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Air Pollution, Reactive Oxygen Species (ROS), and Autonomic Nervous System Interactions Modulate Cardiac Oxidative Stress and Electrophysiological Changes

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1. Introduction

1.1 Air pollution: A global overview

In the last decades we witnessed unprecedented rapid deterioration in the environment as a consequence of intense anthropogenic activity. Air pollution, and more precisely particulate matter (PM) is both an alarming environmental problem and a public health concern that affects many regions of the world. Ambient air pollution has been associated with increased respiratory mucosal symptoms, exacerbation of asthma, chronic obstructive pulmonary disease (COPD) as well as cardiovascular diseases and mortality [World Health Organization (WHO) working group et al., 2003]. This association is based on observational epidemiological studies of disease occurrence in the human populations and in *in-vitro* and *in-vivo* studies of animals and humans [Health Effect Institute [HEI] et al., 2002].

Some groups in the population are more susceptible to air pollution induced health risks. Children are particularly at risk for deleterious respiratory outcomes due to the immaturity of their respiratory organ systems. They spend significantly more time outdoors than adults, especially during the summer months when smog and ozone levels are highest. Elderly, diabetics, people affected by underlying heart or lung disease, lower socioeconomic population living in proximity to sources of pollution [Mohai et al., 2009], are among the groups that experience increased risk for both pulmonary and cardiovascular system diseases.

Sources of pollutants differ in different regions and are influenced by many factors. Air pollution levels are higher in the vicinity of specific sources such as roads, chemical plants, oil refineries, manufacturing facilities, and other industrial facilities. Meteorological factors determine the distribution of pollutants, since they can travel far from the original source, influenced by the wind, along with the topography of the regions and phenomena of temperature inversion, a meteorological condition that traps pollutants at ground level instead of circulating them away, and it is often responsible for episodes of sudden noxious toxin and particle concentrations in small areas. The increase of the world population and the consequent increase in the production and consumption of resources in particular of fossil fuels, influence the concentration and the quality of the

emissions. Urbanization imposes also a significant pressure, by 2025 coastal populations alone are expected to reach six billion while the year 2007 has been the first in human history when more than half of all world population live in cities [United Nation Environment Program (UNEP) 2007].

1.2 Anthropogenic emission in rural areas, industrial environments, and developing countries

Anthropogenic emissions of pollutants in the atmosphere are not only a concern for the industrialized world but also for developing countries and rural areas around the globe, with differences in the sources and in the chemicals dispersed in the environment.

A 2005 World Health Organization (WHO) Global update assessed the burden of diseases due to atmospheric pollution and reported that more than 2 million premature deaths each year can be attributed to air pollution. More than half of this disease burden is borne by the population of developing countries [World Health Organization (WHO) 2005].

In industrialized countries, air pollution is largely due to fossil fuel combustion for transportation, industrial activity and electricity production, while in developing countries (excluding the large metropolitan areas) the air pollution mostly consists of uncontrolled burning of biomass for cooking, heating, farming and deforesting [Armaroli & Balzani 2011].

Rural areas have lower outdoor air pollutants since less vehicular traffic and industrial smoke are present. Nevertheless individual exposures are often huge as a consequence of indoor solid fuel combustion like wood, crop residues, and dung which are extensively used for heating and cooking. The percentage of people using solid fuels varies widely among countries and regions ranging from respectively 77%, 74%, and 74% in sub-Saharan Africa, South-East Asia, and the Western Pacific Region, while in the majority of industrialized countries, the use of solid fuel falls below 5% [Perez-Padilla et al., 2010]. Women are particularly exposed to indoor air pollution, since in rural areas of developing countries they have larger amounts of time for indoor cooking and attending home heating. [Perez-Padilla et al., 2010].

In Europe and North America the introduction of air quality policy, limiting the emissions of the main air pollutants from road transport and large industrial combustion, has significantly improved the air quality and reduced air pollution-induced health effects. However a report from WHO indicated that air pollution contributed annually in Europe to 100,000 premature deaths and the loss of 725,000 working days [Eurostat, 2009].

Data arising from constant monitoring of outdoor pollutants are increasing from many other regions of the world, since the knowledge that air pollutants are dangerous for health is gaining global awareness. The type of pollution and the mix of the gases released in the atmosphere differ among countries. As an example, data from Tokyo showed that sulfur dioxide, one of the main pollutants in urban areas, had and annual average concentration below 10 $\mu g/m^3$, for the years 2000-2005 which corresponds to concentrations recorded in the least polluted cities in Europe such as Copenhagen and Barcelona. On the other hand the annual average ambient nitrogen dioxide concentration in Tokyo was 60 $\mu g/m^3$, a value higher than those in the most polluted cities in Europe such as Paris and Athens [Katanoda et al., 2011].

1.3 Initial reports of health risk associated with air pollution

One of the first recorded episodes that gave undeniable evidence of the potential for atmospheric pollution to cause deaths and diseases was registered in December 1930 in the

Meuse valley in Belgium [Bell & Davis 2001], [Nemery et al., 2001]. The area of Liège on the River Meuse, was once one of the most heavily industrialized areas of continental Europe with steel mills, coke ovens, foundries, smelters, fertilizer and explosives plants established since the industrial revolution. The weather was characterized by temperature inversion conditions, with a very feeble wind that blew from the city of Liège into the narrow valley. The temperature inversion at about 70–80 m above ground, just above the tallest chimneys in the valley, prevented the fumes from rising, trapping the emitted gases and impurities to accumulate in the corridor formed by the valley between Liège and Huy. More than 60 people died which was more than 10 times the normal mortality rate. The average age of the victims was about 62 years and ranged from 20 to 89 years.

The report released by the committee of experts appointed to investigate the disaster, noted that also several hundreds of people were affected severely, with respiratory problems such as "asthma like" symptoms, wheezing, laryngeal irritation, chest pain and coughing. The report postulates that fine soot particles, onto which irritant gases had been adsorbed, had a major role in the noxiousness of the fog. Sulfur compounds proved the most abundant compounds emitted in the valley. Investigators estimated that more than 60,000 kg of sulfur dioxide was produced per day, with a resultant sulfur dioxide (SO_2) concentration of up to 100 mg/m^3 after 4 days of fog. This SO_2 concentration exceeds the current WHO standard for toxicity (20μg/m^3 for daily mean concentration) and could account for the victims' symptoms. Additional components of the thick fog of air pollutants included sulfuric acid mist, and fluoride gases together with carbon soot.

In the United States a similar episode occurred in the town of Donora located in the Monongahela River Valley in Pennsylvania. In late October Donora was submerged in noxious smog. As in the Meuse valley disaster, an intense, meteorological temperature inversion settled on the valley trapping the pollutants. The smog was a deadly mixture of emissions from the steel plants and coal furnaces located in the valley and in the town, consisting of carbon soot, sulfur dioxide, carbon monoxide and metal dust and particulate. The smog was so thick that it was reported that the residents had to keep lights on all day. In the five days between October 26 and 31, twenty people died, some 400 required hospitalization and more than 7,000 in a town of 14,000 residents were sickened before rain dispersed the killing smog on October 30 and 31, 1948 [Helfand et al., 2001].

The investigations following the Donora Smog Disaster once more showed the association between air pollution and increased mortality. The data from the investigations were essential to determine the first federal clean-air act, enacted in 1955 by the Congress, to address the national environmental problem of air pollution and to provide funds to the Public Health Service to conduct research into the causes and the control of air pollution in the United States.

In Europe the deadliest episode that raised the awareness about the threat posed by air pollution, occurred in London in December 1952. The London fog of 1952 is widely regarded as a catalyst for the study of air pollution epidemiology. Again, a temperature inversion settled over windless London and trapped the carbon soot from coal burning, residential heating and cooking, industrial smoke and vehicle exhaust. About 4,000 people died prematurely in a single week followed by another 8,000 deaths during the next few months. The smog-related deaths were primarily attributed to pneumonia, bronchitis, tuberculosis, and heart failure. Many with pre-existing conditions, including asthma, died of respiratory distress. Many others died of cardiac distress and asphyxiation. Non-fatal health effects from the smog included

short-term chest pains, lung inflammation and diminished breathing ability, damaged respiratory cells, permanent lung damage, and increased incidence of asthma attacks [De Angelo, 2009]. In a report of the disaster The Ministry of Health noted that morbidity and mortality remained elevated in the greater London region until March 1953.

The acute air pollution episodes which occurred in the Meuse valley, in Donora and in 1952 London are extreme examples that are unlikely to occur in cities and in countries where the air quality is under constant control. However the risk for acute and chronic exposure to deadly concentration of air pollutants is higher in rapidly economically growing regions or areas where massive urbanization occurs and monitoring for pollution and health is not systematically conducted.

What was observed in those episodes was the first evidence that air pollution and particle pollution, are serious threats to the health of the population, especially for the elderly children and individuals with pre-existing cardiovascular and respiratory conditions. Carbon soot, sulfur dioxide and fine particulate pollution were the major culprits for the adverse health effects occurring in the population in the Meuse valley, Donora and London disasters. Studies in the following decades have identified in air particle pollution one of the major risk for health.

The extent of the effects of air pollution depends on the actual exposure. In a landmark study "The Harvard Six Cities Study", Dockery and colleagues indicated for the first time a strong positive correlation between levels of air pollution and cardiovascular mortality. In this longitudinal cohort mortality study, the morbidity and mortality of the population of six US cities was associated with long-term exposure to fine particulate air pollution including sulfate. The results showed that deaths from lung cancer, pulmonary disease, and heart disease were 26 percent higher in the city with the highest level of pollutants. Rather than indicating sulfur dioxide as the culprit emission, The Harvard Six Cities Study directed the attention to particulate matter. This is another important contribution of the study that identified in particulate matter what is now universally recognized as a dangerous form of air pollution.

2. Sources of air pollution

Air pollution comprises of a very complex mixture of thousands of chemicals and chemicals interactions.

At least six main air pollutants have been identified by the WHO, by the European European Environmental Agency (EEA) and the US EPA agencies, as noxious for human health, these are: lead, carbon monoxide (CO), sulfur dioxide (SO_2), nitrogen dioxide (NO_2), ozone (O_3) and particulate matter (PM). All of these but lead are major components of automotive pollution, and have been included in the guidelines for maximum concentration in urban areas, by the World Health Organization (WHO) and many national environmental agencies around the world.

