Heavy and trace elements are important diagnostic tools during the progression of atherosclerosis; high cholesterol diet supplemented with high zinc level delays or prevents the progression of atherosclerosis

Mohamed Anwar K Abdelhalim[1], Sherif A. Abdelmottaleb Moussa[2], Yanallah Hussain AL-Mohy[1]

[1]Department of Physics and Astronomy, College of Science, King Saud University, Saudi Arabia
[2]Department of Physics, College of Science, Al-Imam Mohammad Ibn Saud Islamic University, P. O. Box 90950, Riyadh, 11623, Saudi Arabia.
abdelhalimmak@yahoo.com

Abstract: The mechanism of atherogenesis has not yet fully understood despite different studies in this area. The effects of high cholesterol diet (HCD) on the changes of trace elements [iron (Fe), copper (Cu), zinc (Zn), cadmium (Cd) and lead (Pb)] in several tissues of rabbits have not been well documented before. Thus, the aim of this study was to elucidate the changes in heavy and trace elements in several tissues of rabbits fed on HCD and HCD + Zn for feeding period of 12 weeks compared with the control rabbits; as a diagnostic tool during the progression of atherosclerosis as well as an early detection for cardiovascular diseases and stroke. The HCD group was fed a NOR rabbit chow supplemented with 1.0% cholesterol plus 1.0% olive oil. The HCD + Zn group was fed on NOR Purina Certified Rabbit Chow plus 1.0% cholesterol and 1.0% olive oil supplemented with 350 ppm Zn (total estimate 470 ppm Zn) for the same feeding period of time. Fe, Cu, Zn and Cd concentrations were measured in five types of tissue (kidney, heart, lung, aorta, and liver) from control, HCD and HCD + Zn rabbits using ICP-ES. Comparing HCD to control rabbits, we found an increase in Fe, Cu, Pb and Cd levels in kidney, heart, lung, aorta, and liver tissues of rabbits; while a decrease observed in Zn level in kidney, heart, lung, aorta, and liver tissues. Comparing HCD + Zn with the control rabbits, we found that supplementation of Zn to the HCD decreased the levels of Fe, Cu, Pb, and Cd in kidney, heart, lung, aorta, and liver tissues of rabbits. These results demonstrate that Fe plays a major role during the progression of atherosclerosis through the production of free radicals, deposition and absorption of intracellular and extracellular lipids in the intima, connective tissue formation, and smooth muscle proliferation. Furthermore, inducing anemia in HCD rabbits may delay or inhibit the progression of atherosclerosis. Cu plays a minor role in atherosclerosis. Zn plays a major role in atherosclerosis; it may act as an endogenous protective factor against atherosclerosis perhaps by reducing lesion Fe content.
[Mohamed Anwar K Abdelhalim[1], Sherif A. Abdelmottaleb Moussa[2], Yanallah Hussain AL-Mohy[1]]. **Heavy and trace elements are important diagnostic tools during the progression of atherosclerosis; high cholesterol diet supplemented with high zinc level delays or prevents the progression of atherosclerosis**. *Life Sci J* 2013;10(4):670-680]. (ISSN:1097-8135). http://www.lifesciencesite.com. 85

Keywords: Heavy elements; trace elements, high cholesterol diet; lipids; rabbits; atherosclerosis; zinc supplementation

Introduction

Atherosclerosis is a complex multifactorial disease, which develops in the arterial wall in response to various stimuli and results in excessive inflammatory and fibro proliferative reactions. Cardiovascular disease (CVD) due to atherosclerosis is the leading cause of morbidity and mortality in westernised countries. Atherosclerosis is a complex disease, involving many cell types and circulating mediators and resulting in an inflammatory state. Atherosclerotic lesions form de novo from focal accumulation of lipoproteins, monocyte-derived macrophages, and lymphocytes within the arterial wall. These lesions can develop as early as the second decade of life and progress into clinical disease over time. The formation of plaque in the arterial intima may be due to hyperlipidemia which may include increased serum concentrations of total cholesterol and low density lipoprotein concentration. However, despite recent advances in cardiology, atherosclerosis remains an important medical problem (Adekunde AS et al., 2013).

In United States of America's data for the year 2004, the first symptom in 62% of man and 47% of women suffering from atherosclerotic cardiovascular disease, is heart attack or sudden death (Nissen SE et al., 2006). In Finland, cardiovascular heart disease is also the cause of every fourth death in the working-range population i.e. 15-64 years, and is the most single death in the whole population. The percentage of premature death from cardiovascular disease ranges from 4% in high-income countries to 42% in low-income countries. This high incidence of death in low-income countries therefore made the study of lipid profile in the general population important in this society. Study had equally shown rise in the level of serum total cholesterol (TC) particularly in urban setting in Asian countries (Khool KL et al., 2003).

Atherosclerosis affects many important biochemical and/or physiological processes in the body. These include loss of ability of intima endothelium to secrete nitric oxide (NO) which is a vasoprotective gas against vascular inflammation. Minerals and trace elements are very important requirements for proper functionality and sustenance of life. For trace elements, they are inorganic compounds that, like vitamins, are essential for health and needed only in small amounts, known as reference nutrient intake. They are needed for normal health and make up less than 0.01 percent of the body's dry weight (Martin AC et al., 2006). Some of them function a cofactors for enzymes, hormones etc. The role of these elements cannot be overemphasized as their deficiencies can lead to unimagined debilitating diseases. Also, the disordered homeostasis of these all important elements have been reported to cause series of human diseases which include bone disease, infertility, hypochromic anemia, macrocytosis etc. In recent years, epidemiological, clinical, pathological and experimental evidence has accumulated which justified undertaking deeper studies of trace elements in cardiovascular diseases.

