muscular effort is related to increased d-ROMs test mean values in softball players

3. 4 Baseball players

Twenty elite baseball players, all members of the Italian National Team were tested (5).

The d-ROMs test was performed in basal conditions, and after an effort test and two official games, respectively. A cycle of 10 days of antioxidant therapy (ARD Stenovit®) between the official games was performed.

The results indicate that compared with situation at rest, the average values of hydroperoxides after training or competitions increased significantly in this baseball players (figure 3. 2).

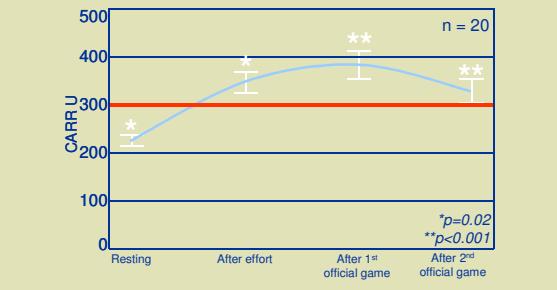

Figure 3. 2 Trend of d-ROMs test mean values after two official games in baseball players

It is interesting to note that the intake of antioxidants decreased significantly the levels of free radicals derivatives after training, thus underlining the usefulness of d-ROMs test in antioxidant therapy management.

3. 5 Triathlon

Ten triathlon athletes were tested after intense training (swimming followed by a race) which lasted 2 hours (5).

The d-ROMs test was performed at rest and after an effort test.

The level of hydroperoxides increased significantly after effort compared to resting values (figure 3. 3).

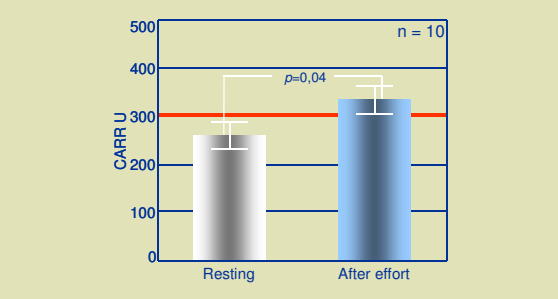

Figure 3. 3 Increased d-ROMs test mean values in triathlon after effort

3. 6 Golf

The d-ROMs test was performed on 12 members of the National Golf team after running for 3,000 meters at maximum speed (5).

The level of hydroperoxides increased from 234.07 (SD 55.30; SE 15.30) at rest to 293,69 (SD 64.10; SE 18.00) after the aerobic activity. The difference was statistically significant (p=0.001).

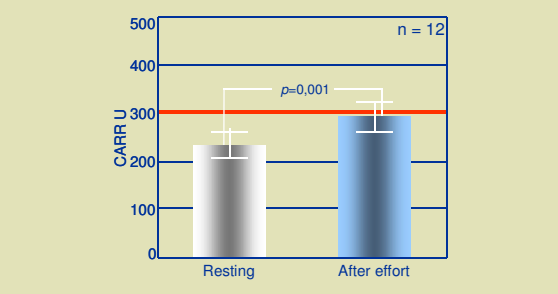

Figure 3. 4 Increased d-ROMs test mean values in golf players after effort

3. 7 Cyclic race

The d-ROMs test was performed on 12 athletes before and after a 150 km endurance cycling race (5).

Six of twelve athletes were also tested after 2 days rest and after 10 days of antioxidant treatment (ARD Stenovit®).

Serum level of hydroperoxides was shown to increase after running and to decrease significantly after resting and treatment with antioxidants (figure 3. 5)

It is very noticeable also in this study the usefulness of d-ROMs test in antioxidant therapy management in cycling races.

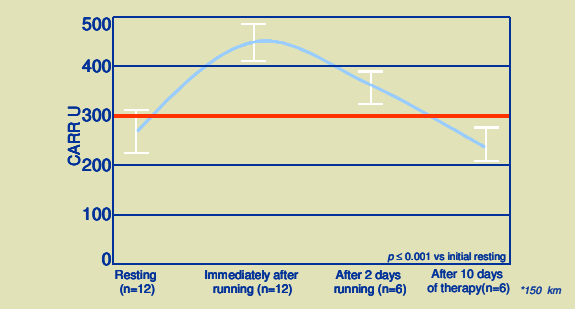

Figure 3. 5 Time-course of d-ROMs test values in a cyclic race

3. 8 Running

In a study 50 healthy men, 25.5±2.7 years aged, participate to a Marathon-like race (6).

The entire route (10.5 km) was cover in 75±15'. Before (t_0), at the end (t_1), and 1 h after the end (t_2) of the competition the serum hydroperoxides were measured according to d-ROMs test.

The level of serum hydroperoxides shifted from 243.4±22.6 (t_0) o 281.2±21.7 (t_1) and 333.2±19.7 (t_2). Changes observed were statistically significant (figure 3. 6).