2.1 Main components of urban air pollution

2.1.1 Lead

In the past, motor vehicles were the biggest source of lead. But since leaded gasoline has been phased out, lead emissions have decreased by about 98 percent. Today, metal processing is the biggest source of atmospheric lead. The highest air concentrations are found in the vicinity of ferrous and nonferrous smelters and battery manufacturers [Clean

Air Trust, 1999]. Lead can harm the kidneys, liver, nervous system and other organs. It may cause neurological impairments such as seizures, mental retardation and behavioral disorders. Even at low doses, lead is associated with damage to the nervous systems of fetuses and young children, resulting in lowered IQ and learning problems. Recent studies also show that lead may be a factor in high blood pressure and subsequent heart disease [Clean Air Trust, 1999].

2.1.2 Carbon monoxide (CO)

Carbon monoxide is a gas emitted directly from vehicles as a result of incomplete combustion of fuel. When it is inhaled in very high doses, CO replaces oxygen in the bloodstream by binding with hemoglobin, thus interfering with normal transport of oxygen to the heart and the brain and to the whole body. Exposure to high CO levels is lethal. Low levels found in ambient settings do not affect healthy individuals and indeed have been shown to have anti-inflammatory effects. Although CO has been shown to hasten the onset of angina (chest pain) in people with coronary artery disease and the incidence of cardiac effects. It is unclear whether this is an effect of CO or traffic related pollutants for which CO is a marker. Some epidemiologic studies have found positive correlation between CO level and morbidity, mortality and adverse pregnancy outcome [HEI, 2004]. *In vivo* experiments showed that urban carbon monoxide air pollution exacerbates rat heart ischemia reperfusion injury. Interestingly, recent observations of CO delivered at very low concentration in a controlled way showed positive beneficial effects in animal models in the modulation of inflammation [Motterlini & Otterbein, 2010]. In another study using a rat model of myocardial infarction and exposure to CO at 35 PPM the current EPA standard, Wellenius and colleagues showed that CO at that concentration reduced arrhythmias observed in the first 24 hrs after the infarction. [Wellenius et al., 2004]

2.1.3 Sulfur oxides (SOx)

Sulfur oxides SOx, comprising sulfur oxide (SO), sulfur dioxide (SO_2), and sulfur trioxide (SO_3), are gaseous by-products of the combustion of fossil fuels that contain sulfur. Clinical studies have shown that exposure to SO_2 at level as low as 0.7 mg/m^3, (0.25 part-per-million (ppm), elicits increased broncho-constriction in people with asthma [Gong H. Jr. et al., 1995]. Acute decline in pulmonary function has also been observed in association with air pollution episodes in which levels of SO_2 were briefly but considerably elevated [Bascom, 1996]. Sulfur dioxide is also associated with increased daily mortality and hospital admissions from respiratory and cardiovascular disease, even at low levels. In an analysis of a large US cohort study long term exposure to SO_2 has been associated with reduced pulmonary function and mortality from cardiovascular disease. Reduction in ambient SO_2 concentrations owing to regulatory action has recently been associated with decreased mortality and improved respiratory health in children in Hong Kong [HEI, 2004].

2.1.4 Nitrogen oxides (NOx)

Nitrogen oxides consisting of nitrogen oxide (NO) and nitrogen dioxide (NO_2) are by-products of fossil fuel combustion in transportation, industrial applications, and waste incineration. Like SO_2 urban cause of outdoor NO_2, are mobile source emissions from both on-road vehicles such as cars, trucks, buses and off-road equipment, consisting mainly of ships, airplanes, locomotives, agricultural and construction machinery. Unlike SO_2, NO_2 is relatively insoluble

and is thus more likely to reach the lower airways. Moreover NO_2 is an oxidant. In clinical experiments it elicits inflammatory responses at levels of 1mg/m^3 (0.9 ppm), and increases responsiveness to ozone and allergens [Bascom, 1996]. In epidemiological studies NO_2 has been associated with increased respiratory morbidity such as asthma exacerbation and reduced lung function and rate of lung growth in children. Short-term increase of NO_2, like SO_2, is associated with increased daily mortality and hospital admission for respiratory and cardiovascular diseases [Zemp et al., 1999; HEI, 2004].

2.1.5 Ozone (O_3)

Ozone and photochemical oxidants are typical pollutants in photochemical smog. Ground level ozone can be formed by sunlight-induced oxidation of precursor pollutants such as NOx and carbon hydroxides in the atmosphere. Ozone is a strong oxidant and an unstable molecule. Important ozone precursors and reactive intermediates are compounds of the organic hydroxyl group, hydroxyl radical (OH), hydroperoxyl radical ($HO_2^{\bullet}$) and singlet oxygen. Volatile organic compounds (VOCs) are a variety of readily evaporable toxic compounds from both anthropogenic sources like unburned fossil fuel, fuel combustion processes and biogenic emission from vegetation. VOCs are responsible for triggering chemical reactions in the atmosphere that contribute to ozone formation. The interaction of these compounds with NOx forms additional ozone in the presence of heat and sunlight, conditions typical of the summer season when ozone tends to accumulate over urban areas.

Studies have shown that ozone has a negative impact on human health, being associated with an increase in pulmonary diseases, reduced pulmonary functions, exacerbation of asthma and increase in hospital admission. Collectively these studies have revealed a small association between daily mortality and ozone level, which is independent of the effect of particulate matter.

There are additional concerns about increased ozone exposure and respiratory morbidity in children. Asthma, the most common chronic childhood disease, has significant public health impacts and is characterized by chronic lung inflammation, reversible airflow obstruction, and immune sensitization to allergens. According to time series evidence, exposure to concentration of ozone below 80 ppb may produce short-term lung function deficits and increased risk of respiratory illnesses including asthma episodes severe enough to require medical attention. Early childhood chronic ozone exposure may produce reduced lung function growth, especially in those parameters reflecting small airway flow rates [Wigle et al., 2007].

2.1.6 Particulate matter (PM)

Particulate matter is a complex mixture of organic and inorganic solid particles and liquid droplets deriving from many different sources and materials. Ambient air particles vary greatly in size and chemical composition depending upon their formation process, the seasonal changes, and the characteristics of the region. PM can be characterized by origin as geogenic, from soil and geological material, as opposed to anthropogenic, when originated by human activities. PM can be discriminated by type of airborne transformation in primary or secondary particles, or can be characterized by physicochemical properties such as solubility, aerodynamic diameter or particle size. In some geographical regions, a single industrial source of PM may dominate, but more commonly the PM is a mixture of local and

transported particulate as well as primary and secondary particles. Because of the variability and complexity of sources and weather patterns, the resultant particulate mixture in any given area has significant daily variability in PM quality.

Primary particles originate directly from a defined source, for example from incomplete combustion of fuels in vehicle exhausts or from the combustion of organic substances. Additional sources are road dust, industrial smoke emissions, smokestacks, forest fires, windblown soil, volcanic dust and sea spray, to mention a few.

Secondary particles are known to make up most of the fine airborne particle pollution mass. These particles are formed in complex reactions in the atmosphere by chemicals such as sulfur dioxides and nitrogen oxides. Organic carbon (OC) and elemental carbon (EC, also called black carbon or soot), form the major fraction of secondary particulate matter. OC and EC are abundant in many source emissions from combustion of organic material. Carbon accounts for 10 to 40% of PM mass on a global scale but this fraction exceeds 50% in many urban atmospheres due to energy-related fossil-fuel and bio-fuel combustion, while in rural areas large-scale wildfires consume millions of acres of forest generating smoke that mostly consists of carbonaceous material [Mauderly & Chow, 2008].

Organic aerosols include also non PM components like volatile organic compounds (VOCs) such as propane and benzene and semi-volatile organic compounds (SVOCs), in equilibrium in the atmosphere between gas and particulate phases, such as polycyclic aromatic hydrocarbons (PAHs), well known carcinogens. VOCs and SVOCs form a dynamic continuum in the atmosphere with particulate and they can be adsorbed on the surface or inside PM [Mauderly & Chow, 2008].

The term PM_{10} refers to all airborne particulate matter less than 10 microns in aerodynamic diameter. The coarse particle fraction has an aerodynamic diameter between 10 μm and 2.5 μm. Fine particles ($PM_{2.5}$) comprise those particles with diameters less than 2.5 μm, while ultrafine particles (UFP or $PM_{0.1}$) are particles with an aerodynamic diameter less than 0.1 μm. In urban areas PM_{10} originates from crushing or grinding operations and dust stirred up by vehicles traveling on roads, with the organic carbon fraction in the form of biological components such as detritus, fungi, pollen, and spores. PM_{10} , once inhaled deposit in the upper part of the respiratory tract such as the nose, trachea, and bronchi. As particles in this fraction land on these mucous and cilia covered surfaces, they may be propelled upwards by the movement of cilia in the upper airways with the mucus and particles moved into the pharynx where it may be swallowed or expectorated. PM_{10} may not cause as extensive cardiovascular effects as $PM_{2.5}$, but has been strongly associated with hospital admissions for respiratory symptoms [Brunekreef & Forsberg, 2005] and there is growing evidence for effects of coarse PM on cardiovascular mortality and morbidity especially in arid regions where coarse PM concentration are relatively high [Gold et al., 1999].

Fine particles, $PM_{2.5}$, consist of a complex mixture of many different constituents, and are often formed from agglomerates of nanosized particles. Foremost among these are secondary particles, formed by secondary sulfate nitrate, and ammonium. Most sulfates derive from the oxidation of sulfur dioxide that is produced by natural sources and by anthropogenic combustion of fossil fuels. Sulfur dioxide when reacting with hydrogen peroxide, ozone, or oxygen results in particulate sulfuric acid. Sulfuric acid can be neutralized by ambient ammonia to ammonium sulfate and bisulfate that contribute to the $PM_{2.5}$ associated acidity. Nitrate associated with $PM_{2.5}$ results largely from the oxidation of nitrogen dioxide derived

primarily from combustion processes of fossil fuels. Nitrogen dioxide is converted into vapor-phase nitric acid by reaction with hydroxyl radicals under daylight conditions, while at night it is oxidized to nitric acid by a sequence of reactions initiated by ozone that produces nitrate. Ammonium nitrate produced by reaction of atmospheric ammonia with nitric acid also contributes to the acidity of the particles [Schlesinger, 2007b].