The present study elucidate the levels of selected elements, hematological and biochemical abnormalities in rabbits fed high cholesterol and saturated fat diet for feeding periods of 12 weeks.

Material and methods:

Rabbit tissue samples

The atherosclerotic model used in this study was the New Zealand white rabbit (male, 12 weeks old), obtained from the Laboratory Animal Center (College of Pharmacy, King Saud University). Fifteen rabbits were individually caged, and divided into control, HCD and (HCD + Zn) groups. The control group (n = 5) was fed on 100 g/day of NOR diet (Purina Certified Rabbit Chow # 5321; Research Diet Inc., New Jersey, USA) for a period of 12 weeks. Chemical composition of the laboratory NOR rabbit diet (Purina Certified Rabbit Chow # 5321) is shown in Table 1 and Table 2. The HCD group (n = 5) was fed on NOR Purina Certified Rabbit Chow # 5321 supplemented with 1.0% cholesterol plus 1.0% olive oil (100 g/day) for the same period of time. The HCD + Zn group was fed on NOR Purina Certified Rabbit Chow plus 1.0% cholesterol and 1.0% olive oil supplemented with 350 ppm Zn (total estimate 470 ppm Zn) for the same feeding period of time.The animals were sacrificed by intravenous injection of Hypnorm (0.3 ml/kg) in accordance with the guidelines approved by King Saud University Local Animal Care and Use Committee.

Biochemical and hematological investigations

The rabbits are anesthetized by inhalation of 5% isoflurane until muscular tonus relaxed. Blood samples (2 ml) were obtained from the rabbits via venepuncture of an antecubital vein. Blood was collected into two polypropylene tubes viz., one for serum and one for plasma. The blood for plasma was collected in heparin. Serum was prepared by allowing the blood to clot at 37º C and centrifugation at 3000rpm for ten minutes. An hematological autoanalyzer (Orphee Mythic 22 Hematological Analyzer; Diamond Diagnostic; USA) is used to determine different hematological and dimensional parameters, such as red blood cells (RBC), white blood cells (WBCs), hemoglobin (HB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), neutrophils%, lymphocytes%, monocytes%, eosinophils%, basophils%, mean platelet volume (MPV), platelet distribution width (PDW)%, plateletcrit (PCT)% and platelets (PLTs). Serum TC and TG levels were analyzed by the enzymatic method used in the clinical laboratory centre of King Khaled Hospital. HDL concentration was determined by the previously reported method (Lee et al., 1998). All experiments are conducted in accordance with the guidelines approved by King Saud University Local Animal Care and Use Committee.

Digestion of rabbit tissue samples

Various rabbit tissue samples were wet digested with nitric acid and converted into acidic digest solutions for analysis by ICP-MS method. The tissue was freeze dried in order to minimize loss of analytes and to facilitate subsequent sample preparation steps, and then homogenized to a fine powder by ball-milling in plastic containers. Approximately 0.20 to 0.25 g of powdered tissue was weighed into a Teflon reaction vessel and 3 ml of HNO_3 were added. The closed reaction vessel was heated in a 130°C oven until digestion was completed.

Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES)

Instrumental preparation

Radiofrequency Power (RF; 1150 W), Nebulizer Gas Flow (0.70 L/min), Argon Gas Pressure (60 psi), Flush Pump Rate (25 rpm), Analysis Pump Rate (50 rpm), Aux. Gas Flow (0.5 L/min), Coolant Gas Flow (12 L/min), Purge Gas Flow (Normal), and Number of Replicates (3).

Samples Preparation

The samples were prepared by accurately weighing a round 200-2000 mg of samples into a dry and clean Teflon digestion beaker, 6 ml of HNO_3, 2 ml H_2SO_4 and 2 ml $HClO_4$ were added to the beaker. Samples were digested on the hot plate at 120-150 °C for approximately 40 minutes. The resulting digest was not clear, so it was filtered through Whatman

filtered paper No.42. The filtered digest was transferred to a 50 ml plastic volumetric flask and made up to mark using deionized water. A blank digest was carried out in the same way.

Figure 1: Biological samples preparation for determination of trace elements using inductively Coupled Plasma/ Emission Spectrometer (ICP/ES)

Reagents

Nitric asid (69% v/v), super Purity grade from Romil, England, Sulfuric acid (98% v/v) and prechloric acid (70% v/v) were supra-pure from Merck Germany. High purity water obtained from Millipore Milli-Q water purification system was used throughout the work.

Calibration

The ICP-ES calibration was carried out by external calibration with the blank solution and three working standard solutions (20, 40, 60, and 80 µg/L), for all elements: Fe, Cu, Zn, Co & Pb starting from a 1000 mg/L single standard solutions for ICP-ES (Aristar grade , BDH laboratory supplies, England for the trace elements; Thermo SCIENTIFIC Model: iCAP 6000 SERIES, United Kingdom, ICP-OES:(Thermo-CAP 6000; Operating Conditions).

