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Exercise and Antioxidants: Current Oxidative Stress Biomarkers: Future challenges and limitations

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ABSTRACT: This review provides an overview on the balance between the production of reactive oxygen species and their removal by antioxidants in living organism in the last few years. The core of the review will discuss the formation of ROS and how it strongly relates to the contribution of redox metals. The impact of oxidative stress on cells and how they respond to stress conditions and current biomarkers of exercise-induced oxidative stress will be reviewed in more details. The current review will also briefly discuss the limitations of analytical techniques used for determination of biological elements as reliable oxidative stress markers.

Keywords: Biological Elements; Oxidative Stress Biomarkers; Antioxidants; Analytical techniques; Biological Samples

1. Introduction and historical overview on oxidative stress

The aim of this review is to review the basic concepts of free radicals that possess one or more unpaired electrons in relation to their formation in living organism along with its control measures and future areas of research. Most of free radicals are oxidants and they are scavenged by antioxidants compounds including vitamins and enzymes [1, 2]. The formation of free radicals in vivo is associated with the consumption of oxygen molecule [3, 4]. Reactive oxygen species such as singlet oxygen, superoxide and hydroxyl radicals are produced by routine cellular metabolism. However, their generation is increased by certain conditions of physical and psychological stress like exercise leading to intracellular oxidative stress. In fact, oxidative stress is imbalance between oxidant and antioxidant levels in living systems [5, 6].

Human cells are protected from excess production of ROS by the body's antioxidant system. This defense system consists of endogenous and exogenous compounds. Superoxide dismutase and glutathione peroxidase are two examples of endogenous enzymatic antioxidants. The exogenous defense compounds are primarily ingested from fruit and vegetables [3, 4, 7]. More importantly, there is a balance between the production level of ROS and their removal by antioxidant system. The physiological function is positively or negatively affected by this important balance and it determines the intracellular redox state of living cells [8]. Chronic exposure to excessive formation of ROS can potentially lead to a shift in the intracellular redox balance towards a more oxidant state. Consequently, oxidative damage of biomolecules, inflammation and many chronic diseases are promoted [3, 4, 7, 9]. Oxidative stress is associated with neurodegenerative diseases [10] like Alzheimer's disease [11-13] and Parkinson disease [14], cardiovascular disease [15], diabetes [16], cancer [17], chronic respiratory diseases [18], and other age-related diseases [7, 19].

Variety of oxidative stressor can cause overproduction of free radicals. There are

numerous exogenous sources of ROS, for example, environmental pollutants, smoking, alcohol, radiation, pesticide and physical activities [3, 4, 7]. However, an acute state of oxidative stress can be generated by any situation in which the consumption of molecular oxygen is increased such as exercise. Moreover, many factors affect the formation of free radical during physical activity such as intensity, duration, type of exercise and the amount of oxygen consumed. Different exercise protocols may result in variable levels of ROS generation as the oxidative stress depends on the duration and the intensity of exercise [4, 7]. The antioxidant defense system is sufficient to scavenge the excessive of ROS production during low intensity and duration exercise [20]. When the intensity and duration of physical activity increase, the oxidative damage to surrounding tissues consequently is elevated as the defense compounds are no longer adequate [4]. The degree of antioxidant defenses is also influenced by extra factors e.g. age [7, 21], training status and dietary ingestion [4, 22, 23].

2. Physical activity and oxidative stress

Regular exercise plays an essential role in human health [24, 25]. It has been well known that a regular exercise can lower the deleterious influence of the modern life style and as a consequence limit the development of chronic diseases [25-27]. It was reported that a daily moderated exercise seemed to be beneficial leading to decrease the damage in vitro in skeletal muscles. Indeed, a regular and moderate exercise should be practiced as it is associated with a lower metabolic rate, a higher activity of antioxidant defense system and thus higher protection against oxidation [28-30]. It is also associated with several physiological body adaptations. It is related to the elevated level of resistance (enhancement of antioxidant compounds) to oxidative stress which subsequently leads to health benefits and prevents the oxidative stress [27, 29, 31, 32]. The formation of ROS during exercise is accompanied with upregulation of antioxidant enzymatic compounds leading to an improvement in intracellular status of antioxidant protection system of trained athletes [33, 34]. Moreover, low to moderate level of oxidants have a beneficial role within cells such as the regulation of gene expressions and signaling pathways [35]. Importantly, to achieve this benefit from exercise the appropriate exercise in terms of intensity and frequency should be determined [29, 36].

Intensive or moderate to long exercise is associated with oxidative cellular damage as it enhances the generation of ROS [24]. ROS are mainly generated from many sources during exercise such as the mitochondrial respiratory chain [29, 33, 37]. During intense exercise the oxygen is increasingly consumed, where the muscles oxygen consumption is increased as much as 100-200 times than at rest [38-40]. This means that the mitochondrial respiration in the active muscles is noticeably increased and as a consequent this rapid respiration can enhance the electron leakage from the electron transfer chain leading to an increase in ROS formation. During or after exercise in working organs or tissues such as muscles, production of ROS is increased [38-40] leading to a disturbance in the oxidant and antioxidant balance [31]. Therefore, the level of antioxidant protection system is increased such as the increasing level of GPX and SOD [27, 28, 41-43]. The response of human organs to oxidative stress by chronic or acute exercise in vitro is different depending on the type of organ and its level of endogenous antioxidant system [44, 45].

In response to exercise, oxidative stress has been reported in numerous studies over the past 30 years as the production of reactive oxygen species is increased by exercise of

sufficient duration and intensity. Such formation of free radicals can cause damage to macromolecules involving DNA, lipids and proteins [5, 6]. This damage has been associated with numerous pathogenesis of human diseases such as cancer [46], diabetes [47], cardiovascular and neurodegenerative diseases [48]. Numerous investigations have been carried out to assess this phenomenon including running [2], cycling [41] swimming [49, 50] and ball games like volley ball [27, 51] and football [52]. On the other hand, it has been shown that only high intensity or long duration exercise can cause a remarkable increase in ROS formation which exceeds the capacity of antioxidant defense system and consequently leads to oxidative stress [53-55]. The mechanism of how the exercise causes oxidative stress has not been understood [54]. But this phenomenon does not occur with a low intensity exercise as the defense system is capable to prevent the damage by scavenging free radicals [29, 41].

3. Free radical generation mechanism

Recently, free radicals induced oxidative stress has been increasingly studied because of their importance and attribution in more than 100 physiological pathways involving cancer and aging [56, 57]. Free radicals can attack and damage all main intracellular components due to their extremely reactivity [58]. However, cells normally regulate the redox state under physiological processes and intracellular free radicals exist continuously at low levels. Free radicals influence on cells harmfully only if the rate of their formation exceeds the protection capacity of the antioxidant system [7, 59]. Normally, living cells generate most energy by consuming oxygen in mitochondria. Therefore, intercellular mitochondria is the main site of endogenous ROS production [28]. Superoxide is generated via electron transfer chain from oxygen locating at the final position of the mitochondrial respiratory chain. Dismutation reaction accelerated by SOD scavenged superoxide radical as demonstrated in the following equations [41, 60-63]:

$$2\ O_2^{\cdot -} + 2\ H^+ \xrightarrow{SOD} O_2 + H_2O_2 \quad \textbf{(1)}$$

$$2\ H_2O_2 \xrightarrow{CAT} 2\ H_2O + O_2 \quad \textbf{(2)}$$

The dismutation occurs very slowly without SOD. At rest, major superoxide radical is reduced by Mn-SOD in mitochondria and the diffused part to cytosol by Cu, Zn-SOD [41]. Hydrogen peroxide formed in the above equation is further converted to water by catalase or GPX [41, 62, 64]. GPX is efficient with high level of ROS [35]. The formation of deleterious free radicals is strongly related to the contribution of free active redox metals such as Fe and Cu [61, 63]. In fact, these redox metals can participate in Fenton reaction producing the extremely destructive hydroxyl radical. Equations summarize how an active redox metal takes part in generation of reactive species can be expressed as follows [62, 64, 65]:

$$M^{n+} + H_2O_2 \longrightarrow M^{(n+1)+} + OH^- + \cdot OH$$
(Fenton reaction) **(3)**

$$M^{(n+1)+} + O_2^{\cdot -} \longrightarrow M^{n+} + O_2 \quad \textbf{(4)}$$

The balance of Haber-Weiss reaction can be expressed by the following equation [62, 64, 65]:

$$H_2O_2 + O_2^{\cdot -} \longrightarrow O_2 + OH^- + \cdot OH \quad \textbf{(5)}$$

Hydrogen peroxide is also considered most suitable for redox signaling due to its physiochemical properties. It is functioning as a messenger to transmit redox signals from the site of its production to a target site. It diffuses across cells' membrane to immediately initiate cellular effects such as proliferation and recruitment of immune cells. It is capable of functioning as a fundamental regulatory metabolite

depending on its concentration ranges in oxidative stress and cellular responses. For more details with respect to the metabolic sources and sinks of H_2O_2 as well as its role in redox signaling see [66, 67] for recent reviews.

Free radicals can be removed by numerous metalloenzymes, for example, catalase, glutathione peroxidase and superoxide dismutase. In addition, they can be eliminated by non-enzymatic antioxidants such as metallothionein, uric acid, ascorbic acid and glutathione [68, 69]. Antioxidants can accept or donate electron to the highly reactive species to neutralize them. They function in three ways: decrease their energy, prevent their formation or stop the oxidation reaction chain [70]. The dismutation of superoxide free radicals is catalyzed by enzymatic antioxidant SOD. SOD is the most essential metalloenzymes as it is in the first protection line against the oxidative damage to macromolecules [71]. Many analytical techniques have been developed [68] because of their great significance and increasing concern on their deleterious oxidative damage. In several applications, separation technique specifically coupled with atomic spectrometric detector has been involved in the speciation analysis where different chemical forms of an analyte existing in the sample can be measured [72].

4. Metal (Fe, Cu, Zn and Mn) induced free radicals' production

A series of transition metals e.g. Fe, Cu, Zn and Mn plays a central role in controlling many metabolic and signaling pathways [73]. These metal ions affect the normal functioning of the cell and can escape from controlling and bind to other cell components because of their rich coordination capability and redox properties [73, 74]. These metal ions can bind with proteins and DNA at specific sites or displace other existing binding elements leading to oxidative damage which eventually result in toxicity [74]. Oxidative stress is also involved in this toxicity oxidative stress is involved. Metals such as Fe and Cu are capable to produce reactive species and consequently cause oxidative damage in humans as illustrated in Figure 1 [73, 74].

Despite the importance of metals in metabolic pathways in living organisms, they are highly toxic if they are present in excess such as iron [75]. The fact that, in many biological processes such as oxygen transportation, Fe plays a basal role where high level of iron causes chronic diseases like cancer [56]. Most diseases are also linked to Cu, Fe and Zn as initiated by mutations in the genes encoding for proteins that are responsible for metal transportation [76]. Thus, living cells have developed mechanisms to regulate the available amount of metals [56]. In metal containing enzymes, metals are isolated and protected from other surrounding cellular constituents to avoid their deleterious effect. Thus, their tight binding to proteins can reduce their toxicity. Conversely, free metal ions or loosely bound to DNA, proteins or lipids are very harmful [56]. Many pathways have been developed by cells to regulate metal homeostasis but under stressed conditions, miss-maintenance can increase the level of free redox metals associated with oxidative stress as demonstrated below [77, 78].