Fine particles also have an organic component of primary and secondary carbonaceous aerosols as well as an inorganic fraction that consists of crustal material derived from soil dust, rocks, or particles produced by mechanical generation from agriculture, mining, construction and road traffic. Aluminum and silicon are the two major crustal derived elements found in $PM_{2.5}$ and, together with calcium, can become aerosolized from various combustion processes, while potassium is a signature for biomass burning such as wood burning and cooking [Schlesinger, 2007b]. Transition metals are an important but relatively small fraction of $PM_{2.5}$. The major metal constituents include Cr, Co, Ni, Mn, Zn, V, Cu, and mainly Fe [Gurgueira et al., 2002].

Ultrafine particles (UFPs or $PM_{0.1}$), with an aerodynamic diameter smaller than 0.1μm, constitute about 10% of fine particulate mass but represent over 85-90% of the total $PM_{2.5}$ particle number. UFP particles are constituted mainly by organic carbon and elemental carbon. The larger UFP numbers in the atmosphere, despite a smaller mass, raise concerns since their exposures have increased dramatically with the increased sources of their emission such as internal combustion engines, power plants, incinerators and many other thermo-degradation practices [Terzano et al., 2010].

How deep a given particle can penetrate into the airways depends largely on its aerodynamic diameter. The alveolar region is of particular interest, as in this region the lungs are most permeable in order to facilitate gas exchange between the blood and the inhaled air. Fine and ultrafine particles dominate the deposited particles in the alveoli both in term of number and mass. They have high deposition efficiencies of 30–60% (between 20 and 40 nm) and 20–30% (between 1 and 3 μm) of the inhaled particles respectively (ICRP 1994 model for particle deposition). In the deeper region of the lungs a major fraction of the deposited particles is engulfed by macrophages, specialized defense cells, which recognize and internalize non-soluble particles which which are either retained or digested and solubilized. Retained fine or ultrafine particles may pass into the lymphatics of the lung, or be taken within specialized macrophages to the lymphatics , and accumulated in the lymphatic nodes of the lung. A remaining small fraction of UFP is retained long term in the epithelium and interstitial spaces [Schmid et al., 2009].

Hypotheses have been proposed suggesting that UFP have the ability to translocate to the blood stream and target other organs in the body [Godleski, 2006]. One of the most important features for particulate toxicity is the surface reactivity that depends on the chemical composition, deposition site, size, shape, and surface area of the particles. The ratio between the surface area and the mass of the particle is an important determinant for the toxicity because chemical reactions and leaking of constituents occurs from the surface. In addition the percentage of surface molecules increases when the particles' diameter decreases, making the fine and ultrafine particles more hazardous.

The following sections will focus mainly on the effects of particulate matter on human health with particular attention on the cardiovascular system. A vast body of literature has shown correlations between fine particulate matter and a wide range of PM-related human

health effects in the general population, including the aggravation of heart diseases, cardiovascular morbidity and mortality. Recent studies have suggested that it is not simply the particulate mass that is responsible for the adverse health effects, but its chemical composition is also an important determinant. Elemental carbon, organic carbonaceous fraction and transition metals are the most frequently cited suspects for the detrimental effects of PM, in particular through their ability to generate free radicals. Moreover, sulfur dioxide and derived sulfates have been associated with health outcomes in the consensus assessments of the WHO [WHO, 2003]. However the causal role of sulfur compounds has been criticized on the basis of their low toxic potency [Schlesinger & Cassee, 2003; Happo et al., 2010]. Thus, it is PM that has become regarded as possible leading mechanisms of disease exacerbation in patients with cardiovascular disorders.

3. Reactive oxygen species, oxidative stress and airborne environmental pollutants

Free radicals and oxidative stress have been implicated in inflammatory response after exposure to particulate matter [Donaldson et al., 2005]. Several studies carried out in cell culture, in animal models as well as in humans have established that once inhaled and deposited in to the lungs, fine and ultrafine particles can trigger pro-inflammatory responses [Gonzalez-Flecha, 2004].

Both organic materials and transition metals present in PM are able to generate free radicals. Electron paramagnetic measurements indicate that $PM_{2.5}$ contains persistent radicals in the concentration range of $1{,}3x10^{16}$ and $1{,}5x10^{17}$ radical/gram ($1{,}0x10^{17}$ = 0,16 mM). This radical concentration is comparable to that found in cigarette smoke particulate matter, which is also in the range of 10^{16} to 10^{17} [Squadrito et al., 2001]. Free radicals are reactive molecules with an unpaired electron. In a stable configuration electrons (e^-) orbit around atoms in pairs with opposite spin. When only one electron is present in the outer orbital the atom has an unstable configuration, and tends to be reactive, attracting one electron from other molecules in order to reorganize itself in a stable configuration.

In biological settings free radicals are potentially very dangerous since they can react indiscriminately with neighboring molecules in order to acquire one electron. This process of electron stealing leads to oxidation and often inactivation of target molecules and cellular damage. In biological systems Reactive Oxygen Species (ROS) or free radicals from oxygen are reactive molecules generated as intermediates in reduction–oxidation (redox) reactions, in which one chemical species is oxidized, and its electrons are passed to another chemical species, which is thereby reduced, leading from molecular oxygen to (O_2) to water (H_2O). Here is the sequence of reactions (1). The donation of a single electron (reduction) to molecular oxygen, results in the formation of a superoxide ($^{\bullet}O_2^-$). Superoxide radical undergoes spontaneously or enzymatically, by the action of superoxide dismutase (SOD) to dismutation, a chemical reaction between two identical molecules to produce two different products, to generate hydrogen peroxide (H_2O_2) and oxygen. The reduction of hydrogen peroxide by a third electron results in the formation of the highly reactive hydroxyl radical ($^{\bullet}OH$). Finally the donation of a fourth electron yields water.

$$O_2 \xrightarrow{e^-} {}^{\bullet}O_2^- \xrightarrow{e^-} H_2O_2 \xrightarrow{e^-} {}^{\bullet}OH \xrightarrow{e^-} H_2O \qquad (1)$$

An additional form of ROS is singlet oxygen, a very short lived and reactive form of molecular oxygen in which the outer electrons are raised to a higher energy state can be formed by a variety of mechanism including the Haber-Weiss reaction (2) occurring in biological systems.

$$H_2O_2 + {}^{\bullet}O_2^- \longrightarrow O_2 + {}^{\bullet}OH + OH^- \quad (2)$$

Another reaction that occurs in biological systems leading to production of highly oxidizing species and formation of $^{\bullet}OH$ radicals is the Fenton reaction (3). Some transition metals including iron can catalyze Fenton reactions.

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + {}^{\bullet}OH + OH^- \quad (3)$$

$$Fe^{3+} + H_2O_2 \longrightarrow Fe^{2+} + {}^{\bullet}OOH + H^+$$

3.1 Cellular sources of ROS and antioxidant defenses

ROS are formed physiologically in biological systems. It is widely accepted that low level of ROS are involved in important physiological functions such as signal transductions to promote cell proliferation and survival. Excessive amounts of ROS, referred to as oxidative stress, contribute to depletion of antioxidants, cause aberrant cell death and have been implicated in a variety of pathological events such as arteriosclerosis, ischemia-reperfusion injury, cardiovascular diseases, inflammation and neurodegenerative diseases [Chen & Nadziejko, 2005].

The mitochondrion is the major cellular source of ROS. In the mitochondria $^{\bullet}O_2^-$ can be generated enzymatically during oxidative phosphorylation in the electron transfer chain, as a byproduct of normal cellular aerobic metabolism, with the rate of respiration responsible for the rate of generation of ROS [Mccarthy et al., 1976]. The mitochondrial enzyme superoxide dismutase (mSOD) rapidly scavenges $^{\bullet}O_2^-$ accelerating the removal of this radical by dismutation to generate H_2O_2, a less reactive molecule.

Mitochondria are involved also in the generation of nitric oxide ($^{\bullet}NO$) via the nitric oxide synthase (NOS) reaction. Superoxide anion and $^{\bullet}NO$ react to form another harmful oxidant, peroxinitrite ($ONOO^-$) which is also a potential source for more powerful and aggressive hydroxyl radical ($^{\bullet}OH$).

Several non-mitochondrial sources of ROS exist in the cell. The endoplasmic reticulum is a source of ROS, where resident cytocrome P-450 oxidizes unsaturated fatty acids and xenobiotics such as pollutants, drugs and toxins to generate $^{\bullet}O_2^-$ and H_2O_2.

Peroxisomes are cytoplasmic membrane-enclosed organelles that contain at least 50 different enzymes involved in a variety of biochemical pathways in different types of cells and oxidation reactions leading to the production of hydrogen peroxide, like the peroxisomal oxidation of fatty acids [Gilca et al., 2007]. The peroxisome also contains catalase, an ubiquitous antioxidant which decomposes H_2O_2 and prevents accumulation of this toxic molecule by either converting it to water or by using it to oxidize other organic compounds.

Membrane associated oxidases such as NADPH oxidases generate ROS by reacting with the intracellular NADPH, to reduce molecular oxygen superoxide anion ($^{\bullet}O_2^-$) [Cash et al., 2007]. This reaction is typical of activated phagosomes in neutrophils and macrophages during the respiratory burst, in response to xenobiotics such as particles of pollution, inflammatory agents or infectious conditions. Upon activation, neutrophils initiate an

oxidative burst by consuming molecular oxygen resulting in the formation ROS as a defense mechanism to achieve localized microbicidal function.

As a consequence of the activation of neutrophils and oxidative burst, myeloperoxidase (MPO), a hemeprotein present in the azurophil granules in neutrophils, is released either into the lysosomes of the phagocytic cell or into the extracellular space. MPO catalyzes the formation of hypochlorous acid (HOCl) from H_2O_2 and chloride ions, a further strong oxidizing and chlorinating species. MPO activation may lead to irreversible protein and lipid modification, increasing levels of oxidized low density lipoprotein, through free radical formation. Elevated MPO is considered as a risk factor for cardiovascular diseases [Al-Hweish et al., 2010].

Cells have a defense mechanism against oxidative stress that is constituted by the endogenous antioxidants. Antioxidants are molecules that protect biological targets against oxidative damage. They play a role in preventing damage by suppressing the formation of ROS or by scavenging them, rapidly before they attack biologically essential molecules. Finally they play a role in the repair of the damage, clearing the waste and reconstructing the lost functions [Niki, 2010].