Diluted suitably using 1% nitric acid, aspirated and then nebulized using a quartz Meinhard micro-concentric type nebulizer into the argon plasma via a peristaltic pump with a flow rate of approximately 0.9–1.0 mL/min. Mass spectral acquisitions are carried out using pulse-counting scanning mode with the following instrumental parameters: mass range scanned 190–220 m/z with 19 channels per mass, three points per peak, and 10.24 ms dwell time on each isotopic mass. The instrument control, methods procedures, and the data system, including calculations and statistics are operated via a personal computer with Plasma Vision Software. Nitric acid (1%) blanks are run in between samples to correct the background levels.

Statistical analysis

The results of the present study were expressed as mean ± SE, statistical analysis for significant differences between the control group and the cholesterol-fed group was done with an ANOVA for repeated measurements.

Results

Figure 2 shows the Fe concentrations in kidney, heart, lung, aorta and liver tissues of control, HCD and HCD + Zn rabbits. The Fe concentration was increased in kidney, heart, lung, aorta and liver of HCD and HCD + Zn rabbits compared with control rabbits.

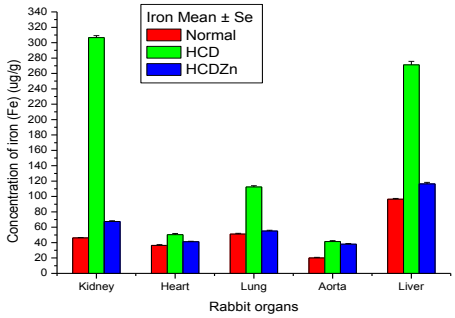

Figure 2: Fe concentrations in kidney, heart, lung, aorta and liver tissues of control, HCD and HCD + Zn rabbits

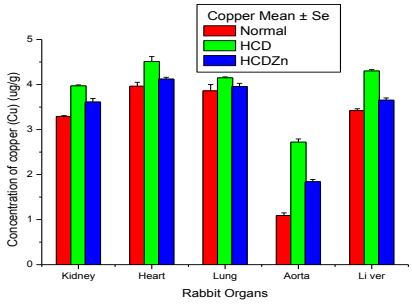

Figure 3: Cu concentrations in kidney, heart, lung, aorta and liver tissues of control, HCD and HCD + Zn rabbits

Figure 3 shows the Cu concentrations in kidney, heart, lung, aorta, and liver tissues of control, HCD and HCD + Zn rabbits. The Cu concentration was increased in kidney, heart, lung, aorta and liver of HCD and HCD + Zn rabbits compared with control rabbits.

Figure 4 shows the Zn concentrations in kidney, heart, lung, aorta and liver tissues of control, HCD and HCD + Zn rabbits. The Zn concentration was decreased in kidney, heart, lung, aorta and liver of HCD and HCD + Zn rabbits compared with control rabbits

Figure 5 shows the Pb concentrations in kidney, heart, lung, aorta and liver tissues of control, HCD and HCD + Zn rabbits. The Pb concentration was increased in kidney, heart, lung, aorta and liver of

HCD and HCD + Zn rabbits compared with control rabbits

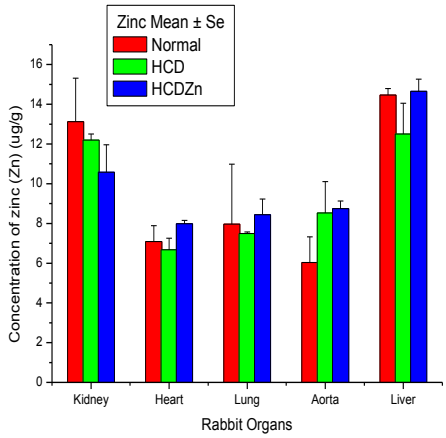

Figure 4: Zn concentrations in kidney, heart, lung, aorta and liver tissues of control, HCD and HCD + Zn rabbits

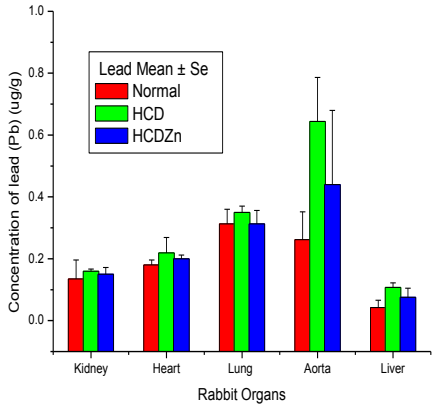

Figure 5: Pb concentrations in kidney, heart, lung, aorta and liver tissues of control, HCD and HCD + Zn rabbits

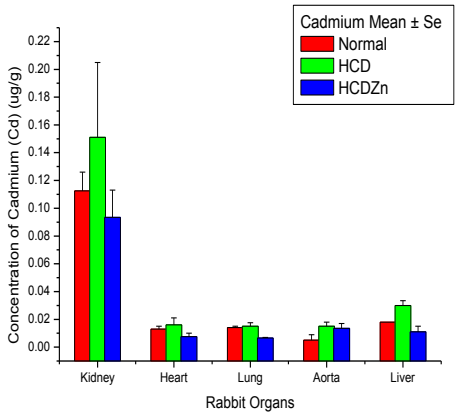

Figure 6: Cd concentrations in kidney, heart, lung, aorta and liver tissues of control, HCD and HCD + Zn rabbits

Figure 6 shows the Cd concentrations in kidney, heart, lung, aorta and liver tissues of control, HCD and HCD + Zn rabbits. The Cd concentration was increased in kidney, heart, lung, aorta and liver of HCD rabbits compared with control rabbits. While the Cd concentration was decreased in all tissues of HCD + Zn rabbits compared with HCD and control rabbits.