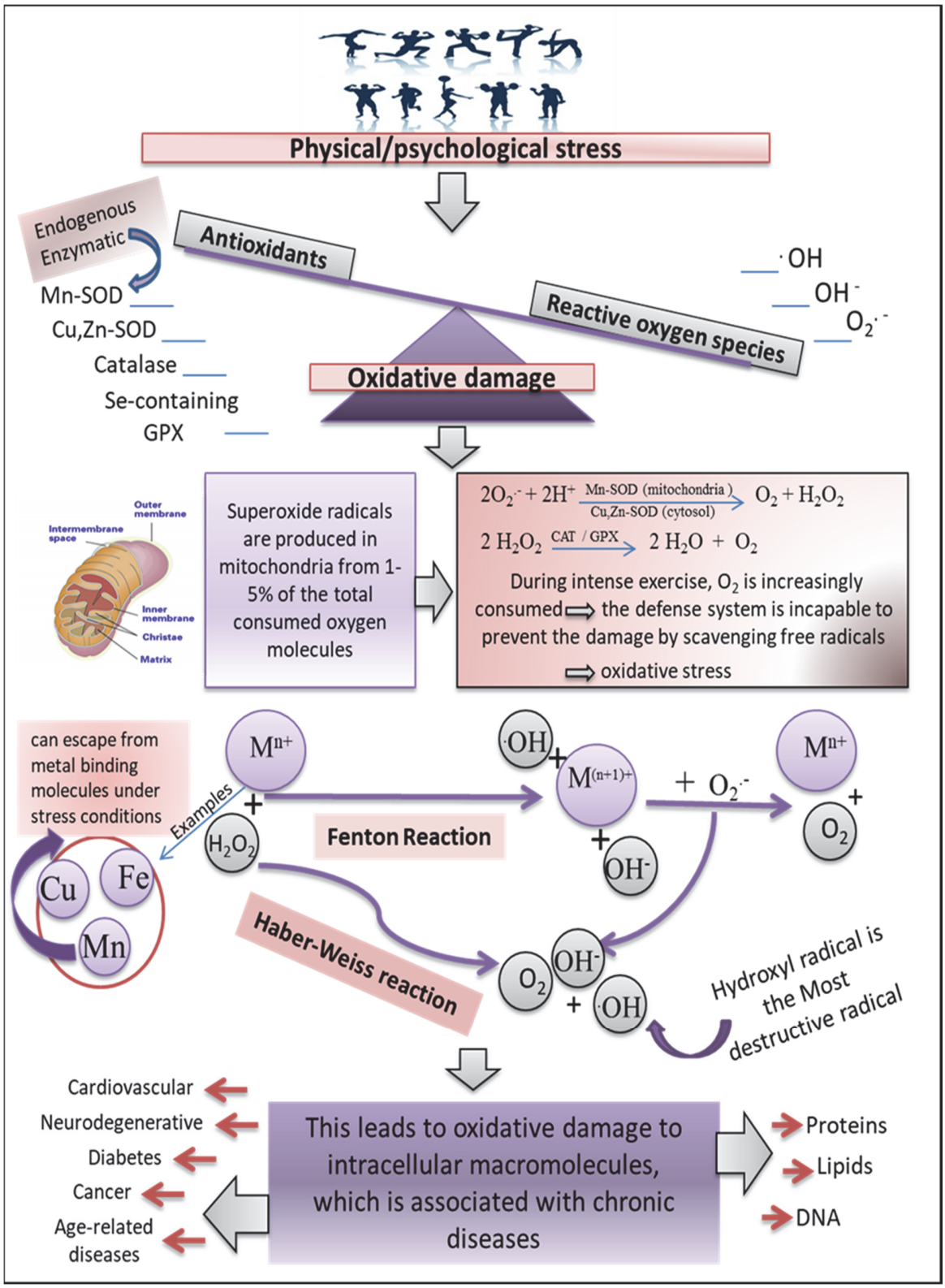

Figure 1: How oxidative stress phenomenon occurs in human cells and its influences on human health [79].

i. Zinc

Human body contains about 1.5-2.5 g of Zn distributed everywhere within the body and 90% of this total amount is contained in skeletal muscles and bones. The concentration of free intracellular Zn is 0.5 nM [74]. Zinc is not considered as an active redox metal under physiological conditions and is an important cofactor for several enzymes and proteins [80]. It has a different function from others cations as it protects intracellular components from the oxidative stress. It is able to displace the other redox active metals from a particular site where the damage occurs to minimize the oxidative stress [56]. For example, it has been revealed that zinc can inhibit the toxic effect of Cu by displacing it from its binding sites [81]. Moreover, zinc can protect DNA from iron induced damage by competing for iron binding sites [56].

Two mechanisms have been proposed for intervention in redox metal induced oxidative stress. The first one is called pull mechanism which implicates the use of particular metal ligands to remove the redox active metals from their binding sites. Push mechanism is the second mechanism involving pushing out the active redox metal from its binding sites by another inactive redox metal like zinc [56]. The removal redox metal can then be pumped out of the cells minimizing its bioavailability to contribute in Fenton reaction and produce hydroxyl radical [74].

Significantly, zinc deficiency can lead to oxidative stress and subsequently cause damage to lipids, proteins and DNA. Inadequate Zn intake may associate with diseases like cancer as it is required in catalytic and structural role within proteins and enzymes like Cu-Zn, SOD. The clear relation between zinc deficiency and diseases is its role as antioxidant. It is believed that zinc indirectly acts as antioxidant as does not contribute to donation or acceptance of electron from oxidants. However, zinc involves in indirect mechanisms providing it with antioxidant properties [80, 82]. Moreover, zinc deficiency minimizes the growth of cultured cells [83]. Cultured cells grown in low Zn containing media are associated with high level of oxidative stress [74].

In contrast, excess amount of such inactive element is linked to Alzheimer disease and chronic kidney disease. Furthermore, the level of iron and copper are subsequently affected [83]. However, the elevated intracellular level of Zn must be prevented as it is a toxic. Zinc homeostasis is strongly regulated to avoid the accumulation of free zinc ions in cells. Free zinc ions are pumped out the cell into particular storage proteins or bound tightly to metallothionein. Metallothionein is capable to bind with 5-7g of Zinc tightly and under high oxidative stress condition Zn is released from this metalloprotein [74].

ii. Copper

Copper plays an essential role as a cofactor in many cellular processes [56], for example, respiration, iron metabolism and connective tissue formation [74]. Human body contains 80 mg of copper. It has a toxic effect if it exists in higher level associated with diseases. Copper-containing functional enzymes require copper such as superoxide dismutase and caeruloplasmine. Similarly to iron, copper is capable to catalyze the production of hydroxyl radical via Haber-Weiss reaction as the following equations show [56]. Cupric ions can be reduced by biological molecules such as ascorbic acid or GSH forming cuprous ions which can react with hydrogen peroxide and produce a destructive hydroxyl radical via Fenton reaction [60, 74, 81].

$$Cu^{1+} + H_2O_2 \longrightarrow Cu^{2+} + OH^- + {\cdot}OH$$

Fenton reaction **(6)**

$$Cu^{2+} + O_2{}^{\cdot -} \longrightarrow Cu^{1+} + O_2 \quad \textbf{(7)}$$

$$H_2O_2 + O_2^{\cdot-} \longrightarrow O_2 + OH^- + {\cdot}OH \quad \textbf{(8)}$$

Hydroxyl free radical is a destructive species and can attack intracellular DNA and cause damage [77]. DNA damage resulting from Cu and hydrogen peroxide can be inhibited by glutathione (GSH). Copper is also capable to oxidize thiol groups and convert them to thiyl radicals [56].

$$RSH + Cu^{2+} \longrightarrow RS^{\cdot} + Cu^{+} + H^{+} \quad \textbf{(9)}$$

Copper and iron are very important metals in living cells as the normal physiological process require a specific level of them. Therefore, the metals homeostasis is controlled within the cells [77]. Copper homeostasis is controlled by copper distribution through the body after it is absorbed in the digestive tract and then mainly excreted into the bile [84]. A constant transit of Cu between low molecular weight pool, functional proteins containing Cu and its storage sites must be maintained [56]. In the blood , Cu is transported to bound with albumin after absorbed from small intestine as cupric form whereas Cu taken up by the liver and stored within hepatocytes is bound to metallothionein [81]. Metallochaperones are required to maintain Cu homeostasis by its safely transporting to particular proteins receptors preventing damage to the cells.

Copper regulation can be disrupted by oxidative stress resulted from excessive amount of free active metals as demonstrated in Figure 1 [77]. Any disruption in this controlled equilibrium in terms of metal distribution and storage can lead to intense imbalance within humans [83]. Importantly, high concentration of copper and iron is related to hemochromatosis, anemia, diabetes, cancer, cardiovascular, Wilson's, Alzheimer's [61, 77] and Parkinson's diseases [61, 81]. Accumulation of copper, iron and zinc in human brain is linked to Alzheimer pathogenesis [81, 83]. Copper accumulation in different tissues is related to Wilson disease [84]. Similarly, the copper deficiency containing enzymes is linked to many diseases. It has been shown that copper deficiency enhances the susceptibility of cell to oxidative stress. This was explained by the fact in which copper deficiency can decrease the cell capability to produce the antioxidant enzyme SOD [60, 74].

iii. Iron

It is one of the most important transition redox metals that have been widely studied. Its amount within human body is 3-4 g. The remaining concentration of iron is normally stored in ferritin within the cell or transferrin transports it as ferric ions [56]. Ferritin is a storage protein in which 4500 iron atoms per a molecule binding weakly with high capacity. On contrary, transferrin is iron transporter protein with high affinity but low capacity of 2 iron atoms in a molecule. It is an essential protein circulating iron through plasma and contributes to iron homeostasis. Liver is the main site for iron storage. Generally, ferritin releases iron when is required to participate in iron containing proteins. Therefore, free active iron is very low as it constantly transports between ferritin (intracellular pool) and functional proteins. In mammals, iron homeostasis must be regulated to prevent the potential health disturbances [74]. On the other hand, the concentration of active redox iron is increased under physiological situation. Ferric ions can be reduced to ferrous in ferritin by superoxide anion and other free radicals which act as electron donors, therefore, iron is released from ferritin. Hydrogen peroxide and other lipid peroxides can release Fe from iron containing proteins by oxidizing it in contrast to donor species. Hence, to prevent metal release induced free radicals' generation, cells regulate the level of superoxide anion and peroxides [56, 60]. The reaction which illustrates the formation of

free radicals can be expressed by Haber-Weiss reaction:

$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + \cdot OH$ (Fenton reaction) **(10)**

$Fe^{3+} + O_2^{\cdot -} \longrightarrow Fe^{2+} + O_2$ **(11)**

The overall reaction can be expressed by the following Haber-Weiss reaction.

$H_2O_2 + O_2^{\cdot -} \longrightarrow O_2 + OH^- + \cdot OH$ **(12)**

Moreover, it is believed that iron takes part in cancer disease as it catalyzes the formation of free radicals which consequently involve in tumor formation [10, 56, 62]. Other redox metals such as Cu and Mn can catalyze such kind of reaction and generate highly reactive hydroxyl radical. Fenton reaction is believed to contribute in free radicals' production in vivo as a result of toxic influence of metals [10, 56].