Among the enzymatic antioxidants is Cu/Zn SOD, a cytoplasmic metalloenzyme that catalyzes the dismutation of superoxide anions to molecular oxygen and hydrogen peroxide and thus is a critical component of the oxidative stress defense mechanism. Mn/SOD is a mitochondrial isoenzyme with the same function but with potentially different sensitivity to soluble metals. In addition, there is a third SOD isoform located on the extracellular surface, EC-SOD. Glutathione peroxidase, an enzyme that catalyzes the reduction of hydroperoxides including hydrogen peroxide using glutathione as reducing substrate and glutathione reductase, a flavoprotein containing redox active disulfide bonds that catalyzes NADPH-dependent reduction of oxidized glutathione and therefore is essential for maintaining levels of reduced glutathione in the cell [Hatzis et al., 2006]. Glutathione (GSH) and the oxidized form gluthatione disulfide (GSSG) are major antioxidants. GSH as the most abundant free thiol in eukaryotic cells maintains an optimal intracellular redox environment. Reduced GSH is the biologically active form that is oxidized to GSSG during oxidative stress [Circu & Aw, 2008]. Thioredoxin and thioredoxin reductase together form an additional redox regulatory system. Thioredoxin and thioredoxin reductase can catalyze the regeneration of many antioxidant molecules including ubiquinone (Q10), lipoic acid and ascorbic acid and, as such, constitute an important antioxidant defense against ROS [Santos et al., 2011]. Catalase is an enzyme comprised in the enzymatic antioxidant protection of the cell, able to metabolize hydrogen peroxide to water and oxygen. The non-enzymatic hydrophilic antioxidant agents include ascorbate, urate, bilirubin, and melatonin. Lipophilic non enzymatic antioxidant radical scavengers include tocopherols (such as α-tocopherol), carotenoids, (such as β-carotene), ubiquinol, and flavonoids.

3.2 Ambient sources of ROS oxidative stress

The human lung with a surface area of 40-120 m^2 is exposed to between 10,000 and 20,000 liters of ambient air each day [Salvi & Holgate, 1999],and ambient air contains a wide range of pollutants. Many of individual pollutants are free radicals or have the ability to promote free radical reactions that can trigger redox cycling.

Several pathways have been considered for inhaled particles to produce ROS and cause oxidative stress. Particles deposited into the lungs can introduce oxidizing species such as transition metals, ozone or organic compounds [Prahalad et al., 1999]. Highly oxidizing

species like PAHs can be adsorbed on the particles surface. These species can be transformed *in vivo* into quinones through the action of cytochrome P450, epoxide hydrolase and oxidoreductase enzymatic pathways. Finally particles can activate inflammatory cells such as phagocytic cells and leukocytes, inducing oxidative burst with the generation of oxidative stress and inflammation [Mudway et al., 2005]. Indeed, it is well known that phagocytosis of even inert particles by lung macrophages will produce a burst of ROS which increases with increasing phagocytic events and is further increased by opsonization of the ingested material [Kobzik et al., 1990].

Several studies suggested the role of metals in the oxidative capacity of PM. Transition metals present in the particles, mostly in the oxidized form as oxides and sulfates, catalyze Fenton-type reactions. Transition metals have been shown in several studies to increase ROS formation in cell free systems, in cell suspension and in animal models [Gonzalez-Flecha 2004]. Wilson and colleagues [Wilson et al., 2002], showed that intratracheal administration of iron iron-bearing with ultra-fine particles amplifies the cell recruitment in the broncho-alveolar lavage fluid (BALF), while Saldiva and colleagues [Saldiva et al., 2002], recognized a significant dose-dependent association between PM metal component especially vanadium, with neutrophils and lymphocytes present in BALF. An *in-vivo* study showed that the inhalation of concentrated respirable size urban particulate matter containing, among other elements Mn, Fe, Cu, and Zn, produced a significant increase of oxidative stress in the lung and in the heart of experimental animals [Gurgueira et al., 2002].

Among organic compounds found on the surface of particles are quinones, a class of cyclic organic compounds. Quinoid redox cycling has been previously implicated in the toxicity of combustion products such as cigarette smoke, and $PM_{2.5}$ has been shown to contain large quantities of semiquinone radicals able to induce redox cycling. During redox cycling the semiquinone intermediate reduces molecular oxygen to the superoxide radical and ultimately yields the hydroxyl radical [Squadrito et al., 2001]. Quinones or hydrogen peroxide applied directly to lung macrophages or macrophage cell-lines in vitro induce production of pro-inflammatory cytokines by these cells.

4. Effects of PM and air pollutants on the cardiovascualr system

"There's so much pollution in the air now, that if it weren't for our lungs, there'd be no place to put it all." (Orben R.). The effects of outdoor air pollution on the pulmonary and cardiovascular systems have been analyzed in both short-term and long term studies using data from population in many regions of the world. In all cases an association has been found for pathologies arising in the pulmonary and in the cardiovascular systems with the severity of concentration of air pollutants and in particular with the exposure to PM.

4.1 Short-term exposure to air pollutants effects on the cardiovascular system

Short-term studies establish association between daily health related events, for example cardiovascular mortality or hospitalization, and daily changes in the levels of ambient air pollutants. Typically associations are evaluated for exposures ranging from the day of the health events to a few weeks prior to the health related event.

Time series studies estimated that an increase of $10\mu g/m^3$ in mean $PM_{2.5}$ concentration during the preceding day contributes on average to the premature death of about 1 susceptible person per day in a region of 5 million people (based on annual US death rates

in 2005). Short-term increase in $PM_{2.5}$ levels lead to the early mortality of tens of thousands of individuals per year in the United States alone [Pope et al., 2006].

Traffic emissions are the major cause for exceedingly high pollutant levels in urban areas. Several studies have found positive association between elevated PM traffic exposures over a period as brief as a few days or even a few hours and elevated risk for myocardial infarction [Peters et al., 2001], [D'Ippoliti et al., 2003], [Zanobetti & Schwartz, 2005]. Acute increases of risk for ischemic heart disease events have been observed consistently even as rapidly as 1 to 2 hours after exposure to elevated PM in case crossover analysis [Symons et al., 2006]. Daily hospitalizations for heart failure have also been associated with short-term changes in $PM_{2.5}$ exposure, in a large daily time-series analysis of and cardiovascular and respiratory hospitalization by use a US Medicare file database [Symons et al., 2006].

Among the groups more susceptible to air pollution are the elderly, and people with preexisting pulmonary and cardiovascular diseases. Peters and colleagues observed an association between exposures to traffic while traveling in cars, buses, and trolley cars and while riding on a bicycle or motorcycle and the increased risk of a myocardial infarction within one hour afterward in a susceptible population [Peters et al., 2004].

The younger, healthier population is not immune to the risks associated with short-term exposures to air pollutants. Some studies have addressed risk for adverse health effect of outdoor air pollution exposure in athletes or subjects exercising in urban areas and cyclist commuters, who often are close to busy traffic. They are often acutely exposed to exhaust from combustion engines during exercising or commuting and this may therefore represent an important fraction of their daily exposure to outdoor particulate pollution. The total number of particles deposited in the lungs increases in proportion to minute ventilation and the deposition fraction nearly doubles from rest to intense exercise [Rundell et al., 2006; Daigle et al., 2003].

In New York City runners, after 30 min of exercise near busy roadways, an acute rise in blood carboxyhaemoglobin from 1.7% to 5.1% occurred, a concentration similar to those found in regular cigarette smokers [Nicholson, 1983]. In a study of marathon runners the levels of PM_{10} were significantly associated with decreased performance for women but not for men. For every 10 ug/m3 increase in PM_{10} women marathon performance can be expected to decrease 1,4%. Men's and women's performances were not associated with any other pollutants. Only PM_{10} was found to correlate with performance.

Barath and colleagues showed that short-term exposure of human subjects to diesel exhaust impairs vasomotor functions of both endothelium dependent and independent vasodilators and endogenous endothelial fibrinolytic function parameters. Inthat study young healthy volunteers exercising on bicycle ergometers were exposed to diesel exhaust derived from engine condition mimicking vehicles functioning in urban environment, with acceleration retardation and variation in engine load as well as periods of constant speed and idling [Barath et al., 2010]. Conversely a similar study found no changes in micro-vascular vasodilation, blood coagulation parameters or markers of systemic inflammation related to particle exposure in healthy volunteers exercising on an ergometer bicycle and breathing air from one of Copenhagen's busiest roads [Brauner et al., 2008].

A study explored the possibility of reducing the cardiovascular effects of short-term air pollution exposure to urban pollution by wearing a simple facemask. Cardiovascular parameters were measured in a group of young subjects, who walked a predefined city center route in Beijing, wearing a facemask. This simple precaution led to a reduction in

systolic blood pressure during exercise and increase heart rate variability. Translated into a susceptible population this finding suggests that wearing a simple facemask has the potential to reduce the incidence of acute cardiovascular events [Langrish & Mills 2009].

4.2 Long-term exposure to air pollutants: evidence for effects on the cardiovascular system

Long-term effects of air pollution are evaluated by monitoring large cohorts of participants over several years. These studies relate progression of disease, hospitalization and long term mortality to average concentration of air pollutants.

The first large prospective cohort study that demonstrated an adverse health impact of long-term air pollution exposure was the Harvard Six Cities study [Dockery et al., 1993]. This study established that chronic exposure to air pollutants is independently related to cardiovascular mortality. Of the 1,401 validated deaths, 646 were due to cardiovascular causes. The American Cancer Society (ACS), Cancer Prevention II study population, the largest study of the long-term health effects of air pollution, included about 500,000 adults resident across the US. Long term exposure to multiple air pollutants was investigated against mortality statistics for a 16 year period [Pope et al., 1995].

The ACS follow-up study increased the degree of control for confounding variables, such as diet and gaseous co-pollutants [Pope et al., 2002]. The primary results showed that each 10μg/m^3 increase in annual $PM_{2.5}$ mean concentration was associated with increases in all-cause, and cardiopulmonary mortality of 4% and 6% respectively. The relationship between $PM_{2.5}$ and adverse health effects was linear and without an apparent lower "safe" threshold. Mortality was most strongly associated with $PM_{2.5}$, sulfate particles, and SO_2. There also appeared to be an association between cardiopulmonary mortality and summertime O_3, when based on mean summer O_3 levels from 1982 to 1998.

Several studies following the lead of these two predecessors, investigated the incidence of specific cardiovascular diseases linked to long term exposure to outdoor pollution. A re-analysis of the ACS study suggested that long term exposure to elevated $PM_{2.5}$ may promote both ischemic and non ischemic cardiovascular events. Ischemic cardiac events occurring when the heart muscle receives insufficient blood, accounted for the largest absolute risk for mortality per 10ug/m^3 elevation in $PM_{2.5}$. Slightly lower was the relative risk for deaths due to arrhythmias, heart failure or cardiac arrest.