Table 3 shows the levels of TC, TG and HDL concentrations in control, HCD and HCD + Zn rabbits. Table 3 indicates significant increases in TC, TG, and HDL in HCD and HCD + Zn compared with control, while significant decreases in TC, TG and HDL in HCD + Zn compared with HCD.

Table 4 shows that RBCs count, hemoglobin, HCT%, monocyte%, neutrophils%, eosinophils%, and basophiles%, decreased in HCD compared with the control; while WBCs count, LYM%, MCV, MCH, MCHC, RDW, PLT, MPV, PCT, and RDW increased in HCD compared with control. The zinc supplementation to HCD improved all blood indices during the progression of atherosclerosis. The deficiency in RBCs, HGB and HCT% are very important factors during the progression of atherosclerosis. The dimensional parameters such as RDW, MPV, PCT, and PDW, and PLT count can also be used as predictor factors for atherosclerosis and cardiovascular diseases.

Discussion

In this study, HCD rabbits were fed normal rabbit chow supplemented with 1.0% cholesterol plus 1.0% olive oil for a period of 12 weeks. We found that in HCD and HCD + Zn rabbits, Fe concentrations were increased in kidney, heart, lung, aorta and liver tissues compared with control rabbits. These results suggest that Fe plays a major role in atherogenesis, probably through the production of free radicals, and that inducing anemia in HCD rabbits may delay or inhibit the progression of atherosclerosis. This study proposes that the increase in Fe in several tissues of rabbit may enhance deposition and absorption of intracellular and extracellular lipids in the intima, promote connective tissue formation, and accelerate smooth muscle proliferation leading to lower matrix degradation capacity and increased plaque stability. Several epidemiological studies have investigated the role of Fe as a potential risk factor in coronary heart disease, although many of this type of study have conflicting conclusions (Liao Y, 1994; Aysegul C, 2011). Any unregulated Fe has the potential to catalyze and generate hydroxyl radicals from superoxide and hydrogen peroxide via the Fenton reaction. The highly reactive hydroxyl radicals

subsequently cause lipid peroxidation, degradation of other macromolecules, leading to cell damage or death (Rice EC, 1993). In the study by Lee et al. 2003 using apo E-deficient mice, vascular Fe deposition was shown to be closely related to the progression of atherosclerosis and LDL oxidation. Watt et al., 2006 have indicated that inducing mild anemia in cholesterol-fed rabbits decreases the progression of atherosclerosis, in conjunction with decreases in lesion Fe content. In another study, rabbits fed with a HFD for 12 weeks, with desferal administration for the final nine weeks, exhibited a significant reduction in average lesion area as compared with 12-week HFD controls (Minqin R, 2003).

In this study, we found that in HCD and HCD + Zn rabbits, Cu concentration was increased in kidney, heart, lung, aorta and liver tissues compared with control rabbits. It was found that serum copper level of HCD rabbits was significantly higher than that of controls. Increased serum copper levels are a part of a specific defense mechanism to provide more copper at the site of infarction to reduce its size and the extent of damage (Gupta R, 1981). Also, the increase of ceruloplasmin, which is a copper containing enzyme and acute phase reactant, may account for the significant increase in serum copper levels (Tan LK, et al., 1992). Ceruloplasmin is an acute phase protein and is synthesized by the liver in response to tissue damage and inflammation. Ceruloplasmin is an important intravascular antioxidant and it protects tunica intima against free radical injury. This phenomenon is the basis for constantly observed sudden increase in serum copper and ceruloplasmin levels (Suciu A, et al., 1992). It has been reported that elevated levels of Fe and Cu were detected in the intima of lesions compared with healthy controls (Stadler N, et al., 2004). Stadler et al. 2004 have reported that Oxidized lipids and proteins, as well as decreased antioxidant levels, have been detected in human atherosclerotic lesions, with oxidation catalyzed by Fe and Cu postulated to contribute to lesion development. It has been proposed that Zn displaces Fe and Cu from oxidation-vulnerable sites, thereby protect against damage. Furthermore, dietary Zn supplementation in cholesterol-fed rabbits decreases the extent of lesion lipid oxidation and attenuates atherosclerotic burden, despite insignificant changes in lesion Zn. It has also been shown that dietary Cu supplementation significantly decreased aorta atherosclerosis in cholesterol-fed rabbits. The lesions from animals that received the Cu supplement contained fewer smooth muscle cells and fewer apoptotic cells (Lamb DJ, et al., 1999). Our findings are therefore consistent with the hypothesis that in our rabbit model, Cu may play a minor role during the progression of atherosclerosis.