Iron overload is associated with significant alteration in cell structure and function resulting in free radical mediated damage. Primarily, iron must be shielded from oxygen and surrounding components to prevent iron induced oxidative stress. Free active iron can participate in one electron transfer chains causing destructive free radical production [74]. Under aerobic condition, Fe^{2+} ions can react with molecular oxygen producing superoxide radical which is not very reactive and cannot attack DNA. In a reaction catalyzed by SOD, two superoxide radicals can react together forming hydrogen peroxide which is capable to react further with Fe^{2+}. Oxidation of biomolecules like ascorbate is initiated by trace level of transition metals like Fe^{3+} which is subsequently reduced to Fe^{2+}. Hence, the redox state of cell is significantly related to iron and strictly maintained in order to prevent the formation of free intracellular iron. It has been reported that under stress condition excessive level of superoxide can release iron from iron binding molecules like ferritin [74].

iv. Manganese

Mn is an important biological element involved in many cellular metabolisms [85]. It has an essential role in cellular adaptation to oxidative stress due to its high reduction potential. This metal can safely act as a cofactor for superoxide dismutase enzymes. Manganese containing antioxidants can effectively protect proteins from oxidative damage. Moreover, manganese can increase the cellular resistance against oxidative stress by substituting as a cofactor for iron in particular enzymes subjected to oxidative damage [86, 87]. However, high level of this element is associated with pathogenesis of a deleterious disease which causes damage to lung and the central nervous system [85].

5. How hypoxia/hyperoxia affect muscle cells

In vitro, cells are commonly grown at 21% O_2 (normoxia conditions) which is the same concentration in air we breathe. The levels of oxygen within our tissues are much lower than its concentration in the atmosphere and they vary deeper in tissues depending on the distance from arterial blood vessels [88]. In vitro, this standard condition of 21% O_2 are considered as hyperoxic [89, 90] because oxygen pressure in human skeletal muscles is about 30-40 mmHg under normoxia (21% O_2) and this approximately equals to 5% O_2 in the cell culture. Accordingly, human skeletal muscle cells should be grown at about 5% O_2 to achieve the physiological normoxic conditions and thus oxygen levels below 5% creates hypoxic conditions [90]. It has been demonstrated that low oxygen culturing environment (hypoxia) increased the proliferation of cells in culture and increased their lifespan by 25% compared to those grown under normal conditions as shown in Figures 2 & 3 [79]. Cells in reduced

oxygen level grow faster and healthier with less DNA damage and less stress response [88]. Under normal and hypoxia conditions, dramatic variations in gene and phenotypic changes in cells cultured [91].

Interestingly, a study was designed to assess the effects of hypoxia (1 and 3% oxygen) on rat smooth muscle cell growth in vitro and compare between them and normoxia (21% O_2). The rate of proliferation of cells exposed to 3% O_2 for 72 hours increased by 42.2% compared to normoxia. On the contrary, cells exposed to 1% O_2 for 72 hours showed a decrease in their proliferation by 21%. This study reported that only the rate of smooth muscle cells proliferation was affected by severe hypoxia (1%) and accordingly decreased without any impact on the cells viability [92]. Additionally, other investigations have revealed that many types of cells proliferate in response to hypoxia, with a maximal response at 3% O_2 [92]. Similarly, another group investigating the proliferation of rat smooth muscle cells under 40 and 70% O_2 (hyperoxia) reported that the proliferation was inhibited by these two oxygen concentrations compared to the normoxic level of 21% [93].

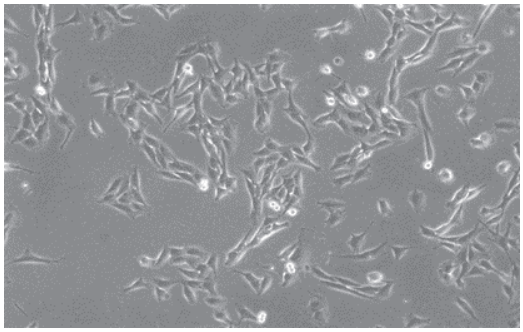

Figure 2: Muscle cells (C2C12) incubated under normal conditions of 21% O_2 and 5% CO_2 for 24 hours [79].

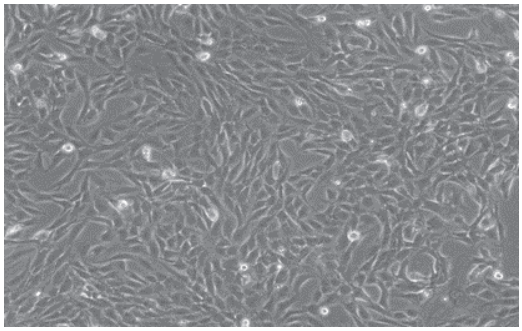

Figure 3: Muscle cells (C2C12) incubated under 5% O_2 (hypoxia conditions) and 5% CO_2 for 24 hours [79].

In vivo, the hypoxia state is originally generated by environmental conditions (high altitude or exercise) or pathological conditions (pulmonary disease or severe anemia) [89]. Oxygen concentration in vivo can range from 1-13% [91]. Hypoxia can occur during exercise and skeletal muscle cells can adapt to deal with this condition of low oxygen availability [89]. It has been reported that hyperoxia (high oxygen availability) can also occur during severe-intensity exercise in humans and affect the muscle metabolic responses [94]. This state of low/high oxygen availability can be either chronic (long lasting for several days) or acute (for several hours). It has been demonstrated that chronic hypoxia results in a negative regulation of protein metabolism and a loss of muscle mass. Other investigations have reported that acute hypoxia enhances the protein balance, between protein synthesis and protein breakdown, in humans and exerts a positive effect on muscle growth. This means that the two kinds of hypoxia regulate the skeletal muscle mass in two different ways. Definitely, if the acute hypoxia exposure improves protein balance within cells, the moderate exercise performed in conditions of low oxygen availability could be extremely useful. However, further investigations are needed to confirm this hypothesis [89].

HIF-1α can help cells to endure this reduced oxygen availability condition. However, in vitro, hypoxia stimulates HIF-1α

more consistently than in-vivo and this is probably due to the high level of hypoxia applied in cell cultures (0-2% O_2). In fact, it has not been confirmed whether HIF-1α is directly responsible for the reduction in protein synthesis detected in vitro [89, 90]. More importantly, mitochondria, where molecular oxygen is consumed, may be assumed to function under even reduced oxygen tensions than these average values in tissues. Consequently, the comparisons of hyperoxic impacts in vitro and in vivo are difficult particularly if quantitative investigations are performed [89].

6. Current biomarkers of exercise-induced oxidative stress

The oxidative damage can be measured by biological biomarkers in which their structures are harmfully affected by ROS and accordingly changed. Recently, analytical assays used to assess the intracellular oxidative damage have been rapidly developed. Such methods rely on relatively stable biological compounds as follows [35, 48]. Representative current biomarkers can be summarized in more details below.

6.1. Measurement of free radicals

Free radicals can negatively effect on biomolecules such as proteins, lipids and DNA. Reactive oxygen species have a short half-life resulting from their highly reactive nature (less than 1 ns). The application of spectroscopic method is not the most suitable on humans as the materials used in this process are toxic. The measurement is performed by processing blood samples and finally the serum is analyzed spectroscopically [19, 23, 32, 68, 95].

6.2. Measurement of oxidative damage on lipids, proteins and DNA modification

6.2.1. Lipids peroxidation

Oxidative stress can be examined by measuring the level of lipid peroxidation in the cell membrane. Lipids molecules decompose into primary oxidative products such as conjugated dienes [23, 96] and secondary products such as malondialdehyde (MDA) [1, 19, 23, 24, 26, 48, 97] and F_2-isoprostane [98]. It has been thought that the high level of MDA in many diseases is linked to free radical species damage. Thus, this biomarker has been widely measured to evaluate the lipid peroxidation in humans. Unfortunately, there is a limitation in using MDA as a biomarker because it is formed in foods and then absorbed by gastrointestinal tract resulting in changing of the background value in vivo [48]. Malondialdehyde is frequently quantified by its reaction with thiobarbituric acid and using spectrophotometrically at 532 nm [23, 33, 48, 68, 96]. However, this method is imprecise as thiobarbituric acid can react with many other substances such as amino acids and carbohydrates leading to errors in measurement. Additionally, there is another source of variability which is due to the potential oxidation of the acid during the sample handling [48]. Alternatively, Many chromatographic techniques e.g. GC-MS [99], HPLC-UV/VIS [100] and HPCE [101] have been developed to measure MDA.

Recently, F_2-isoprostanes serve as a reliable method to quantify oxidative damage on lipids [19, 48, 68, 102-111]. Isoprostanes $F_{2\alpha}$ are 64 isomers compounds to cyclooxygenase-derived $PGF_{2\alpha}$ and they represent the most particular biomarkers of lipid peroxidation in numerous biological samples, e.g. blood, plasma, urine, exhaled breath condensate, bile, seminal fluid and tissues [48, 106]. Importantly, many diseases are associated with the high level of such compounds in urine and serum. Thus, their discovery is beneficial for humans as oxidative stress

status in vivo. They can be reliably assessed. However, these biomarkers are the most complicated to be measured. Analytical assays of MDA and IsoPs require intensive procedures involving sample preparation in terms of purification and extraction [106]. In addition, some considerations should be taken during sample preparation to avoid interferences in IsoPs analysis. Plasma and tissue samples need immediate freezing at -80^{0}C to avoid the autooxidation of samples but urine is more stable as the concentration of IsoPs remains unchanging at room temperature for many days or at -20^{0}C for six months [48]. Recently, several methods have been developed for determination of IsoPs such as gas or liquid chromatography linked to mass spectrometry [106, 112, 113]. Moreover, the analysis of hexane, ethane and pentane in exhaled air is a non-invasive method to measure oxidative stress on lipids. The three alkanes can be produced from another source rather than oxidation and subsequently this method is insufficiently precise [21].

6.2.2. Proteins modification

Oxidative damage on proteins results in generation of carbonyl groups at spots in chains. Thus, the increase in number of carbonyl groups is a result of oxidative stress. Determination of carbonyl groups most widely used for measuring the oxidative damage on proteins [1, 19, 23, 24, 48, 68]. Proteins lose their functional and structural efficiency as a result of oxidative damage. The importance of measuring carbonyl groups as a biomarker is their elevated stability and relatively early production during oxidative stress [48]. Many techniques have been developed to analyse carbonyl groups, for example, spectrophotometric [114] and enzyme-linked immunosorbent assay (ELISA) methods [48]. Assays of protein carbonyls require intensive sample preparations and the samples should be carefully handled and stored [48].

6.2.3. DNA modifications

There are many kinds of DNA modifications resulted from the attack of free radicals to DNA molecules such as protein bonds damage and chain breaks. Many methods have been carried out to determine this damage. The quantification of nucleotide 8-hydroxy-2-deoxyguanosine (8-OHdG), which is the product of guanine oxidation, as a biomarker is the most frequently method to measure DNA modification induced by free radicals [19, 23, 24, 48, 68, 97, 115-124]. The DNA damage is frequently caused by hydroxyl radical. This damage is repaired by specific enzymes and bases are directly expelled into urine. Several analytical methods have been developed for measuring DNA oxidative damage including GC-MS and HPLC with electrochemical detection [19, 48, 122]. However, there is a variable in the background level of 8-OHdG in samples and this variability relies on the technique applied. It is very important to decrease the artifactual oxidation of the base during DNA extraction and derivatisation in order to avoid overestimated baseline level induced by the damage [19, 48].

6.3. Measurement of antioxidants

The activity of enzymatic antioxidants, for example, superoxide dismutase [23, 26, 33, 70, 96, 125-127], catalase [1, 23, 70, 128, 129] and glutathione peroxidase [23, 70, 96], has been widely quantified in many investigations. This method is used to evaluate the antioxidant system at rest as well as after the physical activity [23]. There have been many continuously oxidizing agents the living cells are exposed to. Thus, human antioxidant system possesses the capability to minimize the effect of such agents. The concentration of these antioxidant compounds relies on their formation and consumption during oxidative stress [126].