Survival analysis of US Medicare data from 196,000 survivors of acute myocardial infarction showed that the risk of a post-myocardial death or hospital admission for congestive heart failure was increased with higher exposure to PM_{10} [Zanobetti & Schwartz, 2007]. Data from the Worcester (USA) Heart Attack study also found that long-term exposure to traffic-related air pollution was associated with significantly increased risk of acute myocardial infarction [Tonne et al., 2007].

A number of epidemiological studies using prospective cohort designs suggest a relationship between long-term exposure to sulfate and total cardiopulmonary mortality. Very often however total $PM_{2.5}$ was also associated with these same health endpoints making unclear as to whether effects could be attributed specifically to sulfate [Schlesinger, 2007a].

In the last 40 years the implementation of strict standards for pollutants such as PM, ozone, nitrogen oxides and sulfur oxides by the governments and the environmental agencies of developed countries contributed to reduce their concentration in the cities. A dangerous level of air pollutants in the vast megalopolis is a challenge that developing countries are facing. The first cohort studies have been recently published examining the association of outdoor air

pollution with mortality in China [Cao et al., 2011]. In the study, 70,947 middle-aged men and women in the China National Hypertension Survey and its follow-up study were enrolled; mortality for cardiovascular diseases was associated with total suspended particle concentration, nitrogen dioxide and sulfur dioxide. From 1991 to 2000, a significant association was found between air pollution levels and mortality from cardiopulmonary diseases and from lung cancer. Each 10 $\mu g/m^3$ elevation of total PM, SO_2 and nitric oxides was associated with a 0,9%, 3,2% and 2,3% increased risk of cardiovascular mortality respectively.

Some studies investigated air pollution-induced cardiovascular risk in healthy young adults in their late 20s. A Dutch long term study on exposure to outdoor air pollution that addressed this issue, investigated carotid artery thickening and aortic pulse wave velocity, in a population based prospective cohort study. The aim of the study was to examine early life determinants of cardiovascular risk. Carotid artery intima–media thickness, which is a measure of atherosclerosis, together with pulse wave velocity are intermediate endpoints of cardiovascular diseases and reflect functional and structural arterial characteristics. Accelerating atherosclerosis is one of the consequences of long term exposure to outdoor air pollutants. In the study the estimated individual exposure to traffic related pollution comprising nitrogen dioxide, black carbon, $PM_{2.5}$ and sulfur dioxide showed a positive association with carotid artery intima-media thickness. Furthermore exposure to nitrogen dioxide was associated with a 4,9% increase in pulse wave velocity per 20 mg/m^3 increase in background concentration of sulfur dioxide [Lenters et al., 2010], showing that even low levels of air pollution may cause early vascular damage. Furthermore recent study on young healthy volunteers showed that diesel exhaust impairs vasomotor responses to both endothelium dependent and independent vasodilators [Mills et al., 2005]. On the other hand data exist, showing that in young healthy volunteers, exposure to air pollution particles at outdoor concentrations is not associated with detectable lipid or protein oxidation, altered homeostasis, or micro-vascular activity, all of which may predict cardiovascular events.

Finally the American Heart Association in its Scientific Statement 2010, elaborated by a large number of experts in the field, focusing on major air pollution studies and aiming to provide an updated evaluation linking PM exposure to cardiovascular disease concluded that, considering the available epidemiological studies that reported the associations between PM exposures with specific cardiovascular outcomes such as morbidity, mortality and hospitalization, the existing level of overall evidence is strong for an effect of PM on ischemic heart disease moderate for heart failure and ischemic stroke and modest or mixed for peripheral vascular and cardiac arrhythmia/arrest [Brook et al., 2010].

5. Mechanisms linking air pollution to cardiovascular morbidity and mortality

A number of interrelated pathways have been proposed to explain the epidemiological phenomenon of air pollution related cardiovascular mortality and morbidity. The biological mechanisms underlying cardiovascular alterations induced by both short-term and long-term exposure to airborne pollutants are not entirely clear, neither are fully elucidated the contribution to disease of different air pollution components, nor the mechanisms by which exposures to particulate matter, the component of air pollution most intensively investigated, triggers cardiovascular events.

Air pollutants may directly affect cardiac tissue via the generation of pulmonary and systemic inflammation or by direct translocation of oxidant components of the particles into the systemic circulation.

Extensive literature shows that inhalation of particulate matter may eventually lead to endothelial cell dysfunction and the release of blood and systemic factors increasing platelet aggregability, that increased vascular oxidative stress, accelerate the progression of atherosclerosis and the development of acute coronary syndromes. Decreases of cardiac blood flow and increases in coronary vascular resistance with inhalation of concentrated ambient particles have been shown in a canine model of coronary ischemia [Bartoli et al., 2009].

In addition, an accumulating body of data suggests that particulate matter triggers pulmonary nerve reflexes, which can activate and unbalance the autonomic nervous system, resulting in alterations of heart rate variability and induction of arrhythmias, leading to cardiomyopathy.

5.1 Pulmonary and systemic oxidative stress and inflammation: potential for cardiovascular system harm by air pollution

Inflammation is a common feature in most diseases that have been associated with PM exposures and several studies indicate that PM could cause both lung and systemic inflammation [Scapellato & Lotti, 2007]. While inhaled coarse particles are mostly removed by ciliary clearance, fine and ultrafine particles have the ability to reach the lower airways, be deposited in the alveolar space and trigger local inflammation. Alveolar macrophages exposed in vitro to PM have been shown to undergo intracellular oxidation, become apoptotic, have impaired phagocytosis, depressed respiratory burst responses and release pro-inflammatory cytokines [Tao et al., 2003].

Experimental animals exposed to titanium dioxide fine and ultrafine particles, which are able to generate free radicals, have shown that more ultra-fine particles are retained in the interstitial tissue of the lung, developing a marked airspace inflammatory response [Ferin et al., 1992].

The lungs can be both a target and a source of systemic inflammation and oxidative stress that can induce and worsen pre-existing chronic inflammatory conditions [Gomez-Mejiba et al., 2009]. In a model of short-term exposure to urban concentrated air particles (CAPs) experimental animals showed two-fold increase in the steady state concentration of oxidants in the lungs and heart. The increase in oxidative stress was associated with CAPs' content of iron, manganese, copper, and zinc in the lung and with iron, aluminum, silicon and titanium in the heart. CAPs exposure was associated with significant lung and heart edema and increased serum lactate dehydrogenase indicating mild damage to both tissues [Gurgueira et al., 2002]. Inflammation produced in the lung has a number of effects in organs, such as the cardiovascular system and the nervous system. The mechanism by which inhaled ambient particles are sensed and how these effects are systemically transduced remain elusive.

Ozone, a major component of air pollution, has a considerable impact on public health. Besides the well-described respiratory tract inflammation and dysfunctions, there is accumulating evidence indicating that ozone exposure affects brain functions. In a recent published study it is showed that, similarly to with lung inflammation, ozone exposure caused a sustained time- and dose-dependent neuronal activation in the hypothalamus, amigdala and dorso-lateral regions of the nucleus tractus solitarius overlapping terminal lung afferents running in vagus nerves. [Gackiere et al., 2011].

In a study where spontaneously hypertensive rats underwent a single-inhalation exposure to residual oil fly ash (ROFA) which are particles rich in transition metals, cardiovascular

toxicity was induced in a concentration-dependent manner. Exposure to the highest concentration of PM impaired heart functions, reduced heart rate, and increased arrhythmias. These changes were accompanied by increased pulmonary inflammation, increased lung resistance, and increased vagal tone, [Farraj et al., 2011].

Among postulated mechanisms aiming to explain links among oxidative stress, inflammation and the effects on the cardiovascular system is the hypothesis that fine and ultrafine particles containing metals and organic components with oxidant potential, translocate from the alveoli to the blood and lymphatic circulation and then deposited in many compartments of the organism including the liver, the kidneys, the heart and the brain where they exert the inflammatory potential.

Diesel exhaust, the major source of fine and ultrafine emission particulate in urban environments, has been shown to generate high concentrations of superoxide anion in solution as measured by electron paramagnetic resonance. The result highlights the innate potential of these particles to generate oxygen-centered free radicals and importantly, shows that combustion derived nanoparticles do not necessarily require recruitment of inflammatory cells from the lung to cause oxidative stress and damages [Miller et al., 2009], and that particles can induce directly oxidative stress if translocated to other body regions, although the mechanism by which ultra-fine particles can penetrate through pulmonary tissue and enter capillaries remains to be shown. In addition to translocation through blood circulation, particles once phagocytized by macrophages and dendritic cells may be transported and accumulated in lymph nodes in the lung or to those closely associated with the lungs [Peters et al., 2006].

Accumulation of macrophages containing ultrafine carbonaceous particles has been shown in the bronchoalveolar lavage of children with no respiratory symptoms, with the percentage of cells containing particles ranging between 1% and 16% [Bunn et al., 2001]. In addition, PM can impair macrophages phagocytic activity. Alveolar macrophages exposed to aggregates of ultrafine carbon particles showed less ability to kill bacteria as compared to macrophages loaded with washed diesel exhaust particles. Conversely, lipid peroxidation of lung surfactants was significantly increased either with washed diesel-loaded macrophages or with carbon-loaded macrophages with a predominance of diesel particle-induced lipid peroxidation, probably caused by 10 to 100 fold higher concentration of transition metal, in washed diesel than in carbon particles [Lundborg et al., 2007].

A recently published study evaluated the inflammatory response systemically and in the lung, for a panel of elderly volunteers upon exposure to PM. The study considered the effect of the organic fraction of fine particles, comprising primary organics from combustion sources, and secondary organics from photochemically oxidized volatile organic compounds. The differences in lung versus systemic inflammatory responses to primary and secondary organic particle components, particle size fractions, and the potential to induce reactive oxygen species were evaluated. Exhaled nitric oxide (NO), which is an airway inflammation marker and plasma interleukin-6 (IL-6) which is a systemic inflammation marker were measured. Secondary particles, organic markers, fine particles and O_3 were positively associated with exhaled NO. Primary particles, organic markers, ultrafine particles, CO, and NO_x were positively associated with IL-6. Reactive oxygen species were associated with both outcomes [Delfino et al., 2010].