In this study, we found that in HCD and HCD + Zn rabbits, Zn concentration was decreased in kidney, heart, lung, aorta and liver tissues compared with control rabbits. These results suggest that Zn may act as an endogenous protective factor against atherosclerosis, perhaps by reducing lesion Fe content, intracellular and extracellular lipids in the intima, connective tissue formation, and smooth muscle proliferation. Furthermore, our results suggest that Zn supplements may completely inhibit the progression of atherogenesis, perhaps by reducing the percentage change of Fe in most of the tissues of HCD rabbits. A study has shown that Zn can reduce the effects of carotid artery injury induced in rats by balloon dilatation, by reducing smooth muscle cell proliferation and intimal thickening (Berger M, et al., 2004). Zn is a co-factor of many enzymes and has been shown to have anti-inflammatory and anti-proliferatory properties. Studies also indicate that Zn is vital to vascular endothelial cell integrity and Zn deficiency causes severe impairment of the endothelial barrier function (Reiterer G, et al., 2004) Zn is believed to have specific anti-atherogenic properties by inhibiting oxidative stressresponsive transcription factors which are activated during an inflammatory response in atherosclerosis (Beattie JH, et al., 2004). In other work, test rabbits received a high cholesterol diet with Zn supplements for eight weeks and control rabbits were fed with a high cholesterol diet only for the same period of time. Lesion area analyses showed that the average lesion area was significantly reduced for the rabbits on the Zn-supplement diet (Ren MQ, et al., 2005). Jenner et al. (Jenner A, et al., 2007) have reported that Zn has an antiatherogenic effect, possibly due to a reduction in iron-catalyzed free radical reactions. In cholesterol-fed animals, Zn supplementation significantly reduced the accumulation of total cholesterol levels in aorta which was accompanied by a significant reduction in average aorta lesion cross-sectional areas of the animals. Elevated levels of cholesterol oxidation products in aorta of rabbits fed a cholesterol diet were significantly decreased by zinc supplementation. Alissa EM, et al., 2004 found that when rabbits were fed dietary supplements of Cu or Zn separately in conjunction with a HCD, aorta atherogenesis was inhibited. It becomes evident from this study that the changes in trace elements would alter the initiation and progression of atherosclerosis in HFD rabbits. The evidence for the same can be found only in aorta tissue [Watt et al., 2006), but not in several tissues as in our study. Watt et al. (Watt et al., 2006) have elucidated the role of trace elements Fe, Zn, Cu and Ca in induced atherosclerosis rabbits. Fe was present in early lesions at concentrations around seven times higher than in normal artery wall. Measurements of

localized lesion Fe concentrations were observed to be highly correlated with the depth of the lesion in the artery wall for each individual animal, implying that local elevated Fe concentrations may provide an accelerated process of atherosclerosis in specific regions of the artery. When Fe levels were reduced in the lesion, the progression of the disease was significantly slowed. Zn is depleted in the lesion and is also observed to be anti-correlated with local lesion development. Feeding the rabbits on a HCD with Zn supplements inhibited lesion development, although since no significant increase in lesion Zn levels was measured, this anti-atherosclerotic effect may be indirect. Xi-Ming and Li (Yuan XM, et al., 2003) has reported that published data from 11 countries clearly indicate that the mortality from cardiovascular diseases is correlated with liver iron. It proposes that redox active iron in tissue is the atherogenic portion of total iron stores. Further studies are required to clarify any change in the excretion of trace elements in the stools or urine, and to get the degree of atherosclerosis in HFD rabbits and to correlate the degree of atherosclerosis with the tissue concentration of various trace elements.

Serum zinc levels of AMI patients were significantly decreased than that of control group (Abdullah KH, et al., 2009). Recently, Kodavanti et al., 2003 reviewed associations between cardiovascular morbidity and mortality and air pollution indices, and have implicated particulate matter containing highly bioavailable zinc. Low serum Zn levels in patient group have been related to excess release of steroids due to the release of leukocyte endogenous mediators which redistributes the body Zn from serum and may cause a drop in serum Zn and also due to elevated levels of α2-macroglobulin which is a transport protein containing large amounts of Zn (Samal KK, et al., 1992). Recent characterization, suggest that zinc may share absorptive mechanisms with a variety of divalent cations, including cadmium, copper, iron, and lead **(**Fleming RE, et al., 1999).
Induction of metallothionein by zinc has been shown to alter the physiological disposition of copper (Waalkes MP, et al., 2000) and metallothionein has a greater binding capacity for copper than for zinc (Walsh CT, 1994), so causing elevation of serum copper level and lowering serum zinc level. Issa et al., 2001 proved that total mean level of serum zinc for patients with atherosclerosis was significantly lower than that of controls and total mean copper was significantly higher in those patients. Tan IK et al. (**1**992) found significantly elevated copper while serum magnesium and zinc slightly lower than in control group in a study carried out on 41 AMI patients and 41 healthy controls matched subjects. Metwalli et al**.** (1998) showed serum copper and magnesium levels in all patients with AMI were significantly higher than the corresponding values of controls and serum zinc level was not significantly different from that of control group. Ali et al**. (**1974) showed serum zinc concentration in AMI fall sharply within a day of onset.