There are three isomers of superoxide dismutase enzyme. Mn-SOD is located in

mitochondrial matrix and Cu, Zn-SOD located in the cytosol and the mitochondrial intermembrane space. Copper and zinc are also required for the third type of SOD which is located in extracellular space [35]. Specifically, in skeletal muscle, 15–35% of the total SOD activity within the mitochondria, and the remaining 65–85% is in the cytosol [35]. It is essential to measure SOD in human tissues particularly patients with diseases promoted from insufficient quality of antioxidants. The deficiency of this enzyme can induce the accumulation of superoxide radical [126]. Cu, Zn-SOD catalyzes the conversion of superoxide radical into oxygen and hydrogen peroxide radicals, which are decayed by catalase and glutathione peroxidase. Copper ion has the role to dismutase the radical whereas zinc ion is responsible for the structural function [127]. Indeed, quantification of SOD can reveal the amount of free radicals formed. Numerous spectrophotometric methods [130] and electrochemical techniques [126, 131] have been reported for measuring this antioxidant enzyme [126, 132, 133]. Unfortunately, some studies have reported that endurance exercise does not affect or increase the activity of SOD in muscle. In contrast, most researchers have indicated that the activity of SOD has been increased (20-122%) by endurance exercise in muscles. This variation in findings is due to the sensitivity of the techniques used to measure the differences in SOD activity [35].

It has been identified that there are five kinds of selenium containing GPX enzymes. Their function is to accelerate the reduction of hydrogen peroxide to water by converting the reduced form of glutathione (GSH) to the oxidised form of glutathione disulfide (GSSG). It is very important antioxidant compound against ROS damage to intracellular macromolecules. Identical to SOD, numerous studies have demonstrated that endurance exercise can lead to increase the activity of GPX in muscles by 20-177% and this increase depends on the intensity and duration of exercise [35].

Thiol protein GSH and its oxidised form GSSH have been used to measure the oxidative stress as GSH is the most essential protein in humans [23, 24, 31, 128, 134]. It has a significant ability to protect the cell against oxidative stress and maintain its redox state by trapping radicals and minimize the formation of peroxides [74]. Glutathione has a significant role in intracellular copper metabolism and suppression of copper toxicity. It directly chelates copper and keeping it away from participating in redox cycle [60].

Non-enzymatic antioxidant vitamins such as A, C and E have been commonly quantified in order to assess the quality of antioxidant defense system and vitamins deficiency [41]. Similarly to enzymatic antioxidant, the amount of antioxidant vitamins is varied as a result of oxidative stress. This change in the concentration of vitamins can be subsequently used as indirect biomarker to assess the oxidative damage [23]. For example, it has been shown that vitamin E can defend against copper that causes oxidative stress [60, 61, 81]. Also, Uric acid, which is non-enzymatic antioxidant [135], can be determined to examine the oxidation but it lacks of reliability. Finally, the total antioxidant capacity (TAC) has been quantified in many studies to evaluate all antioxidants compounds [23, 70, 128, 135].

6.4. Redox elements in muscle cells as oxidative stress biomarkers

Unfortunately, none of those biomarkers mentioned above is sufficient to discover the potential role of oxidative stress involved in pathogenic mechanism of human diseases [13, 35, 48]. There are no ideal biomarkers and optimal methods but some biomarkers are more reliable and stable during the analysis than others [48]. Oxidative stress biomarkers

were also considered to be too variable and highly unspecific for utilization in medical diagnostics of a specific disease [17]. They should also maintain their composition on storage and not be lost during sample preparation. Nevertheless, there might be a variable response of the biomarkers to different macromolecules between humans and even in the same subject. Additionally, the biomarkers may not represent the extent of this oxidative damage. Consequently, the selection of appropriate biomarkers should depend on the study aim. Careful consideration is necessary for even the most developed biomarkers prior to applying them to human research [48, 136]. Surprisingly, some investigations have failed to prove exercise induced oxidative stress [137]. It has been suggested that there might be several reasons for these findings. For example, the variability in subjects; their gender, age and physical condition may all influence study results. Furthermore, a wide variety of exercise protocols (type, intensity and duration) have been used resulting in variation in results [55]. Several biomarkers' level used in the studies may not change and reach their maximum magnitude during or immediately after exercise. They may need hours or days to rise to a noticeable level to be measured [54]. Detailed studies have been carried out on trained endurance athletes who are adapted to exercise influence [41].

This variation in detection may explain the contradictory results within the literature [4]. The results have been conflicting regarding the influence of exercise on selected biomarkers [52, 138]. In addition, analytical methods applied for their measurement should be chemically robust, repeatable and with high sensitivity and low detection limit to compare between reference and changed values in living organisms [110]. Thus, a reliable biomarker, an accurate method and a sensitive technique are required to measure oxidative stress in human cells. Biological elements like Zn, Cu, Mn and Fe at trace levels can be used as oxidative stress markers due to their vital role as cofactors of the antioxidant enzymes. Cu, Zn-superoxide dismutase and Fe catalase are two examples of the most important enzymatic antioxidants that protect the human cells against the intracellular oxidative stress. Their level relies on their formation and consumption during oxidative stress resulting in an increase/decrease in their corresponding cofactors' level [126].

Numerous analytical techniques have been developed for qualitative and quantitative study of metals in biological systems (Table 1) [139-147].

Table 1: Qualitative and quantitative analytical techniques employed for trace determination of biological elements*

Method	Advantages	Disadvantages
FAAS	• high sensitivity • inexpensive • rapid • does not require high level of operator skill • range value of the measurement (50 ppb-500 ppm) [139]	• not simultaneous • different cathode lamp for different elements • measures only metals and some non-metals • low precision (0.1-1%) • only for liquid samples • requires big sample quantity • issue with refractory elements • interferences from matrix [139]
ICP-MS	• capability to measure isotopes • detection limits (0.01-0.1 µg/L for many elements • simple sample preparation • high throughput • ability to measure many elements simultaneously [83, 139, 140]	• strong digestion of the sample is needed prior to analysis which may result in metal contamination • relatively large sample quantity • influences of dissolved solids/matrix (need to dilute samples) [83, 139, 140]
ETAAS	• very small sample volume (as low as 0.5µL) • low spectral interferences because of elevated temperature • low detection limit (<pg and ng) • high precision and good accuracy • for quantitative and qualitative analyses [83, 139, 141]	• depends on the sample mass, the instrument calibration and the sample homogeneity • expensive • low precision for few elements • low sample throughput • high interferences from matrix • not easy to use, needs a skillful operator [83, 139, 141]
TXRF	• simultaneous measurement • detection limit is pg/g with pre-concentrated samples • for quantitative and qualitative studies • surface sensitive multielement analysis [142, 143]	• strong digestion of mammalian cells sample is needed before analysis which leads to sample manipulation resulting in possible metal contamination or loss of the analyte during evaporation [83] • matrix interferences [142]
LA-ICP-MS	• direct elemental analysis of solid samples • very low detection limit >0.1 ppm	• elemental fractionation • non-stoichiometric effects because of preferable ablation of more volatile compounds

	• linear dynamic range of up to 10 orders of magnitude • enabling microanalysis, depth profiling analysis, and 2-dimensional elemental mapping • simple sample preparation • high sample throughput • capability of isotopes measurement • ability to analyze many types of materials, conductive and non-conductive and opaque and transparent [139, 144]	• differences in gravitational settling between smaller and larger particles when they are transported from the ablation chamber into ICP • evaporation, atomisation and ionisation in ICP (less effective for larger particles) • matrix interferences • lack of standard reference materials • interaction differences between the laser beam and sample surface [139, 144]
PIXE	• high sensitivity and spatial resolution • Low detection limit (usually μ/g) • quantitative analysis • simultaneous trace elements analysis • a very low background signal due to great ionization cross sections [144] • metal speciation technique without the use of separation methods [83]	• poor depth resolution • it is a surface technique • inter-element interference • determination of light elements with low X-ray energies is difficult because of X-ray attenuation in thick samples • inaccurate estimation of concentration due to the volatility of some elements by boiling [145]
ICP-AES	• multi-elemental analysis • rapid • detection limit (1 ppb-1000 ppm) • good linear dynamic range (10^6-10^8) [139, 146]	• relatively poor detection limits • interferences • high sample volume (1-5 mL) • samples pretreatment is required [139, 146]
SXFM	• high sensitivity • for elemental bioimaging with submicron resolution • nondestructive technique • visualization of the metal ion distribution in biological samples (chemical elements speciation) [144, 147]	• lack of standard reference materials • expensive due to the application of X-ray • exposure to X-ray radiation • requires to preserve samples by flash freezing and freeze-drying [144]

*FAAS: Flame atomic absorption spectrometry, ICP-MS: Inductively coupled plasma-mass spectrometry, ETAAS: Electrothermal atomic absorption spectrometry, TXRF: Total reflection X-ray fluorescence, LA-ICP-MS: Laser ablation-

inductively coupled plasma-mass spectrometry, PIXE: Particle-induced X-ray emission, ICP-AES: Inductively coupled plasma-atomic emission spectroscopy, SXFM: Synchrotron X-ray fluorescence microscopy.

A precise and reproducible quantitative analysis of some biological elements like Zn, Cu and Fe in mammalian cells in culture or from tissues is very important in order to determine the effect of such elements on physiological process and pathology of diseases. Therefore, an accurate method and a sensitive technique must be selected for a successful biological study [83]. However, many factors should be considered before selecting the appropriate method such as timing, accuracy and the cost. In addition, the intensive manipulation of a sample analysed for trace metals is a key issue in elemental analysis resulting in contamination throughout the digestion and dilution of the sample. Metal determination process can be also performed via sub-cellular fractionation of the cell contents in which cross contamination may occur. Elemental analysis cannot provide accurate results without appropriate sample preparation procedures. Separation methods utilized in some metal speciation work can result in loss of metals of interest during sample treatments steps [79].

By considering all factors mentioned above, correct analytical techniques must be selected prior to the study in order to obtain accurate data. Currently, the most common analytical methods published so far in the literature are ICP-MS (Inductively coupled plasma-mass spectrometry), ETAAS (Atomic absorption spectrometry with electrothermal atomization) and TXRF (total reflection X-ray fluorescence) due to their high sensitivity and low sample amount requirements. It is worth mentioning, atomic absorption spectrometry with flame ionization (FAAS) is also widely used for elemental analysis. However, it requires a relatively big quantity of analyte compared to the available amount of biological samples. It has also limited sensitivity due to a limited sampling efficiency (short residence time of analyte in optical path) [79]. The beneficial properties of ICP-MS open the doors to use this powerful technique to measure the most important biological elements. However, at worst, some metallocompounds are difficult to be measured due to the weakness of metal coordination with biomolecules which leads to loss of such metals during analysis. At best, some other elements like sulfur and phosphorous which are covalently bound to proteins allow the analysis to be safely performed without releasing such bioelements [148]. However, such elements are not efficiently ionized in ICP due to their high ionization energy [149] and in any case, would not provide a useful measure of anti-oxidant enzymes.