5.2 Vascular changes and alterations in blood-borne factors induced by air pollutants affect the cardiovascular system

In addition to promoting exacerbations of lung disease and inflammation, air pollution and especially PM have been associated with damage of the vasculature and exacerbation of cardiovascular diseases. Recent controlled exposures have demonstrated that air pollution inhalation causes vascular endothelial dysfunction [Langrish et al., 2009], arterial vasoconstriction, increased blood pressure [Brook et al., 2011], myocardial ischemia [Nuvolone et al., 2011] and attenuates micro vascular endothelium-dependent dilation [Nurkiewicz et al., 2004; Hildebrandt et al., 2009]. Ambient air pollution is also associated with increased concentration of blood markers of inflammation and coagulation such as fibrinogen, in patients with chronic pulmonary disease [Hildebrandt et al., 2009]. Fibrinogen is an acute phase reactant and coagulation factor synthesized by hepatocytes and released in large amounts into the circulation in response to inflammatory factors. Increased in concentrations of fibrinogen are considered a risk factor for arterial occlusive disorders such as myocardial infarction.

One investigation in particular, examined the relationship of outdoor pollution and *in vivo* width of the human microvasculature. In a large population-based cohort study of adults without pre-existing cardiovascular disease, independent association was found between long-term and short-term concentrations of fine particulate air pollution and vessel diameters of the retinal microvasculature, as measured using a standardized photographic methods. Increased air pollution concentrations were associated with retinal arteriolar narrowing, an outcome that has been connected with increased risk of myocardial infarction, stroke, hypertension and cardiovascular mortality independent of other risk factors [Adar et al., 2010].

A study was undertaken to investigate possible links between air pollution and changes in blood indices in elderly people. Exposure to fine particles from two UK cities demonstrated associations between air pollution and hematological indices such as hemoglobin content, and concentration of red blood cells, including mean cell volume, mean cell hemoglobin, and mean cell hemoglobin concentration. The changes in hemoglobin adjusted for albumin suggests that inhalation of fine particles may cause the decrease of red cells in the circulation and that an action of such particles either on lung endothelial cells or on erythrocytes themselves may be responsible for changing red cell adhesiveness [Seaton et al., 1999].

Particle pollution has been shown in several investigations to affect the progression of atherosclerosis. Atherosclerosis can remain asymptomatic for years and is widespread in populations consuming high fat diets. In atherosclerosis fatty material such as cholesterol collects along the walls of arteries, eventually stiffening and blocking the arteries. A chronic inflammation in the wall of arteries is a typical characteristic of atherosclerosis. Fragments of plaque can break off and move through the affected artery to smaller blood vessels, blocking them and causing tissue damage or death mostly due to heart attack and stroke.

Animal studies has first established a causal association between exposure to PM and atherosclerosis. The effects of inhaled fine particles on aortic atherosclerosis have been assessed in a mouse model of hypercholesterolemia. Long-term exposures of the ApoE mice to concentrated ambient air particles, showed a 57% increase in the percentage of atherosclerotic plaque area in the aortic root, as compared to the control animals [Chen & Nadziejko, 2005; Araujo & Nel, 2009].

In a recent study ApoE mice receiving tracheal instillation of diesel exhausts particles showed for the first time that pulmonary exposure to the PM within diesel exhaust enhances atherogenesis. Plaque area was significantly increased in diesel exhaust-exposed mice as

compared with controls. Interestingly there was no evidence of systemic inflammation, increased circulating blood lipids or endothelial dysfunction in the diesel exhaust-exposed animals [Miller et al., 2009]. Studies on human populations have evidenced the association between PM exposures and the progression of atherosclerosis. Hoffmann and colleagues investigated the associations of long-term residential exposure to traffic and fine particulate matter with the degree of coronary atherosclerosis in a population-based cohort in Germany. The study demonstrated that residential exposure to highly trafficked roads is associated with coronary atherosclerosis measured as coronary artery calcification, with a positive association between fine particulate and the progression of the disease. A reduction in the distance between the residence and a major road by half was associated with a 7.0% higher coronary artery calcification [Hoffmann et al., 2007].

A cross-sectional study conducted among young participants aged 10-18 years, randomly selected from different urban areas of Isfahan city, the second largest and air-polluted city in Iran, showed a higher correlation between fine particulate matter and platelet tissue factor expression, a coagulation factor associated with atherosclerotic cardiovascular disease. This finding is consistent with various experimental studies that showed exposure to PM increases platelet tissue factor expression in atherosclerotic lesions [Poursafa et al., 2011].

Despite accumulating data indicating that particulate matter exposure contributes to the progression of atherosclerosis in animal models and in humans, the strength of associations between PM and atherosclerosis in humans is still being debated, and the strength and relative importance of the mechanisms in contributing to cardiovascular morbidity and mortality is still to be ascertained.

5.3 Disturbances of the autonomic nervous system: Impacts on the cardiopulmonary system

The interplay between nerves and vasculature is very complex and not yet completely clarified. Developmental studies in mice show that smooth muscle cells surrounding main vessels, release diffusible factors that direct growth of sympathetic nerves along arteries. In general, nerves and vasculature course along each other forming a neurovascular bundle, with blood vessels being not only routes for sympathetic axon guidance but also themselves, final sympathetic neuron targets [Larrivee et al., 2009].

Sympathetic and parasympathetic nervous system activity have complex effects on vascular tone.

Whereas increased parasympathetic activity normally leads to coronary vasodilation, in the presence of coronary artery disease, parasympathetic stimulation may lead to net coronary constriction [Pope et al., 2004]. Additionally, the stimulation of nervous C-fiber endings in the lungs may result in an axon local reflex with release of neuropeptides, which in turn may act on the mucosal vasculature, to promote vasodilatation and micro-vascular leakage. Neuropeptides may further interact with sensory neurons, propagating neurogenic inflammation within the airways.

Neurogenic inflammation has a potential importance in airway diseases; for instance neurogenic inflammation triggers a vicious cycle of neuro-immune interactions that amplify airway inflammation and airway hyperresponsiveness in allergic asthma [Pisi et al., 2009a]. In response to inspired air conditions, the sensory nerves in the lungs recruit appropriate reflexes, which induce different vascular processes, such as vasodilatation, vasoconstriction, plasma extravasation and exudation [Pisi et al., 2009b]. Observations have been reported on

the significant narrowing of the pulmonary vasculature in rats exposed to urban fine particulate aerosol, with the effect being more prominent in the intra-acinar arterioles [Rivero et al., 2005]. Thus suggesting that exposure to environmental particles may modify the balance between vasoconstriction and vasodilation at pulmonary level.

The sympathetic and parasympathetic nerve controls vital functions such as heart rate (HR), heart rate variability (HRV), and heart rate recoveries, parameters linking autonomic changes with mortality and morbidity. Many studies reported to date, have shown associations between air pollution levels and changes in heart rate variability, propensity to ischemia and arrhythmias, in elderly subjects or in patient with apparent cardiovascular diseases [Godleski, 2006].

HR is normally determined by spontaneous and periodic depolarizations of the sinoatrial node, the frequency of which is modulated by the sympathetic and parasympathetic divisions of the autonomic nervous system. HRV is controlled by the cardiac autonomic nervous system, and refers to the beat-to-beat difference or R-R interval change in the intrinsic rhythm of the heart.

Assessment of HRV provides a measure of the degree of balance between sympathetic and parasympathetic (vagus nerve) activity. Decreased HRV has been considered to reflect a diminished vagal and increased sympathetic modulation of the sinus node. Sympatho-exitation has been shown to exert pro-arrhythmic effects and has been implicated in the development of heart failure, myocardial infarction and hypertension. Aberrant sympatho-activity and reduced vagal modulation of sinus node lead to an autonomic imbalance favoring cardiac electrical instability, which is predictive of negative cardiac outcomes. Additional findings has been shown that decreased HRV can be influenced by a greater complexity in the relationship between neural input and sinus node responsiveness as well as the possible interference with non neural mechanisms [Lombardi, 2002].

HRV can be assessed by time or frequency domain indices. Time domain measures the amount of time in milliseconds in the beat to beat intervals of the heart; frequency domain provides information about the frequency distribution of the components of HRV. HRV is often express as low frequency, high frequency ratio (LF/HF) [Feldman et al., 2010]. HRV parameters offer a non invasive method to estimate autonomic control mechanisms and to identify patients with an increased cardiac mortality.

Gathering data show a correlation between exposure to particulate matter and triggering of autonomic nervous system reflexes, eventually altering the cardiac frequency and function. It has been shown in the Framingham Heart Study that in elderly subjects decreased heart rate variability is associated with substantially increased occurence of major cardiac events. Studies on animals exposed to particle pollution support the hypothesis of the autonomic nervous system imbalance as a consequence of particles inhalation. Dogs exposed to concentrated air particles (CAPs) revealed LF and HF powers during HRV that were significantly higher for CAPs exposure as compared to sham exposure. The cardiac and respiratory changes recorded in the study, suggest an effect mediated via both the sympathetic nervous system and the vagus nerve [Godleski et al., 2000]. In animals exposed ROFA particles, associations have been reported between these fine particles and ventricular arrhythmia with changes of the autonomic regulation of the heart including a decrease in parasympathetic modulation [Wellenius et al., 2002].

Similar evidence has been reported from an epidemiological study, in patients with a history of serious arrhythmias, carrying an Implanted Cardioverter Defibrillator (ICD). ICD device monitors electrocardiographic (ECG) abnormalities that may occur in the heart

rhythm. On detection of ventricular arrhythmia or rapid atrial fibrillation the defibrillator initiates pacing, an electrical shock therapy, in order to restore a normal cardiac rhythm. The device provides objective and accurate records of the occurrence and timing of arrhythmic events, and it found correspondence between increased risk of cardiac arrhythmias and elevated concentration of air pollutants. The association was stronger to nitrogen dioxide, carbon monoxide, black carbon, and fine particle mass, suggesting autonomic dysregulation as presumed pathways to these clinical endpoints [Peters et al., 2000].

Clinical exposure studies in young healthy individual have shown variable effects. The influence of air pollution on ECG parameters in young healthy individuals has been investigated in controlled human exposures to elemental carbon ultrafine particles, using set parameters that describe the autonomic regulation of the heart. As expected, young healthy individuals did not show dramatic changes in the ECG parameters. Interestingly, exposure to 10μm/m^3 ultrafine particles was associated with changes in parameters indicating an increase in parasympathetic tone, either with exposures at rest as well as during exercising, furthermore the heart repolarization parameter, was influenced even if not significantly, by air pollution exposure [Zareba et al., 2009]. Conversely adverse effects on ventricular repolarization were assessed in a community-based study of adult healthy individuals. The adverse effects were associated with $PM_{2.5}$ particle upon 3 to 4 hours of outdoor activity as recorded with a PM and ECG portable Holter recorder [Liao et al., 2010].