In this study, we found that in HCD and HCD + Zn rabbits, Pb concentration increased in kidney, heart, lung, aorta and liver tissues compared with control rabbits. The blood Pb level was not being associated with coronary heart disease incidence (Kromhout D, 1988). The car-diovascular effects of Pb have been associated with increased blood pressure and hypertension. Studies in general populations have identified a positive association of Pb exposure with clinical cardiovascular (coranary artery disease: CAD), stroke mortality, and peripheral arterial disease, but the number of studies is small (Navas--Acien A, 2007; Lustberg M, 2002). Numerous experimental studies in animals have shown irrefutable evidence that chronic exposure to low Pb levels results in arterial hypertension that persists long after the cessation of Pb exposure (Navas--Acien A, 2007). We couldn't found information about Pb levels among patients with CAD having no history of Pb exposure in literature. In the present study, it was found that mean levels of serum Pb tended to be higher in CAD patients.

In this study, we found that in HCD and HCD + Zn rabbits, the Cd concentration increased in kidney, heart, lung, aorta and liver tissues compared with control rabbits.

The toxic mechanisms of Cd are not clear, as it is unable to generate free radicals directly. Indirect formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) involving the superoxide radical, hydroxyl radical and nitric oxide has been reported (Waisberg et al., 2003) and it is known to act intracellularly, mainly via free radical-induced damage, particularly to the heart, lungs, kidneys, bone, central nervous system and reproductive organs and (Waalkes, 2000). Cd can replace Fe and Cu in various cytoplasmic and membrane proteins (e.g. ferritin, apoferritin), thus increasing the amount of unbound free or poorly chelated Cu and Fe ions participating in oxidative stress via Fenton reactions (Price DJ et al., 1983;Watjen W et al., 2004).

Cd and Pb increased with age, whereas Cr, Mn and Ni tended to decrease, smoking induced elevation both in Cd and in Pb (Davies et al., 1997; Ikeda et al., 2011).Increased levels of Cd and Hg, and reduced levels of Zn, were related to a thickened IMT (Messner et al., 2009: Skoczyńska et al., 2009) or severity of coronary lesions at angiography (Giannoglou et al., 2010). Furthermore, exposure to

Cd increased, while administration of Zn and Cr reduced atherosclerosis development in the hypercholesterolemic rabbit and ApoE knock-out mice (Messner et al., 2009: Price Evans et al., 2009), giving further support to the theory that metals might be involved in the pathogenesis of atherosclerosis. Increased levels of Cd and Hg, and reduced levels of Zn, were related to a thickened IMT (Messner et al., 2009: Skoczyńska et al., 2009) or severity of coronary lesions at angiography (Giannoglou et al., 2010). Furthermore, exposure to Cd increased, while administration of Zn and Cr reduced atherosclerosis development in the hypercholesterolemic rabbit and ApoE knock-out mice (Messner et al., 2009: Price Evans et al., 2009), giving further support to the theory that metals might be involved in the pathogenesis of atherosclerosis.

It has been shown in different studies that Pb, Cd, Ni, Co and Hg levels are increased in patients with coronary artery disease, or in subjects developing myocardial infarction during a certain follow-up in cohort studies. In contrast, Cu, Zn and Cr were reported to be reduced in such studies (Afridi et al., 2010; Afridi et al., 2008: Alissa et al., 2009). Some essential metals can give adverse effects if the levels are decreased, but can also accumulate in the body, yielding higher levels, especially in the elderly (Afridi et al., 2010; Dar et al., 2008; Kazi et al., 2008). The hallmark of myocardial infarction is atherosclerosis. In the case of myocardial infarction, an atherosclerotic plaque in the coronary arteries ruptures and gives rise to an occluding thrombus. A number of studies have reported associations between metals and atherosclerosis, evaluated by carotid artery intima-media thickness (IMT).

This study suggests that additional experiments are needed to avoid or prevent the high toxicity induced by heavy and trace elements and studying the role of oxygen free radicals and antioxidants during the progression of atherosclerosis. These results also suggest that it may be possible to use the measurement of changes in trace elements in different tissues of rabbits as an important diagnostic tool during the progression of atherosclerosis.

Table 1: Chemical composition of laboratory NOR diet (Purina Certified Rabbit Chow # 5321)