7. Limitations with possible solutions in the analysis of biological elements as oxidative stress biomarkers in biological samples

The variation in findings of oxidative stress measurements in literature is due to the sensitivity of the techniques used to measure biomarkers of interest. As discussed, the quality of trace and sub levels determination and chemical speciation of biological elements in biological samples is crucial. Limitations with possible solutions in the analysis of such biological elements as oxidative stress biomarkers in biological samples can be summarized as follows:

i. Biological elements are routinely detected using ICP-MS which requires centralized laboratories, trained personnel and are considerably costly. Thus, in this scenario fast and robust methods of BAs analysis will be required for future regulations in fermented products.

ii. The complex matrix of biological samples for quantifying biological elements has been the main barrier in its analysis. Sample clean-up or sample pre-concentration steps are used commonly; however, matrix effect is also addressed by allowing the quantification of ultra-traces of biological elements by the use of internal standards.
iii. Moreover, these analytical techniques cannot be carried out on site, and there is a considerable time-lag between sample procurement and carrying out the analysis and relaying the result, which is incompatible with a timely intervention. Alternative methods that facilitate rapid, reliable, cost-effective, and easy-to-use devices for use at the point-of-need are urgently required.
iv. On the other hand, considerable interest has been witnessed in extraction procedures such as DLLME, its automation, and mixed mode coupling with other pretreatment steps to more reliability deal with the desired target. It has been extensively applied in various industrial, food, and clinical applications.
v. An automated green preconcentration method for biological elements in future will serve as a standard in biological samples and will serve as a milestone in the development of an ideal green analytical method for the target elements in complex matrices. Similarly, an excellent ion-pair-assisted extraction combined with spectrochemical and/or voltammetric at surface modified electrode as alternative tools for the determination of target elements in biological samples. There are relatively no DLLME studies reported for biological samples in complex combined with spectrofluorimetry and stripping voltammetry.
vi. The absence of electrochemical techniques for analysis of target biological elements attributes to the feasibility of the electron transfer in their structures. Surface-modified electrodes in stripping voltammetry are of great interest to be used in combination with DLLME and other microextraction techniques for the analysis of target biological elements. Research in developing novel methods for analysis of biological elements is an emerging area of research as most of the methods either suffer interference issues or they were complicated and expensive.

8. Conclusion and future prospective

The present review covers recent information's regarding chronic exposure to excessive formation of ROS. ROS can potentially lead to a shift in the intracellular redox balance towards a more oxidant state which is accompanied with upregulation of antioxidant enzymatic compounds which are no longer adequate to scavenge the excessive ROS production. The review also discusses the increase in ROS generation by certain conditions of physical and psychological stress like an intensive exercise. Metals such as Fe and Cu are capable to produce reactive species by escaping from controlling and bind to other cell components resulting in toxicity associated with oxidative stress. Consequently, oxidative damage of biomolecules, inflammation and many chronic diseases can be promoted. Due to the importance of the intracellular oxidative stress and the increasing concern on its deleterious damage, several different biomarkers and many analytical techniques have been used to measure the oxidative damage. None of these biomarkers is sufficient to discover the potential role of oxidative stress involved in pathogenic mechanism of human diseases. It is highly valuable to identify an ideal biomarker as well as an optimal method to assess oxidative

stress. It greatly helps in predicting the progression of diseases and the therapy required to prevent the damage. Therefore, the availability of a reliable non-invasive biomarker to measure oxidative stress is the ultimate challenge in the biological field. Further investigations in vitro and vivo are of prime importance to fully study the oxidative damage within biological environments. Separation techniques coupled with ICP-MS can also be employed for quantitative/qualitative elemental analysis. In vivo, bioimaging/mapping of metallic elements in biological samples can be quantitatively used for the study mechanisms of many diseases and physiological processes. To achieve such goals, the overall hypothesis and future work most likely will be focused on:

i. Coupling the dispersive liquid -liquid microextraction (DLLME) with the ICP-MS for proper separation and chemical speciation of the various forms (oxidation states) of biological elements (Fe, Cu, Zn, Mn, etc) at trace and sub trace levels.

ii. Coupling the dispersive solid phase microextraction (DSPME) in flow mode with the ICP-MS for proper separation and chemical speciation of the various forms of the essential elements in biological systems at trace and sub trace levels.

iii. Coupling DLLME and/or DSPME with ICP-MS minimizes the possible spectral interference from the matrix and will improve the lower limits of detection (LOD) and quantification (LOQ), linear dynamic range (LDR), sensitivity, elemental selectivity, low detection and isotope ratio capability of the ICP-MS.

iv. Lateral flow protocols for the rapid separation and speciation are model strategies, applicable for clinical use, if highly sensitive, on-site and timely determination is desired.

v. Extensive modification in advance samples preparation techniques-in particular automation- would eventually results in exceptional enrichment of trace and ultra-trace analysis of biological elements in complex matrices.

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References

[1] **Radovanovic, D., Bratic, M., Nurkic, M., Cvetkovic, T., Ignjatovic, A., and Aleksandrovic, M.** "Oxidative stress biomarker response to concurrent strength and endurance training". *Gen. Physiol. Biophys,* 28, p. 205-211 (2009).

[2] **Bloomer, R. J., Falvo, M. J., Fry, A. C., Schilling, B. K., Smith, W. A., and Moore, C. A.** "Oxidative stress response in trained men following repeated squats or sprints". *Med Sci Sports Exerc,* 38**(8)**, p. 1436-42 (2006).

[3] **Bloomer, R. J. a. F.-W., K.H.** . "Blood Oxidative Stress Biomarkers: Influence of Sex, Exercise Training Status, and Dietary Intake". *GENDER MEDICINE,* 5**(3)**, p. 218-228 (2008).

[4] **Fisher-Wellman, K. and Bloomer, R. J.** "Acute exercise and oxidative stress: a 30 year history". *Dyn Med,* 8, p. 1 (2009).

[5] **Bloomer, R. J. and Smith, W. A.** "Oxidative stress in response to aerobic and anaerobic power testing: influence of exercise training and carnitine supplementation". *Res Sports Med,* 17**(1)**, p. 1-16 (2009).

[6] **Bloomer, R. J., Ferebee, D. E., Fisher-Wellman, K. H., Quindry, J. C., and Schilling, B. K.** "Postprandial oxidative stress: influence of sex and exercise training status". *Med Sci Sports Exerc,* 41**(12)**, p. 2111-9 (2009).

[7] **Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., Gargiulo, G., Testa, G., Cacciatore, F., Bonaduce, D., and Abete, P.** "Oxidative stress, aging, and diseases". *Clinical Interventions in Aging,* 13, p. 757-772 (2018).

[8] **Belinskaia, D. A., Voronina, P. A., Shmurak, V. I., Vovk, M. A., Batalova, A. A., Jenkins, R. O., and Goncharov, N. V.** "The Universal Soldier: Enzymatic and Non-Enzymatic Antioxidant Functions of Serum Albumin".

Antioxidants (Basel, Switzerland), 9**(10)**, p. 966 (2020).

[9] **Cutler, R. G., Plummer, J., Chowdhury, K., and Heward, C.** "Oxidative stress profiling: part II. Theory, technology, and practice". *Ann N Y Acad Sci,* 1055, p. 136-58 (2005).

[10] **Del Rio, M. J. and Velez-Pardo, C.** "Transition metal-induced apoptosis in generation, mitochondria dysfunction lymphocytes via hydroxyl radical, and caspase-3 activation: An in vitro model for neurodegeneration". *Archives of Medical Research,* 35**(3)**, p. 185-193 (2004).

[11] **Huang, X., Moir, R. D., Tanzi, R. E., Bush, A. I., and Rogers, A. T.** "Redox-Active Metals, Oxidative Stress, and Alzheimer's Disease Pathology". *Ann. N.Y. Acad. Sci.,* 1012, p. 153-163 (2004).

[12] **Nunomura, A., Castellani, R. J., Zhu, X., Moreira, P. I., Perry, G., and Smith, M. A.** "Involvement of oxidative stress in Alzheimer disease". *Journal of Neuropathology and Experimental Neurology,* 65**(7)**, p. 631-641 (2006).

[13] **Collin, F., Cheignon, C., and Hureau, C.** "Oxidative stress as a biomarker for Alzheimer's disease". *Biomarkers in Medicine,* 12**(3)**, p. 201-203 (2018).

[14] **Bjorkblom, B., Adilbayeva, A., Maple-Grodem, J., Piston, D., Okvist, M., Xu, X. M., Brede, C., Larsen, J. P., and Moller, S. G.** "Parkinson disease protein DJ-1 binds metals and protects against metal-induced cytotoxicity". *The Journal of Biological Chemistry,* 288**(31)**, p. 22809-20 (2013).

[15] **El Assar, M., Angulo, J., and Rodriguez-Manas, L.** "Oxidative stress and vascular inflammation in aging". *Free Radical Biology and Medicine,* 65, p. 380-401 (2013).

[16] **Pouvreau, C., Dayre, A., Butkowski, E. G., de Jong, B., and Jelinek, H. F.** "Inflammation and oxidative stress markers in diabetes and hypertension". *Journal of Inflammation Research,* 11, p. 61-68 (2018).

[17] **Andrisic, L., Dudzik, D., Barbas, C., Milkovic, L., Grune, T., and Zarkovic, N.** "Short overview on metabolomics approach to study pathophysiology of oxidative stress in cancer". *Redox Biology,* 14, p. 47-58 (2018).

[18] **Dua, K., Malyla, V., Singhvi, G., Wadhwa, R., Krishna, R. V., Shukla, S. D., Shastri, M. D., Chellappan, D. K., Maurya, P. K., Satija, S., Mehta, M., Gulati, M. et al.** "Increasing complexity and interactions of oxidative stress in chronic respiratory diseases: An emerging need for novel drug delivery systems". *Chemico-Biological Interactions,* 299, p. 168-178 (2019).

[19] **Peoples, M. C. and Karnes, H. T.** "Recent developments in analytical methodology for 8-hydroxy-2'-deoxyguanosine and related compounds". *J Chromatogr B Analyt Technol Biomed Life Sci,* 827**(1)**, p. 5-15 (2005).

[20] **Ammar, A., Trabelsi, K., Boukhris, O., Glenn, J., Bott, N., Masmoudi, L., Hakim, A., Chtourou, H., Driss, T., Hökelmann, A., and Abed, K.** "Effects of Aerobic-, Anaerobic- and Combined-Based Exercises on Plasma Oxidative Stress Biomarkers in Healthy Untrained Young Adults". *International Journal of Environmental Research and Public Health,* 17, p. 2601 (2020).

[21] **JI, L. L., LEEUWENBURGH, C., LEICHTWEIS, S., GORE, M., FIEBIG, R., HOLLANDER, J., and BEJMA, J.** "Oxidative Stress and Aging Role of Exercise and Its Influences on Antioxidant Systems". (2000).

[22] **Nijhawan, P., Arora, S., and Behl, T.** "Intricate role of oxidative stress in the progression of obesity". *Obesity Medicine,* 15, p. 100125 (2019).

[23] **Stanković, M. and Radovanović, D.** "Oxidative stress and physical activity". *Sportlogia,* 8**(1)**, p. 1-11 (2012).

[24] **Bloomer, R. J., Goldfarb, A. H., Wideman, L., McKenzie, M. J., and Consitt, L. A.** "Effects of acute aerobic and anaerobic exercise on blood markers of oxidative stress". *Journal of Strength and Conditioning Research,* 19**(2)**, p. 276-285 (2005).

[25] **Paul, Y., Ellapen, T. J., Barnard, M., Hammill, H. V., and Swanepoel, M.** "The health benefits of exercise therapy for patient with Down syndrome: A systematic review". *African Journal of Disability,* 8, (2019).

[26] **de Gonzalo-Calvo, D., Fernandez-Garcia, B., de Luxan-Delgado, B., Rodriguez-Gonzalez, S., Garcia-Macia, M., Suarez, F. M., Solano, J. J., Rodriguez-Colunga, M. J., and Coto-Montes, A.** "Chronic training increases blood oxidative damage but promotes health in elderly men". *Age (Dordr),* 35**(2)**, p. 407-17 (2013).

[27] **Djordjevic, D., Cubrilo, D., Macura, M., Barudzic, N., Djuric, D., and Jakovljevic, V.** "The influence of training status on oxidative stress in young male handball players". *Mol Cell Biochem,* 351**(1-2)**, p. 251-9 (2011).

[28] **Poljsak, B.** "Strategies for reducing or preventing the generation of oxidative stress". *Oxid Med Cell Longev,* 2011, p. 194586 (2011).