Finally, several studies showed that short-term exposure to concentrated $PM_{2.5}$ raises diastolic blood pressure and trigger vasoconstriction in healthy adults [Brook et al., 2002; Urch et al., 2005]. A study provided additional insight into the acute cardiovascular effects of air pollution exposure and the autonomic nervous system control. In the study PM2.5 and not ozone was responsible for a diastolic blood pressure and only transiently during the actual period of inhalation. The result implicates autonomic nervous system imbalance, as the most pro-hypertensive response. The magnitude of diastolic BP elevation during exposures was most strongly associated with decreases in HRV markers of parasympathetic autonomic nervous system withdrawal [Brook, 2008]. This hypothesis is supported by the fact that human airways are lined with receptors and nerve endings that, after stimulation by inhaled particulate matter, may be capable of altering reflexes in the autonomic nervous system pathways, leading to a blunting of cardiovascular parasympathetic tone and relative favoring of sympathetic activity [Widdicombe & Lee, 2001]. A further study with dogs exposed to particle pollution, has contributed to the evidence that diastolic BP, acutely increases during short term concentrated ambient particles inhalation. Also show in these studies was that homeostatic mechanisms such as baroreceptor reflex sensitivity was activated during the inhalation exposure in order to acutely lower the blood pressure response to pollutants, thus limiting the height of the blood pressure response. Alpha-adrenergic receptor antagonist mitigated this response. [Bartoli et al., 2009] suggesting activation of the sympathetic branch of the autonomic nervous system.

6. Evidence for the autonomic nervous system and ROS participation in the alteration of cardiovascular system

Air pollution especially the particulate fraction, has been shown to trigger or exacerbate pathological cardiovascular conditions in short-term and long term exposure studies. Free radicals play a role in the cardio-toxicity induced by air pollution since particulate matter is rich in organic and inorganic molecules able to trigger free radical reactions. Among the

hypothesized pathways for the onset and the exacerbation of cardiovascular pathologies are the stimulation of the nerve fibers and the imbalance of the autonomic nervous system. Studies presented in this section show that concentrated air pollutants are able to trigger an oxidative response in the heart of experimental animals and to stimulate both the limbs of the autonomic nervous system. Furthermore PM induced oxidative stress lead to heart damages and alteration of cardiac parameters controlled by the autonomic nervous system.

6.1 Role of ROS in the autonomic nervous system related cardiac changes upon exposure to air pollution

Ambient particles may elicit cardiovascular effects in part, through the stimulation of nervous fibers of the autonomic system. Support for this hypothesis has come from a number of studies showing that short-term exposure to particles is associated with changes in the autonomic functions as assessed by heart rate (HR) and heart rate variability (HRV) [Delfino et al., 2005] in a wide population range, from healthy adults [Wu et al., 2010] to older people and in patients with current or underlying cardiovascular diseases [Riojas-Rodriguez et al., 2006; Park et al., 2005; Schwartz et al., 2005]. HRV is the variability between the R-to-R waveform intervals, allowing a quantitative non-invasive measure of the cardiac autonomic nervous system control. Reduced heart rate variability has been linked to increased risk of myocardial infarction and it has been considered a predictor of increased risk of mortality in patients with heart failure [Tsuji et al., 1996].

The PM concentration and composition in the urban environment varies hourly during the day. Experimental models using animals exposed to outdoor airborne pollutants are a valuable representation to appreciate the activity of the cardiac autonomic nervous system, in a realistic representation of the urban population exposure.

Airborne particles can be collected by means of virtual impactor technology, which selects, concentrate and deliver outdoors particle pollution to the experimental setting. In the virtual impactor the airborne particles are separated by size, and redirected into two main airstreams, according to cut off characteristics. The fine particles follow that streamlines of the major air flow, while the coarse particles pass into the forward minor flow. In this way only the particle size of interest is kept and delivered for inhalation. Once selected, the fine particles are concentrated and maintained in suspension without physical or chemical alteration so that the quality of the complex mixtures of pollutants present in the urban environment is preserved. Concentrated ambient particles (CAPs) delivered to experimental animals have been shown to produce ROS and induce oxidative stress [Gurgueira et al., 2002]. The decay of excited oxygen molecules and carbonyl groups, generated during the lipid peroxidation induced by ROS, can be measured as light emission that can be detected as chemiluminescence (CL). CL constitutes a quantitative measure of the oxidative stress generated in whole intact organ, in real time, and in a non-invasive manner, after CAPs inhalation.

Gurgueira and colleagues [Gurgueira et al., 2002] showed that the CL in the heart and lung of rats increases two-fold, when the animals undergo to short-term CAPs exposure. Interestingly upon inhalation of pollutants, the oxidative stress in lungs increased immediately, while a significant level of oxidative stress in the heart was developed only after one-hour lag time, suggesting pulmonary to cardiac signaling, via either nervous or systemic mediators. In addition these changes were associated with oxidants dependent lung and heart edema, and with significant increase in serum levels of lactate dehydrogenase and creatine phosphokinase activities, measures of tissue damage, detected in both heart and lung. Interestingly, the level of activity of several antioxidants enzymes

such as SOD and catalase, was up regulated in both organs. The increased oxidative stress in heart and lung and the increased concentration of antioxidants shows that CAPs induces ROS activity, and that in turn, ROS trigger an adaptation/protection mechanism in those organs, increasing the concentration of antioxidants.

The exciting data showing the time delay, between the rise of oxidative stress in rat's lung versus rat's heart, implies that a mechanism of pulmonary to cardiac system signaling through the autonomic nervous system or through systemic metabolites is in action; in this mechanism ROS, may play a determinant role. The signaling to the heart may be triggered in the lungs. The bronchial airways autonomic neural control includes both sympathetic and parasympathetic innervations. In response to inspired air conditions, the sensory nerves can recruit appropriate reflexes, causing afferent transmission, and release of neurotransmitters from peripheral endings, probably via local axon reflexes with antidromic conduction down afferent nerve collaterals [Pisi et al., 2009a]. Sensory nerves can also recruit appropriate reflexes which can induce different vascular processes such as vasodilatation, increase in vascular permeability and bronchoconstriction [Saria et al., 1988].

When rats undergo short-term exposures to CAPs, the CL measured from the whole heart is significantly higher than in the rats exposed to filtered air, and it shows signs of tissues edema indicating heart tissue damage. If the animals are pretreated with N-acetyl-cisteine (NAC), an antioxidant precursor of glutathione, both the increase in heart CL and the development of edema in the cardiac tissue are abrogated, confirming that PM is able to generate oxidative stress in the heart, and that pretreatment with antioxidants exert a protection against PM inhalation. The damages detected in the heart of rats after CAPs exposures, suggest the possibility of alterations in cardiac function. Experimental animals in which a controlled and steady suspension of standard Urban Ambient Particles (UAP) rich in S, Fe, Br, and Pb, was instilled intratracheally, showed significant changes in the autonomic control of the heart. Although UAP instillation did not change the HR, it significantly altered the HRV, with increases in both the high frequency (HF) and the low frequency (LF) components of HRV, which signify the parasympathetic and sympathetic limbs of the autonomic nervous system respectively. In this model the pre-treatment of the rats with the adrenergic receptor antagonist atenolol and with the muscarinic receptor antagonist glycopirrolate, prevents UAP-induced increase CL in the heart, suggesting that cardiac oxidants are mediated through the two limbs of the autonomic nervous system [Rhoden et al., 2005].

The result is confirmed in experimental setting in which rats are short-term exposed to CAPs. Indeed in CAPs model as for the UAP model, both atenolol and glycopirrolate pre-treatment, effectively prevents the increase of heart CL. The analysis of HRV and the HF and LF components in the rats exposed to CAPs, shows increases in both sympathetic and parasympathetic stimulation, with a predominance of the parasympathetic limb. In addition both sympathetic and parasympathetic antagonists prevented PM-induced oxidative stress in the heart. Taken together these outcomes suggest that deposition of PM in the lung is rapidly sensed, resulting in an imbalance in cardiac autonomic activity. The prevention of oxidant accumulation exerted by the pretreatment with NAC indicates that this pathway uses ROS as intermediates [Rhoden et al., 2005]. Interestingly, a recent paper by Rimmer and colleagues [Dyavanapalli et al., 2010], showed that ROS and especially singlet oxygen and hydroxyl radicals, block the ganglionic transmission in rats' intra-cardiac ganglion neurons, with consequences for the parasympathetic regulation of the cardiac functions, the

parasympathetic unbalance, and a predominance of sympathetic pro-arrhythmic activity. In this model the administration of catalase, prevented the blockage of the transmission in intra-cardiac ganglion by ROS. These results further indicate that ROS plays a determinant role in the autonomic control of the heart.

6.2 CAPs upregulate parasympathetic cardiac tone through stimulation of lung's afferent nervous fibers

Inhaled toxicants trigger cardiopulmonary reflexes aimed to reduce the amount of inspired pollutants transported into the blood stream. Pulmonary chemoreflexes including apnea, bradycardia and hypotension were first reported by Brodie in the 1900 [Brodie 1900]. Extensive evidence indicates that bronchopulmonary C-fiber afferents are responsible for these responses and that these afferents are extremely sensitive to chemical irritants. Approximately 75% of the afferent fibers in the vagal branches innervating the respiratory tract are nonmyelinated C-fibers. A variety of inhaled toxicants such as ozone, sulfur dioxide, ammonia, tobacco, wood smoke diesel exhaust, PM, acrolein, volatile anesthetics, and capsaicin, have been shown to stimulate bronchopulmonary C-fiber afferents.

A proposed hypothesis suggests that PM deposition in the lung triggers neural reflexes mediated by vagus nervous unmyelinated C-fibers. In this approach PM could modulate the sympathetic/parasympathetic tone in the heart increasing the oxidative stress and leading to functional cardiac alterations. The Transient Receptor Potential Vanilloid Receptor 1 (TRPV1) is pervasive on parasympathetic fibers in the lung and in particular on C-fibers. The TRPV1 receptor plays a role in initiating inflammatory processes and integrating painful stimuli and it is known to be broadly expressed in all "port of entry" tissues such as the skin, the gut, the conjuntiva, and the airways [Veronesi & Oortgiesen, 2006]. TRPV1 is activated by various ligand-like agents and a plethora of seemingly unrelated stimuli such as chemical irritants, inflammatory mediators, and tissue damaging stimuli and by nonselective stimuli such as high temperature (>43°C), acidic pH (<5.3), intracellular redox states, and electrostatic charge.