Nutrients		Minerals		Vitamins	
Protein%	16.20	Ash%	7.30	Carotene, ppm	28.00
Arginine%	0.84	Calcium%	1.10	Vitaimn K, ppm	2.90
Cystine%	0.25	Phosphorus%	0.50	Thiamin Hydrochloride, ppm	4.80
Glycine%	0.77	Phosphorus (non-phytate)%	0.27	Riboflavin, ppm	5.00
Histidine%	0.38	Potassium%	1.20	Niacin, ppm	54.00
Isoleucine%	0.88	Magnesium%	0.25	Pantothenic Acid, ppm	19.00
Leucine%	1.30	Sulfur%	0.24	Choline Chloride, ppm	1600.00
Lysine%	0.78	Sodium%	0.30	Folic Acid, ppm	8.40
Methionine%	0.35	Chlorine%	0.66	Pyridoxine, ppm	4.50
Phenylalanine%	0.80	Fluorine, ppm	11.00	Biotin, ppm	0.20
Tyrosine%	0.50	Iron, ppm	340.00	B_{12} mcg/kg	6.60
Threonine%	0.64	Zinc, ppm	120.00	Vitamin A, IU/gm	20.00
Tryptophan%	0.14	Manganese, ppm	121.00	Vitamin D, IU/gm	1.10
Valine%	0.84	Copper, ppm	17.00	Vitamin E, IU/gm	44.00
Serine%	0.85	Cobalt, ppm	0.50	Ascorbic Acid, mg/gm	-
Aspartic Acid%	.87	Iodine, ppm	1.10		
Glutamic Acid%	3.33	Chromium, ppm	0.70		
Alanine%	0.85	Selenium, ppm	0.25		
Proline%	1.31				
Taurine%	<0.01				
Fat (ether extract)%	2.50				
Fat (acid hydrolysis)%	4.00				
Cholesterol, ppm	0.00				
Linoleic Acid%	1.31				
Linolenic Acid%	0.08				
Arachidonic Acid%	0.00				
Omega-3 Fatty Acids%	0.08				
Total Saturated Fatty Acids%	0.43				
Total Monounsaturated Fatty Acids%	0.70				
Fiber (Crude)%	14.00				
Neutral Detergent Fiber%	27.40				
Acid Detergent Fiber%	17.10				
Nitrogen-Free Extract (by difference)%	50.00				
Starch%	21.50				
Glucose%	0.34				
Fructose%	0.90				
Sucrose%	2.44				
Lactose%	0.00				
Total Digestible Nutrients%	66.00				

Gross Energy, kcal/gm	3.81				
Physiological Fuel Value, kcal/gm	2.88				
Metabolizable Energy, kcal/gm	2.49				

Table 2. Chemical composition of laboratory NOR diet (Minerals and Vitamins; Purina Certified Rabbit Chow # 5321)

Minerals		**Vitamins**	
Ash%	7.30	Carotene, ppm	28.00
Calcium%	1.10	Vitaimn K, ppm	2.90
Phosphorus%	0.50	Thiamin Hydrochloride, ppm	4.80
Phosphorus (non-phytate)%	0.27	Riboflavin, ppm	5.00
Potassium%	1.20	Niacin, ppm	54.00
Magnesium%	0.25	Pantothenic Acid, ppm	19.00
Sulfur%	0.24	Choline Chloride, ppm	1600.00
Sodium%	0.30	Folic Acid, ppm	8.40
Chlorine%	0.66	Pyridoxine, ppm	4.50
Fluorine, ppm	11.00	Biotin, ppm	0.20
Iron, ppm	340.00	B_{12} mcg/kg	6.60
Zinc, ppm	120.00	Vitamin A, IU/gm	20.00
Manganese, ppm	121.00	Vitamin D, IU/gm	1.10
Copper, ppm	17.00	Vitamin E, IU/gm	44.00
Cobalt, ppm	0.50	Ascorbic Acid, mg/gm	-
Iodine, ppm	1.10	-	-
Chromium, ppm	0.70	-	
Selenium, ppm	0.25	-	-

Table 3: Concentrations of lipids and lipoproteins in control, HCD and HCD+Zn rabbits

Biochemical parameters (mg/dl)	**Control (n=5)**	**HCD (n=5)**	**HCD + Zn (n= 5)**
Total Cholesterol	65.2 ± 8.71	658.95 ± 95.27	93.12 ± 5.5
Triglyceride	89.24 ± 9.71	197.25 ± 32.25	109 ± 10.25
Low density lipoprotein (LDL)	43.6 ± 7.41	677.2 ± 11.1	95 ± 24.13
High density lipoprotein (HDL)	12.37 ± 1.65	17.32 ± 1.55	13 ± 1.60

Table 4: Complete blood picture of control, HCD and HCD + Zn rabbits

Blood index	**Control (n=5)**	**HCD (n= 5)**	**HCD + Zn (n= 5)**
WBC (K/UL)count	6.95 ± 0.44	13.9 ± 0.57	8 ± 0.52
LYM %	43.73 ± 2.02	60 ± 2.00	50 ± 2.00
MON %	2.23 ± 1.27	1.15 ± 0.15	2.26 ± 0.97
NEU %	46.55 ± 2.69	35.7 ± 2.10	43.21 ± 2.33
EOS %	2.0 ± 0.57	1.33 ± 0.66	1.6 ± 0.50
BAS %	0.50 ± 0.18	0.45 ± 0.05	1 ± 0.13
RBCs (K/UL) count	6.10 ± 0.14	4.23 ± 0.48	5.64 ± 0.50
HGB (g/dl)	12.53 ± 0.28	10.56 ± 0.31	12.47 ± 0.83
HCT (%)	40.41 ± 0.81	32.42 ± 2.05	34.34 ± 5.19
MCV	66.27 ± 0.77	79.62 ± 6.75	70.56 ±6.20
MCH	20.43 ± 0.40	26.8 ± 4.14	28.62 ± 6.36
MCHC	31.01 ± 0.15	32.92 ± 1.98	33.98 ± 2.56
RDW	13.2 ± 0.34	16.36 ±1.09	14.42 ± 1.87
PLT	398.83 ± 50.00	609.65 ± 70.23	328.87 ± 52.23
MPV	6.6 ± 0.21	8.7 ± 0.44	6.1± 0.29
PCT	0.25 ± 0.03	0.35 ± 0.06	0.22 ± 0.05
PDW	9.97 ± 0.55	11.1 ± 0.76	9.23 ± 0.82

Conclusions

The aim of this study was to elucidate the changes in heavy and trace elements in several tissues of rabbits fed on HCD and HCD + Zn for a period of feeding of 12 weeks compared with control rabbits.