[29] **Gacitua, T., Karachon, L., Romero, E., Parra, P., Poblete, C., Russell, J., and Rodrigo, R.** "Effects of resistance training on oxidative stress-related biomarkers in metabolic diseases: a review". *Sport Sciences for Health,* 14**(1)**, p. 1-7 (2018).

[30] **Hall, M. E. and Church, F. C.** "Exercise for Older Adults Improves the Quality of Life in Parkinson's Disease and Potentially Enhances the Immune Response to COVID-19". *Brain Sci,* 10**(9)**, (2020).

[31] **Escribano, B. M., Tunez, I., Requena, F., Rubio, M. D., De Miguel, R., Montilla, P., Tovar, P., and Aguera, E. I.** "Effects of an aerobic training program on oxidative stress

biomarkers in bulls". *Veterinarni Medicina,* 55**(9)**, p. 422-428 (2010).

[32] **Leeuwenburgh, C. and Heinecke, J. W.** "Oxidative Stress and Antioxidants in Exercise". *Current Medicinal Chemistry,* 8, p. 829-838 (2001).

[33] **Martinovic, J., Dopsaj, V., Dopsaj, M. J., Kotur-Stevuljevic, J., Vujovic, A., Stefanovic, A., and Nesic, G.** "Long-term effects of oxidative stress in volleyball players". *Int J Sports Med,* 30**(12)**, p. 851-6 (2009).

[34] **Bogdanis, G. C., Stavrinou, P., Fatouros, I. G., Philippou, A., Chatzinikolaou, A., Draganidis, D., Ermidis, G., and Maridaki, M.** "Short-term high-intensity interval exercise training attenuates oxidative stress responses and improves antioxidant status in healthy humans". *Food Chem Toxicol,* 61, p. 171-7 (2013).

[35] **Powers, S. K. and Jackson, M. J.** "Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production". *Physiol Rev,* 88**(4)**, p. 1243-76 (2008).

[36] **Ceci, R., Beltran Valls, M. R., Duranti, G., Dimauro, I., Quaranta, F., Pittaluga, M., Sabatini, S., Caserotti, P., Parisi, P., Parisi, A., and Caporossi, D.** "Oxidative stress responses to a graded maximal exercise test in older adults following explosive-type resistance training". *Redox Biology,* 2, p. 65-72 (2014).

[37] **Memme, J. M., Erlich, A. T., Hood, D. A., and Phukan, G.** "Exercise and mitochondrial health". *Journal of Physiology-London*, p. 1-15 (2019).

[38] **Chevion, S., Moran, D. S., Heled, Y., Shani, Y., Regev, G., Abbou, B., Berenshtein, E., Stadtman, E. R., and Epstein, Y.** "Plasma antioxidant status and cell injury after severe physical exercise". *Proc Natl Acad Sci U S A,* 100**(9)**, p. 5119-23 (2003).

[39] **Nikolaidis, M. G., Jamurtas, A. Z., Paschalis, V., Fatouros, I. G., Koutedakis, Y., and D., K.** "The Effect of Muscle-Damaging Exercise on Blood and Skeletal Muscle Oxidative Stress". *Sports Med* 38**(7)**, p. 579-606 (2008).

[40] **Sjodin, B., Westing, Y., and Apple, F.** "Biochemical Mechanisms for Oxygen Free Radical Formation During Exercise". *Sports Medicine,* 10**(4)**, p. 236-254 (1990).

[41] **Finaud, J., Lac, G., and Filaire, E.** "Oxidative Stress, Relationship with Exercise and Training". *Sports Med,* 36**(4)**, p. 327-358 (2006).

[42] **LL, J.** "OXIDATIVE STRESS DURING EXERCISE: IMPLICATION OF ANTIOXIDANT NUTRIENTS". *Free Radic Biol Med,* 18**(6)**, p. 1079-1086 (1995).

[43] **Azizbeigi, K., Stannard, S. R., Atashak, S., and Mosalman Haghighi, M.** "Antioxidant enzymes and oxidative stress adaptation to exercise training: Comparison of endurance, resistance, and concurrent training in untrained males". *Journal of Exercise Science & Fitness*, p. 1-6 (2014).

[44] **Liu, J. K., Yeo, H. C., Overvik-Douki, E., Hagen, T., Doniger, S. J., Chu, D. W., Brooks, G. A., and Ames, B. N.** "Chronically and acutely exercised rats: biomarkers of oxidative stress and endogenous antioxidants". *Journal of Applied Physiology,* 89**(1)**, p. 21-28 (2000).

[45] **Man, A. W. C., Li, H., and Xia, N.** "Impact of Lifestyles (Diet and Exercise) on Vascular Health: Oxidative Stress and Endothelial Function". *Oxid Med Cell Longev,* 2020, p. 1496462 (2020).

[46] **Klaunig, J. E. and Wang, Z.** "Oxidative stress in carcinogenesis". *Current Opinion in Toxicology,* 7, p. 116-121 (2018).

[47] **Sagoo, M. K. and Gnudi, L.** "Diabetic nephropathy: Is there a role for oxidative stress?". *Free Radical Biology and Medicine,* 116, p. 50-63 (2018).

[48] **Mateos, R. and Bravo, L.** "Chromatographic and electrophoretic methods for the analysis of biomarkers of oxidative damage to macromolecules (DNA, lipids, and proteins)". *Journal of Separation Science,* 30**(2)**, p. 175-191 (2007).

[49] **Ramos, D., Martins, E. G., Viana-Gomes, D., Casimiro-Lopes, G., and Salerno, V. P.** "Biomarkers of oxidative stress and tissue damage released by muscle and liver after a single bout of swimming exercise". *Applied Physiology Nutrition and Metabolism-Physiologie Appliquee Nutrition Et Metabolisme,* 38**(5)**, p. 507-511 (2013).

[50] **Varamenti, E. I., Kyparos, A., Veskoukis, A. S., Bakou, M., Kalaboka, S., Jamurtas, A. Z., Koutedakis, Y., and Kouretas, D.** "Oxidative stress, inflammation and angiogenesis markers in elite female water polo athletes throughout a season". *Food and Chemical Toxicology,* 61, p. 3-8 (2013).

[51] **Martinovic, J., Dopsaj, V., Kotur-Stevuljevic, J., Dopsaj, M., Vujovic, A., Stefanovic, A., and Nesic, G.** "Oxidative stress biomarker monitoring in elite women volleyball athletes during a 6-week training period". *J Strength Cond Res,* 25**(5)**, p. 1360-7 (2011).

[52] **Costa, C. S. C. d.** "Oxidative Stress Biomarkers Response to Exercise in Brazilian Junior Soccer Players". *Food and Nutrition Sciences,* 02**(05)**, p. 407-413 (2011).

[53] **Deminice, R., Sicchieri, T., Payao, P. O., and Jordao, A. A.** "Blood and Salivary Oxidative Stress Biomarkers Following an Acute Session of Resistance Exercise in Humans". *International Journal of Sports Medicine,* 31**(9)**, p. 599-603 (2010).

[54] **Cooper, C. E., Vollaard, N. B. J., Choueiri, T., and Wilson, M. T.** "Exercise, free radicals and oxidative stress". *Biochemical Society Transactions,* 30**(2)**, (2002).

[55] **Sacheck, J. M., Milbury, P. E., Cannon, J. G., Roubenoff, R., and Blumberg, J. B.** "Effect of vitamin E and eccentric exercise on selected

biomarkers of oxidative stress in young and elderly men". *Free Radical Biology and Medicine,* 34**(12)**, p. 1575-1588 (2003).

[56] **Galaris, D. and Evangelou, A.** "The role of oxidative stress in mechanisms of metal-induced carcinogenesis". *Critical Reviews in Oncology/Hematology 42 (2002) 93–103,* 42, p. 93-103 (2002).

[57] **Warraich, U. E., Hussain, F., and Kayani, H. U. R.** "Aging - Oxidative stress, antioxidants and computational modeling". *Heliyon,* 6**(5)**, p. e04107 (2020).

[58] **Di Meo, S. and Venditti, P.** "Evolution of the Knowledge of Free Radicals and Other Oxidants". *Oxidative Medicine and Cellular Longevity,* 2020, p. 9829176-9829176 (2020).

[59] **Halliwell, B.** "Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life". *Plant Physiol,* 141**(2)**, p. 312-22 (2006).

[60] **Jomova, K. and Valko, M.** "Advances in metal-induced oxidative stress and human disease". *Toxicology,* 283**(2-3)**, p. 65-87 (2011).

[61] **Jomova, K., Vondrakova, D., Lawson, M., and Valko, M.** "Metals, oxidative stress and neurodegenerative disorders". *Molecular and Cellular Biochemistry,* 345**(1-2)**, p. 91-104 (2010).

[62] **Lee, J.-C., Son, Y.-O., Pratheeshkumar, P., and Shi, X.** "Oxidative stress and metal carcinogenesis". *Free Radical Biology and Medicine,* 53**(4)**, p. 742-757 (2012).

[63] **Valko, M., Rhodes, C. J., Moncol, J., Izakovic, M., and Mazur, M.** "Free radicals, metals and antioxidants in oxidative stress-induced cancer". *Chemico-Biological Interactions,* 160**(1)**, p. 1-40 (2006).

[64] **Kasprzak, K. S.** "Oxidative DNA and protein damage in metal-induced toxicity and carcinogenesis". *Free Radical Biology and Medicine,* 32**(10)**, p. 958-967 (2002).

[65] **Leonard, S. S., Harris, G. K., and Shi, X.** "Metal-induced oxidative stress and signal transduction". *Free Radic Biol Med,* 37**(12)**, p. 1921-42 (2004).

[66] **Marinho, H. S., Real, C., Cyrne, L., Soares, H., and Antunes, F.** "Hydrogen peroxide sensing, signaling and regulation of transcription factors". *Redox Biology,* 2, p. 535-562 (2014).

[67] **Sies, H.** "Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress: Oxidative eustress". *Redox Biology,* 11, p. 613-619 (2017).

[68] **Rimbach, G., Höhler, D., Fischer, A., Roy, S., Virgili, F., Pallauf, J., and Packer, L.** "Methods to assess free radicals and oxidative stress in biological systems". *Archiv für Tierernaehrung,* 52**(3)**, p. 203-222 (1999).

[69] **Giménez-Egido, J. M., Hernández-García, R., Escribano, D., Martínez-Subiela, S., Torres-Luque, G., Ortega-Toro, E., and Cerón, J. J.** "Changes in Markers of Oxidative Stress and α-Amylase in Saliva of Children Associated with a Tennis Competition". *International Journal of Environmental Research and Public Health,* 17**(17)**, p. 6269 (2020).

[70] **Trivić, T., Drid, P., Obadov, S., and Ostojic, S.** "Effect of endurance training on oxidative stress in male wrestlers". *Journal of Martial Arts Anthropology,* 11**(2)**, p. 6- 9 (2011).

[71] **Wei, W., Wang, H., and Jiang, C.** "Spectrofluorimetric determination of superoxide dismutase using a Europium-tetracycline probe". *Spectrochim Acta A Mol Biomol Spectrosc,* 70**(2)**, p. 389-93 (2008).

[72] **Carter, S., Fisher, A. S., Hinds, M. W., Lancaster, S., and Marshall, J.** "Atomic spectrometry update. Review of advances in the analysis of metals, chemicals and materials". *Journal of Analytical Atomic Spectrometry,* 28**(12)**, p. 1814-1869 (2013).

[73] **Flora, S. J. S., Mittal, M., and Mehta, A.** "Heavy metal induced oxidative stress & its possible reversal by chelation therapy". *Indian J Med Res* 128, p. 501-523 (2008).