The activation of TRPV1 receptors on sensory fibers, such as C-fibers and some non-neuronal cells like respiratory epithelia, produces calcium and sodium influx and the corresponding release of tachykinin neuropeptide, such as substance P and inflammatory cytokines, initiating and modulating neurogenic inflammation. Studies on human respiratory epithelial cells show that PM-induced inflammatory cytokine release is initiated by activation of TRPV1 receptors. Reflexes generated by nocireceptors such as TRPV1 affect cardiovascular function by increasing sympathetic and decreasing parasympathetic activity [Klabunde, 2005].

Capsazepine (CPZ) is a selective pharmacological antagonist of TRPV1. The administration of CPZ in rats before short-term exposure to CAPs, blocks the TRPV1 receptor in the lungs and effectively prevents CAPs induced increase of oxidative stress in the heart. In animals not pretreated with CPZ, CAPs exposure induces increase of CL together with changes in cardiac functions measured as HR and ECG waveform morphology. The triggering of TRPV1-mediated autonomic reflexes in the lungs during CAPs exposure induces changes in the cardiac rhythm, stimulated cardiac current abnormalities and led to changes in conduction velocity and ventricular repolarization. In particular the functional cardiac electrophysiologic changes elicited by the reflex activation consisted in increased P-wave duration, a marker of intra-atrial conduction time, which is influenced by changes in the autonomic tone, and increase of QT interval, a measure of ventricular depolarization and repolarization. On the other hand the QRS interval that reflect, the rapid depolarization of

the right and left ventricles and T_{pe} duration, a marker of transmural dispersion and repolarization, were decreased upon exposure to CAPs.
Notably all of these changes were abrogated by the pretreatment with the TRPV1 antagonist CPZ. Therefore this suggests a central role for TRPV1 receptor in eliciting the observed responses while the complete abrogation, of the CAPs induced increase in cardiac oxidative stress, by the pretreatment with CPZ provides evidences in support of the primary role of pulmonary irritant receptors in mediating the response of autonomic nervous system PM mediated oxidative stress.
The depolarization repolarization equilibrium producing the ECG waveform is influenced by ions channels [Zareba & Cygankiewicz, 2008]. The inhibition of sodium and calcium channels by pharmacological intervention has been shown to shorten the QT interval and increases the risk of ventricular fibrillation. Given the results from animal CAPs exposures, it is tempting to speculate that the mechanism by which CAPs leads to shortening of the QT interval, involves increased vagal tone to the heart and inhibition of sodium, calcium or potassium channels. This hypothesis is supported by the changes in vagal tone and by the prevention of these changes by CPZ. Interestingly recent data from Whyte and colleagues show that ROS alter the depolarization-activated calcium and potassium conductance, which underlie neuronal excitability of intra-cardiac neurons in the intra-cardiac ganglion plexus [Whyte et al., 2009]. This notion is particularly interesting since it has been reported that some interventions terminating or evoking arrhythmias may produce their effects also via modification of intra-cardiac neural activity [Kukanova & Mravec, 2006].

6.3 Role of ROS pressor stimuli in the autonomic nervous system cardiac: Changes upon exposure to air pollution.

Cohort and panel studies have found that exposure to PM is associated not only with changes in HR and decreased heart rate variability, but also with changes in vascular parameters, and increased arterial blood pressure [Nurkiewicz et al., 2004; Brook et al., 2002]. One study in particular [Bartoli et al., 2009] showed that short-term exposure to CAPs increased heart rate, systolic blood pressure, diastolic blood pressure and mean arterial pressure in experimental animals. These changes were accompanied by increase in baroceptor reflex sensitivity after CAPs exposure while blockade by alpha adrenergic inhibitor completely abrogated those changes.
The autonomic nervous system is the most rapidly responding regulator of blood pressure. It receives continuous information from the baroreceptors, which are pressure sensitive nerve endings, situated in the carotid sinus and the aortic arch. A decrease in blood pressure causes activation of the sympathetic nervous system resulting in increased contractility of the heart (beta receptors) and vasoconstriction of both the arterial and venous side of the circulation (alpha receptors). Other factors contributing to blood pressure control are: the exchange of fluid that occurs across the capillary membrane between the blood and the interstitial fluids, hormonal responses, and the kidneys through the renin-angiotensin pathway.
Angiotensin-II (ang-II), the final active messenger of the renin–angiotensin system, has multiple biological actions including vasoconstriction, stimulation of myocytes, and facilitation of norepinephrine release from sympathetic neurons.
Furthermore ang-II interacts with the sympathetic nervous system both peripherally and centrally to increase vascular tone. Animal studies show that ang-II has effects on both limbs of the autonomic nervous system, simultaneously facilitating sympathetic activity and inhibiting vagal activity on the heart. Interestingly ang-II increases the production of

superoxide anion via stimulation of NAD(P)H oxidase, and the resulting oxidative stress has been postulated as an important mediator of ang-II signaling, in the central nervous system. A study on rats has shown that ang-II activates sympathetic outflow by stimulation of superoxide anion in the paraventricular nucleus [Patel et al., 2011]. Thus ang-II is a possible important link between the pulmonary and cardiovascular effects of PM mediated by the autonomic nervous system.

Short exposures of rats to CAPs have been shown to significantly increase the blood levels of ang-II. Pretreatment of the animals with angiotensin converting enzyme (ACE) inhibitor, significantly reduced the circulating concentration of ang-II, while the blockade of the ang-II receptor AT-1, further increased the ang-II blood level as compared to the CAPs only group. Plasma creatinine levels in the experimental animals were within the physiological range, showing that no significante change in the renal system has occurred.

Increased levels of circulating ang-II were accompanied by increased production of ROS in the heart of rats exposed to CAPs, leading to altered ventricular repolarization as an effect of possible changes in ion currents . On the other hand, heart CL assessment after CAPs exposures in rats treated with ACE and AT1 inhibitors showed a significantly decreased level of oxidants, suggesting a direct role of ang-II in the rise of the cardiac oxidative stress.

The evaluation of ECGs in CAPs exposed animals for changes in the waveforms showed a lengthening of the QRS, QT, T_{pe} and RT_p intervals and a decrease in T_{pe} and increase in P_{dur} and RT_p intervals, while blockade of angiotensin synthesis with ACE inhibitor reversed the effect of CAPs exposure. Consistently following the blockade of AT1 receptor, CAPs exposure was not associated with statistically significant changes with T_{pe} , P_{dur} or RT_p conduction velocity and ventricular depolarization intervals.

This model of CAPs short- term exposure has shown a significant increase of ROS in the cardiac tissue as measured as CL. The increase in ROS, possibly mediated by receptor AT1 /NAD(P)H oxidase activation, could lead to electrophysiological changes, in the duration of cardiac waveform and possibly arrhythmia, by mechanisms similar to those initiated by activation of pulmonary reflexes.

Interestingly, exposure to fine PM may potentiate hypertension generating ROS through a NAD(P)H oxidase and eNOS dependent mechanism, with important implications for PM mediated cardiovascular effects, suggesting that PM my induce cardiac damage by modulating sensitivity to pressor stimuli [Sun et al., 2008].

7. Conclusion

Air pollution and in particular particulate matter, has been recognized as a risk for human health. PM inhalation has been positively associated with the onset and exacerbation of cardiovascular pathologies. The risk for cardiovascular outcomes has been found to rise among susceptible population such as individuals with preexisting pulmonary and cardiovascular conditions and the elderly. The mechanism(s) by which PM elicit cardiovascular diseases are not fully elucidated but scientific evidences showed that PM-induced free radicals are responsible for triggering systemic inflammation, impairing vascular parameters and influenceing the cardiac autonomic nervous system. In our researches we measured *in vivo* free radicals formation in rats exposed to concentrated ambient particles (CAPs). We showed that CAPs increased free radical induced oxidative stress in lung and heart and that antioxidants prevented it. CAPs inhalation increased both sympathetic and parasympathetic stimulation, with a predominance of the parasympathetic

limb while pretreatment with antagonists of both limbs restored the autonomic cardiac control and prevented PM-induced oxidative stress in the heart. Taken together these outcomes suggest that deposition of PM in the lung is immediately sensed, resulting in an imbalance in cardiac autonomic activity. The prevention of oxidants accumulation exerted by the pretreatment with NAC indicates that this pathway uses free radicals as intermediates. Furthermore CAPs inhalation through the stimulation of TRPV1 receptors in the lungs induced changes in the cardiac rhythm, stimulated cardiac current abnormalities and led to changes in conduction velocity and ventricular repolarization. Finally we showed that CAPs exposure significantly increased the blood levels of angiotensin-II accompanied by increased production of ROS in the heart of rats. Treatment with ACE and AT-1 receptor inhibitors prevented CAPs effects in experimental animals. Further researches are needed to elucidate which component(s) of PM trigger the pathological cardiac effects and the increased oxidative stress, which are the consequences of angiotensin-II rise on the vascular tone, blood pressure and cardiac rhythm and how PM, through pulmonary reflexes, influence the autonomic nervous system.

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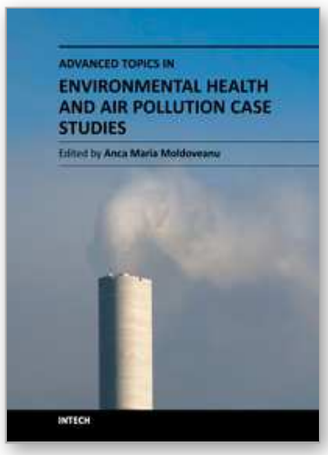

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The book describes the effects of air pollutants, from the indoor and outdoor spaces, on the human physiology. Air pollutants can influence inflammation biomarkers, can influence the pathogenesis of chronic cough, can influence reactive oxygen species (ROS) and can induce autonomic nervous system interactions that modulate cardiac oxidative stress and cardiac electrophysiological changes, can participate in the onset and exacerbation of upper respiratory and cardio-vascular diseases, can lead to the exacerbation of asthma and allergic diseases. The book also presents how the urban environment can influence and modify the impact of various pollutants on human health.

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