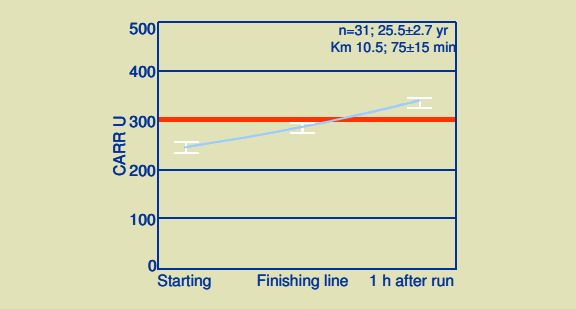

Figure 2. 2 Time-course of d-ROMs test values in a maraton-like race

3. 8 Miscellanea

In an other study (7), the level of hydroperoxides ranged within 110-516 CARR U in 407 athletes (332 professional football players of 7 Italian elite teams, 24 cross-country skiers ad 51 skyrunners). An increase >300 CARR U was shown in 382/1071 (35.66%) samples.

TAS levels ranged within 1.06-1.69 mmol/L and appeared under the cut-off (1.30 mmol/L) in 84/954 (8.80%). Correlation between d-ROMs and TAS shown a slight, inverse trend, as expected.

Chapter 4. Concluding remarks

The peroxidation of organic substrates (i. e. lipids, amino acids, proteins, nucleotides, etc.) by ROS is related to an impaired balance between the radical formation and antioxidant defences. During exercise, peroxidation can take place not only in the contracting muscle but also in engaged connective tissue compartments, and in the leukocyte and erythrocyte plasmamembranes. The result of this process could be: muscle inflammation, inflammation of connective tissues and related organs (including bursitis and tendinitis), reduced number of leukocytes and rupture of their membranes (with subsequent reduced immune activity and increased susceptibility to infectious diseases) and hemolysis (with subsequent reduced arterial blood oxygen content and oxygen transport capacity). Any of these consequences could result in reduced physical performance directly or indirectly in elite athletes and those enrolled in daily fitness programs. Therefore, it's very important that all athletes and subjects practising sports at amateur levels monitor their free radical levels in order to avoid the damage induced by the oxidative stress.

The d-ROMs test (Diacron International sas patent) is the only test actually available to evaluate the serum/plasma level of hydroperoxides, which are considered not only the “witnesses” but also the “amplifiers” of the oxidative stress damage.

Healthy peoples were shown to have in their serum a value of d-ROMs test between 250 CARR U (i. e. 20.00 mg H_2O_2/dL) and 300 CARR U (i. e. 24.00 mg H_2O_2/dL). The distribution of these values follows a Gauss-like curve, thus indicating that every individual have an own “oxidative trait” depending on own life-style (a complex of factors which are able to modulate the balance between free radical production and antioxidant defences).

Moreover, according to the data of scientific literature, sport amateurs and elite athletes were shown to exhibit a lower serum level of hydroperoxides compared to “general population”. This fact likely reflects an optimal balance between production and elimination of free radicals, a postulate consequence of the training programs.

The results of the studies above reported indicate also that the serum level of hydroperoxides increases after exercise respect to basal values, as measured at rest. This fact certainly reflects the increased production of oxygen free radical and their derivatives, such as hydroperoxides, after effort, a well documented consequence of strenuous aerobic activity. However, trained individuals were shown to have lower values of d-ROMs test when compared with non-trained peoples (which are prone to have a less efficacious antioxidant system respect to their trained colleagues). In this picture, it is interesting to note that the values of d-ROMs test directly correlated to the intensity of exercise performed. Indeed, highest levels of hydroperoxides were observed after a great endurance cycling race. Such values could indicate an alteration in health condition and may also reveal poor recovery levels or even overtraining conditions.

Finally, a decreased level of serum hydroperoxides was observed amongst athletes taking antioxidants. This evidence confirms that specific and effective antioxidant treatment in athletes may be important in order to compensate the alterations created during conditions of physical stress between the production of free radicals and the efficiency of endogenous antioxidant mechanisms.

On the basis of such evidences it is clear that d-ROMs test provide an easy and very suitable method non only to prevent and to monitor the oxidative stress but also to “personalize” training programs and antioxidant therapy either in athletes or peoples performing training programs. In this picture, the values provided by d-ROMs test are strictly related to those of other tests which evaluated the antioxidant capacity, i. e. FRAP test (Diacron International), TAS test (Randox. UK). In fact, a slight, inverse, but statistically significant trend, as expected, was observed in elite players between the two test.

In conclusion, since oxidative stress causes damages to cells which may lead to an alteration of the DNA, as seen during recent investigations, it is of vital importance that athlete who are involved in sporting activity at amateur levels monitor their level of ROMs. The determination of serum hydroperoxides by d-ROMs test may be useful in order to evaluate weather training regimen are suitable and, thereby improve training and also in order to change dietary habits and administrate antioxidants in cases of proven oxidative stress. The measurement of hydroperoxides by the determination of d-ROMs can be particularly interesting also to obtain an index of psycho-physical condition of an athlete during normal and resting period, and, therefore, the oxidative stress condition of an athlete after a competition. In fact, high values after a race could indicate an alteration in healthy condition and may also reveal poor recovery levels or overtraining conditions. Finally, the results above discussed suggest that d-ROMs test is a useful test to monitor and/or to personalize the antioxidant treatment either in athletes or in healthy peoples. In this field further studies are in progress.

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Either abstracts or full text of papers
above listed are available.
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