[74] **Valko, M., Morris, H., and Cronin, M. T.** "metal, toxicity and oxidative stress". *Current Medicinal Chemistry*, p. 1161-1208 (2005).

[75] **Sun, H. and Chai, Z.-F.** "Metallomics: An integrated science for metals in biology and medicine". *Annual Reports Section "A" (Inorganic Chemistry),* 106, p. 20 (2010).

[76] **Mounicou, S., Szpunar, J., and Lobinski, R.** "Metallomics: the concept and methodology". *Chem Soc Rev,* 38**(4)**, p. 1119-38 (2009).

[77] **Battin, E. E., Zimmerman, M. T., Ramoutar, R. R., Quarles, C. E., and Brumaghim, J. L.** "Preventing metal-mediated oxidative DNA damage with selenium compounds". *Metallomics,* 3**(5)**, p. 503-12 (2011).

[78] **Pisoschi, A. M., Pop, A., Iordache, F., Stanca, L., Predoi, G., and Serban, A. I.** "Oxidative stress mitigation by antioxidants - An overview on their chemistry and influences on health status". *European Journal of Medicinal Chemistry,* 209, p. 112891 (2021).

[79] **Taghreed M., F.** "ICP-MS determination of Zn, Cu, Fe and Mn in muscle cells as potential markers of oxidative stress," Ph.D, Chemistry, Loughborough University, 2017.

[80] **Eide, D. J.** "The oxidative stress of zinc deficiency". *Metallomics,* 3**(11)**, p. 1124-9 (2011).

[81] **Gaetke, L. M. and Chow, C. K.** "Copper toxicity, oxidative stress, and antioxidant nutrients". *Toxicology,* 189**(1-2)**, p. 147-63 (2003).

[82] **Oteiza, P. I.** "Zinc and the modulation of redox homeostasis". *Free Radical Biology and Medicine,* 53**(9)**, p. 1748-1759 (2012).

[83] **Cerchiaro, G., Manieri, T. M., and Bertuchi, F. R.** "Analytical methods for copper, zinc and iron quantification in mammalian cells". *Metallomics,* 5**(10)**, p. 1336-45 (2013).

[84] **M-M, P., Weiskirchen, R., Gassler, N., Bosserhoff, A. K., and Becker, J. S.** "Novel bioimaging techniques of metals by laser ablation inductively coupled plasma mass spectrometry for diagnosis of fibrotic and cirrhotic liver disorders". *PLoS One,* 8**(3)**, p. e58702 (2013).

[85] **Wehe, C. A., Bornhorst, J., Holtkamp, M., Sperling, M., Galla, H. J., Schwerdtle, T., and Karst, U.** "Fast and low sample consuming quantification of manganese in cell nutrient solutions by flow injection ICP-QMS". *Metallomics,* 3**(12)**, p. 1291-6 (2011).

[86] **Aguirre, J. D. and Culotta, V. C.** "Battles with iron: manganese in oxidative stress protection". *J Biol Chem,* 287**(17)**, p. 13541-8 (2012).

[87] **McEwan, A. G.** "New insights into the protective effect of manganese against oxidative stress". *Mol Microbiol,* 72**(4)**, p. 812-4 (2009).

[88] Bates, M. K. (2012, 2/6/2016). *Culturing Cells Under Hypoxic Conditions for Biologically Relevant Results*. Available: http://www.americanlaboratory.com/913-Technical-Articles/123131-Culturing-Cells-Under-Hypoxic-Conditions-for-Biologically-Relevant-Results/

[89] **Jamieson, D., Chance, B., Cadenas, E., and Boveris, A.** "THE RELATION OF FREE RADICAL PRODUCTION TO HYPEROXIA". *Ann. Rev. Physiol,* 48, p. 703-19 (1986).

[90] **Deldicque, L. and Francaux, M.** "Acute vs. chronic hypoxia: What are the consequences for skeletal muscle mass?". *Cellular and Molecular Exercise Physiology,* 2**(1)**, (2013).

[91] **Andrew, T.** "Advantages of Oxygen Control in CO2 Incubation for Research and Clinical Applications". *BioTechniques Protocol Guide*, (2009).

[92] **Cogo, A., Napolitano, G., Michoud, M. C., Ramos Barbon, D., Ward, M., and Martin, J. G.** "Effects of hypoxia on rat airway smooth muscle cell proliferation". *J Appl Physiol,* 94, p. 403–1409 (2003).

[93] **Absher, M., Makrides, W., Shapiro, P., and Evans, J. N.** "Hyperoxia inhibits proliferation of cultured rat tracheal smooth muscle cells". *American Journal of Physiology - Lung Cellular and Molecular Physiology,* 267**(2)**, p. L101-L105 (1994).

[94] **Vanhatalo, A., Fulford, J., DiMenna, F. J., and Jones, A. M.** "Influence of hyperoxia on muscle metabolic responses and the power–duration relationship during severe-intensity exercise in humans: a 31P magnetic resonance spectroscopy study". *Exp Physiol,* 95**(4)**, p. 528–540 (2010).

[95] **Tony, A., Young, I. S., Peters, J. R., Jones, E., Jackson, S. K., Davies, B., and Rowlands, C. C.** "Electron spin resonance spectroscopy, exercise, and oxidative stress: an ascorbic acid intervention study". *J. Appl. Physiol.,* 87**(6)**, p. 2032–2036 (1999).

[96] **Arcari, D. P., Porto, V. B., Varalda Rodrigues, E. R., Martins, F., de Lima, R. J., Helena Frankland Sawaya, A. C., Ribeiro, M. L., and Carvalho, P. d. O.** "Effect of mate tea (Ilex paraguariensis) supplementation on oxidative stress biomarkers and LDL oxidisability in normo- and hyperlipidaemic humans". *Journal of Functional Foods,* 3**(3)**, p. 190-197 (2011).

[97] **Muniz, J. F., McCauley, L., Scherer, J., Lasarev, M., Koshy, M., Kow, Y. W., Nazar-Stewart, V., and Kisby, G. E.** "Biomarkers of oxidative stress and DNA damage in agricultural workers: a pilot study". *Toxicol Appl Pharmacol,* 227**(1)**, p. 97-107 (2008).

[98] **Halliwell, B. and Lee, C. Y.** "Using isoprostanes as biomarkers of oxidative stress: some rarely considered issues". *Antioxid Redox Signal,* 13**(2)**, p. 145-56 (2010).

[99] **Fan, X.** "Measurement of malonaldehyde in apple juice using gc-ms and a comparison to the thiobarbituric acid assay". *Food Chemistry,* 77, p. 353-359 (2002).

[100] **Mateos, R., Goya, L., and Bravo, L.** "Determination of malondialdehyde by liquid chromatography as the 2,4-dinitrophenylhydrazone derivative: a marker for oxidative stress in cell cultures of human hepatoma HepG2". *Journal of Chromatography. B, Analytical technologies in the biomedical and life sciences,* 805**(1)**, p. 33-39 (2004).

[101] **Wilson, D., Metz, H., Graver, L., and Rao, P.** "Direct method of quantification of free malondialdehyde with high-performance capillary electrophoresis in biological samples". *Clinical Chemistry,* 43, p. 1982-4 (1997).

[102] **Comporti, M., Signorini, C., Arezzini, B., Vecchio, D., Monaco, B., and Gardi, C.** "F2-isoprostanes are not just markers of oxidative stresse". *Free Radical Biology and Medicine,* 44**(3)**, p. 247-256 (2008).

[103] **Cracowski, J. L., Durand, T., and Bessard, G.** "Isoprostanes as a biomarker of lipid peroxidation in humans: physiology, pharmacology and clinical implications". *Trends in Pharmacological Sciences,* 23**(8)**, p. 360-366 (2002).

[104] **Dorjgochoo, T., Gao, Y.-T., Chow, W.-H., Shu, X.-o., Yang, G., Cai, Q., Rothman, N., Cai, H., Li, H., Deng, X., Franke, A., Roberts, L. J. et al.** "Major metabolite of F-2-isoprostane in urine may be a more sensitive biomarker of oxidative stress than isoprostane itself". *American Journal of Clinical Nutrition,* 96**(2)**, p. 405-414 (2012).

[105] **Halliwell, B. and Lee, C. Y. J.** "Using Isoprostanes as Biomarkers of Oxidative Stress: Some Rarely Considered Issues". *Antioxidants & Redox Signaling,* 13**(2)**, p. 145-156 (2010).

[106] **Janicka, M., Kot-Wasik, A., Kot, J., and Namiesnik, J.** "Isoprostanes-biomarkers of lipid peroxidation: their utility in evaluating oxidative stress and analysis". *Int J Mol Sci,* 11**(11)**, p. 4631-59 (2010).

[107] **Liu, W., Morrow, J. D., and Yin, H.** "Quantification of F-2-isoprostanes as a reliable

index of oxidative stress in vivo using gas chromatography-mass spectrometry (GC-MS) method". *Free Radical Biology and Medicine,* 47**(8)**, p. 1101-1107 (2009).

[108] **Milne, G. L., Sanchez, S. C., Musiek, E. S., and Morrow, J. D.** "Quantification of F-2-isoprostanes as a biomarker of oxidative stress". *Nature Protocols,* 2**(1)**, p. 221-226 (2007).

[109] Milne, G. L., Yin, H., Brooks, J. D., Sanchez, S., Roberts, L. J., and Morrow, J. D., "Quantification of F2-isoprostanes in biological fluids and tissues as a measure of oxidant stress," in *Lipidomics and Bioactive Lipids: Specialized Analytical Methods and Lipids in Disease*, vol. 433, H. A. Brown, Ed. (Methods in Enzymology, 2007, pp. 113-126.

[110] **Nikolaidis, M. G., Kyparos, A., and Vrabas, I. S.** "F-2-isoprostane formation, measurement and interpretation: The role of exercise". *Progress in Lipid Research,* 50**(1)**, p. 89-103 (2011).

[111] **ROBERTS, L. J. and MORROW, J. D.** "MEASUREMENT OF F2-ISOPROSTANES AS AN INDEX OF OXIDATIVE STRESS IN VIVO". *Free Radical Biology & Medicine,* 28**(4)**, p. 505-513 (2000).

[112] **Lee, C. Y., Jenner, A. M., and Halliwell, B.** "Rapid preparation of human urine and plasma samples for analysis of F2-isoprostanes by gas chromatography-mass spectrometry". *Biochem Biophys Res Commun,* 320**(3)**, p. 696-702 (2004).

[113] **Ohashi, N. and Yoshikawa, M.** "Rapid and sensitive quantification of 8-isoprostaglandin F2alpha in human plasma and urine by liquid chromatography-electrospray ionization mass spectrometry". *J Chromatogr B Biomed Sci Appl,* 746**(1)**, p. 17-24 (2000).

[114] **Levine, R., Garland, D., Oliver, C., Amici, A., Climent, I., and Lenz, A.** "Determination of carbonyl content in oxidatively modified proteins". *Methods Enzymol,* 186, p. 464-78 (1990).

[115] **Barregard, L., Moller, P., Henriksen, T., Mistry, V., Koppen, G., Rossner, P., Jr., Sram, R. J., Weimann, A., Poulsen, H. E., Nataf, R., Andreoli, R., Manini, P. et al.** "Human and methodological sources of variability in the measurement of urinary 8-oxo-7,8-dihydro-2'-deoxyguanosine". *Antioxid Redox Signal,* 18**(18)**, p. 2377-91 (2013).

[116] **Crow, B., Bishop, M., Kovalcik, K., Norton, D., George, J., and Bralley, J. A.** "A simple and cost effective method for the quantification of 8-hydroxy-2'-deoxyguanosine from urine using liquid chromatography tandem mass spectrometry". *Biomed Chromatogr,* 22**(4)**, p. 394-401 (2008).

[117] **FRAGA, C. G., SHIGENAGA, M. K., Park, J. W., DEGAN, P., and Ames, B. N.** "Oxidative damage to DNA during aging: 8-Hydroxy-2'-deoxyguanosine in rat organ DNA and urine". *Proc. Nadl. Acad. Sci. USA,* 87, p. 4533-4537 (1990).

[118] **Henriksen, T., Hillestrom, P. R., Poulsen, H. E., and Weimann, A.** "Automated method for the direct analysis of 8-oxo-guanosine and 8-oxo-2 '-deoxyguanosine in human urine using ultraperformance liquid chromatography and tandem mass spectrometry". *Free Radical Biology and Medicine,* 47**(5)**, p. 629-635 (2009).

[119] **Hu, C. W., Huang, Y. J., Li, Y. J., and Chao, M. R.** "Correlation between concentrations of 8-oxo-7,8-dihydro-2'-deoxyguanosine in urine, plasma and saliva measured by on-line solid-phase extraction LC-MS/MS". *Clin Chim Acta,* 411**(17-18)**, p. 1218-22 (2010).

[120] **Komatsu, T., Duckyoung, Y., Ito, A., Kurosawa, K., Maehata, Y., Kubodera, T., Ikeda, M., and Lee, M. C.** "Increased oxidative stress biomarkers in the saliva of Down syndrome patients". *Arch Oral Biol,* 58**(9)**, p. 1246-50 (2013).

[121] **Loft, S., Vistisen, K., Ewertz, M., Tjonneland, A., Overvad, K., and Poulsen, H. E.** "Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index". *Carcinogenesis,* 13**(12)**, p. 2241-7 (1992).

[122] **Mei, S., Yao, Q., Wu, C., and Xu, G.** "Determination of urinary 8-hydroxy-2'-deoxyguanosine by two approaches-capillary electrophoresis and GC/MS: an assay for in vivo oxidative DNA damage in cancer patients". *J Chromatogr B Analyt Technol Biomed Life Sci,* 827**(1)**, p. 83-7 (2005).

[123] **Renner, T., Fechner, T., and Scherer, G.** "Fast quantification of the urinary marker of oxidative stress 8-hydroxy-2 '-deoxyguanosine using solid-phase extraction and high-performance liquid chromatography with triple-stage quadrupole mass detection". *Journal of Chromatography B,* 738**(2)**, p. 311-317 (2000).

[124] **Valavanidis, A., Vlachogianni, T., and Fiotakis, C.** "8-hydroxy-2' -deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis". *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev,* 27**(2)**, p. 120-39 (2009).

[125] **Deitrich, C. L., Braukmann, S., Raab, A., Munro, C., Pioselli, B., Krupp, E. M., Thomas-Oates, J. E., and Feldmann, J.** "Absolute quantification of superoxide dismutase (SOD) using species-specific isotope dilution analysis". *Anal Bioanal Chem,* 397**(8)**, p. 3515-24 (2010).

[126] **Girotti, S., Fini, F., Ferri, E., Budini, R., Piazzi, S., and Cantagalli, D.** "Determination of superoxide dismutase in erythrocytes by a chemiluminescent assay". *Talanta* 51, p. 685-692 (2000).

[127] **Ordóñez, Y. N., Deitrich, C. L., Montes-Bayón, M., Blanco-González, E., Feldmann, J., and Sanz-Medel, A.** "Species specific isotope

dilution versus internal standardization strategies for the determination of Cu, Zn-superoxide dismutase in red blood cells". *Journal of Analytical Atomic Spectrometry,* 26**(1)**, p. 150-155 (2011).

[128] **Firuzi, O., N., S., Amoozgar, H., Borzoee, M., Abtahi, S., Ajami, G., Ardi, P., and Miri, R.** "Effects of Omega-3 Polyunsaturated Fatty Acids on Heart Function and Oxidative Stress Biomarkers in Pediatric Patients with Dilated Cardiomyopathy". *Int Cardivasc Res J.,* 7**(1)**, p. 8- 14 (2013).

[129] **PerrinNadif, R., Dusch, M., Koch, C., Schmitt, P., and Mur, J. M.** "Catalase and superoxide dismutase activities as biomarkers of oxidative stress in workers exposed to mercury vapors". *Journal of Toxicology and Environmental Health,* 48**(2)**, p. 107-119 (1996).

[130] **Spitz, D. R. and Oberley, L. W.** "An assay for superoxide dismutase activity in mammalian tissue homogenates". *Anal Biochem,* 179**(1)**, p. 8-18 (1989).

[131] **Wang, L., Liu, S., Zheng, Z., Pi, Z., Song, F., and Liu, Z.** "Rapid assay for testing superoxide anion radical scavenging activities to natural pigments by ultra-high performance liquid chromatography-diode-array detection method". *Analytical Methods,* 7**(4)**, p. 1535-1542 (2015).

[132] **Usui, A., Kato, K., Tsuboi, H., Sone, T., Sassa, H., and Abe, T.** "Concentration of Mn-superoxide dismutase in serum in acute myocardial infarction". *Clin Chem,* 37**(3)**, p. 458-61 (1991).

[133] **Laihia, J. K., Jansen, C. T., and Ahotupa, M.** "Lucigenin and linoleate enhanced chemiluminescent assay for superoxide dismutase activity". *Free Radic Biol Med,* 14**(5)**, p. 457-61 (1993).

[134] **da Costa, C. M., dos Santos, R. C. C., and Lima, E. S.** "A simple automated procedure for thiol measurement in human serum samples". *j Bras Patol Med Lab,* 42**(5)**, p. 345- 350 (2006).

[135] **Kondakova, I., Lissi, E. A., and Pizarro, M.** "TOTAL REACTIVE ANTIOXIDANT POTENTIAL IN HUMAN SALIVA OF SMOKERS AND NON-SMOKERS". *BIOCHEMISTRY and MOLECULAR BIOLOGY INTERNATIONAL,* 47**(6)**, p. 911-920 (1999).

[136] **Clouse, A., Deo, S., Rampersaud, E., Farmer, J., Goldschmidt-Clermont, P. J., and Daunert, S.** "Defining a molecular portrait of physical fitness". *Anal Bioanal Chem,* 405**(1)**, p. 21-6 (2013).

[137] **Shi, M., Wang, X., Yamanaka, T., Ogita, F., Nakatani, K., and Takeuchi, T.** "Effects of anaerobic exercise and aerobic exercise on biomarkers of oxidative stress". *Environmental Health and Preventive Medicine,* 12**(5)**, p. 202-208 (2007).

[138] **Dawson, E. A., Green, D. J., Cable, N. T., and Thijssen, D. H. J.** "Effects of acute exercise on flow-mediated dilatation in healthy humans". *Journal of Applied Physiology,* 115**(11)**, p. 1589-1598 (2013).

[139] **Gątarek, P., Pawełczyk, M., Jastrzębski, K., Głąbiński, A., and Kałużna-Czaplińska, J.** "Analytical methods used in the study of Parkinson's disease". *TrAC Trends in Analytical Chemistry,* 118, p. 292-302 (2019).

[140] Calderon-Cells, F. and Ruiz Encinar, J., "A reflection on the role of ICP-MS in proteomics: Update and future perspective," vol. 198, ed, 2019, pp. 11-17.

[141] **Javier, A. A.** *Fundamentals of Electrothermal Atomic Absorption Spectrometry: A Look Inside the Fundamental Processes in ETAAS*. World Scientific Publishing Company, 1998.

[142] **Marcó P, L. M. and Hernández-Caraballo, E. A.** "Direct analysis of biological samples by total reflection X-ray fluorescence". *Spectrochimica Acta Part B: Atomic Spectroscopy,* 59**(8)**, p. 1077-1090 (2004).

[143] Schmeling, M., "X-Ray Fluorescence and Emission | Total Reflection X-Ray Fluorescence☆," in *Encyclopedia of Analytical Science (Third Edition)*, P. Worsfold, C. Poole, A. Townshend, and M. Miró, Eds. Oxford: Academic Press, 2019, pp. 449-458.

[144] **Jurowski, K., Buszewski, B., and Piekoszewski, W.** "Bioanalytics in Quantitive (Bio)imaging/Mapping of Metallic Elements in Biological Samples". *Critical Reviews in Analytical Chemistry,* 45**(4)**, p. 334-347 (2015).

[145] **Clayton, E.** "Sample characterization by proton induced x-ray emission analysis". *Applications of Surface Science,* 13**(1)**, p. 136-158 (1982).

[146] Ulrich, N., "SPECTROSCOPY | Atomic Emission and Absorption," in *Encyclopedia of Food Sciences and Nutrition (Second Edition)*, B. Caballero, Ed. Oxford: Academic Press, 2003, pp. 5441-5447.

[147] **Banci, L.** *Metallomics and the Cell*. Springer, Dordrecht, 2013.

[148] **Pereira Navaza, A., Ruiz Encinar, J., and Sanz-Medel, A.** "Quantitative protein phosphorylation analysis: the role of ICP-MS". *Journal of Analytical Atomic Spectrometry,* 22**(10)**, p. 1223-1237 (2007).

[149] **Szpunar, J.** "Advances in analytical methodology for bioinorganic speciation analysis: metallomics, metalloproteomics and heteroatom-tagged proteomics and metabolomics". *Analyst,* 130**(4)**, p. 442-65 (2005).

الرياضة ومضادات الأكسدة: المؤشرات الحيوية الحالية للإجهاد التأكسدي: القيود والتحديات المستقبلية

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المستخلص: ازداد الإهتمام بشكل كبير في السنوات الحالية بدراسة الإتزان بين مستوى انتاج الشقوق الحرة في الكائنات الحية وإزالتها بواسطة نظام مضادات الأكسدة والذي يعرف بالإجهاد التأكسدي. وبالتالي تقدم هذه الدراسة لمحة عامة عن هذا الإتزان في الكائنات الحية في السنوات القليلة الماضية. وسيتم التركيزعلى كيفية توليد الشقوق الحرة بواسطة بعض الظروف البدنية والنفسية التي تؤدي إلى هذا الإجهاد داخل خلايا الكائنات الحية والذي يؤدي بدوره إلى الضرر بالجزيئات الحيوية داخل الخلايا والإصابة بالأمراض المزمنة. وسوف يناقش جوهر هذه الدراسة العوامل التي تؤثر على إنتاج هذه الشقوق أثناء القيام بالأنشطة الرياضية البدنية مثل نوع التمرين الرياضي ومدته الزمنية ومقدار ما يستهلك من الأكسجين اثناء التمرين. كما تهدف الدراسة الحالية إلى تلخيص مدى ارتباط تكوين الجذور الحرة الضارة بالمعادن النشطة التي تساهم في عملتي الأكسدة والاختزال داخل الخلايا. وسوف تتناول ايضا بشكل مفصل مدى تأثير هذا الإجهاد التأكسدي على خلايا الكائنات الحية وكيفية استجابتها له في المختبروعلى وجه الخصوص الخلايا العضلية. وكذلك المؤشرات الحيوية المستخدمة حاليا لدراسة وقياس هذا النوع من الإجهاد التأكسدي في العينات الحيوية والناجم من التمارين الرياضية. وسوف تناقش هذه الدراسة بإيجاز قصور بعض تقنيات التحليل الكيميائي المستخدمة في تعيين بعض العناصر الحيوية (الخارصين والنحاس والحديد والمنجنيز) اثناء استخدامها كواسم حيوي لقياس الإجهاد التأكسدي داخل الخلايا.

كلمات مفتاحية: مضادات الأكسدة، العناصر الحيوية، مؤشرات الإجهاد التأكسدي، تقنيات التحليل الكيميائي، العينات الحيوية

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