The HCD group fed on a NOR rabbit chow supplemented with 1.0% cholesterol plus 1.0% olive oil. The HCD + Zn group was fed on NOR Purina

Certified Rabbit Chow plus 1.0% cholesterol and 1.0% olive oil supplemented with 350 ppm Zn (total estimate 470 ppm Zn) for the same feeding period of time. Fe, Cu, Zn and Cd concentrations were measured in kidney, heart, lung, aorta, and liver tissues in control, HCD and HCD + Zn rabbits using ICP-ES.

Comparing HCD to control rabbits, we found an increase in Fe, Cu, Pb and Cd levels in kidney, heart, lung, aorta, and liver tissues of rabbits; while a decrease observed in Zn level in kidney, heart, lung, aorta, and liver tissues.

Comparing HCD + Zn with the control rabbits, we found that supplementation of Zn to the HCD decreased the levels of Fe, Cu, Pb, and Cd in kidney, heart, lung, aorta, and liver tissues of rabbits. These results demonstrate that Fe plays a major role during the progression of atherosclerosis through the production of free radicals, deposition and absorption of intracellular and extracellular lipids in the intima, connective tissue formation, and smooth muscle proliferation. Furthermore, inducing anemia in HCD rabbits may delay or inhibit the progression of atherosclerosis. Cu plays a minor role in atherosclerosis. Zn plays a major role in atherosclerosis; it may act as an endogenous protective factor against atherosclerosis perhaps by reducing lesion Fe content.

Acknowledgements
The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for its funding of this research through the research Group Project No. RGP-VPP-285

Corresponding Author:
Prof Dr. Mohamed Anwar K Abdelhalim
Department of Physics and Astronomy
College of Science
King Saud University
P.O. 2455, Riyadh 11451
E-mail: abdelhalimmak@yahoo.com
mabdulhleem@ksu.edu.sa

References

1. Abdelhalim MAK, Alhadlaq HA, Moussa SA. Elucidation of the effects of a high fat diet on trace elements in rabbit tissues using atomic absorption spectroscopy. Lipids in Health and Disease 2010, 9:2
2. Abdelhalim MAK. Atherosclerosis can be strongly influenced by iron and zinc overload or deficiency in the lung and kidney tissues of rabbits. African Journal of Microbiology Research Vol. 4(24) pp. 2748-2753, 18 December, 2010
3. Abdelhalim MAK. The changes of iron and zinc concentrations in heart and aortic tissues of rabbits fed on high fat diet during the progression of atherosclerosis. African Journal of Microbiology Research Vol. 4(15), pp. 1670-1675, 4 August, 2010
4. Abdullah Kh. Ibrahem, M.B.,Ch.B., M Sc Clinical Biochemistry, Serum copper, zinc, and magnesium in acute myocardial infarction in Ramadi municipality, Vol.7,No.1,June 2009 , ISSN: 2070-8882
5. Adekunle AS, Adelusi T I, Fatoki1 J O and Oyedokun B, A Diet-induced Atherosclerosis in Rabbit Model Provides an Insight into Essential Elements Concentrations in Cardiovascular Disease, British Journal of Medicine & Medical Research, 3(3): 517-531, 2013.
6. Afridi HI, Kazi TG, Kazi GH, Jamali MK, Shar GQ. Essential trace and toxic element distribution in the scalp hair of Pakistani myocardial infarction patients and controls. Biol Trace Elem Res 2008;113:19–34.
7. Afridi HI, Kazi TG, Kazi N, Kandhro GA, Baig JA, Shah AQ, et al. Evaluation of toxic elements in scalp hair samples of myocardial infarction patients at different
8. Ali M, Handjani J, Cecil SJ, John B et al. Serum zinc concentration in acute myocardial infarction. Chest 1974; 65: 185-187
9. Alissa EM, Bahijri SM, Lamb DJ, Ferns GAA: A nuclear microscopy study of trace elements Ca, Fe, Zn and Cu in atherosclerosis. Int J Exp Pathol 2004,85(5):265-275.
10. Alissa EM, Bahjri SM, Ahmed WH, Al-Ama N, Ferns GA. Chromium status and glucose tolerance in Saudi men with and without coronary artery disease. Biol Trace Elem Res 2009;131:215–28.
11. Aysegul C, Yuksel K, Hasan G, Halit D, Ibrahim H, Nihat S, Yilmaz G, Mustafa T., Trace Elements, Heavy Metals and Vitamin Levels in Patients with Coronary Artery Disease, International Journal of Medical Sciences tertilrlfeiclcieces 2011; 8(6):456-460
12. Beattie JH, Kwun IS: Is zinc deficiency a risk factor for atherosclerosis?. Br J Nutr 2004, 91(2):177-181.
13. Berger M, Rubinraut E, Barshack I, Roth A, Keren G, George J: A nuclear microscopy study of trace elements Ca, Fe, Zn and Cu in atherosclerosis. Atherosclerosis 2004, 175(2):229-234
14. Dar NA, Mir MM, Salam I, Malik MA, Gulzar GM, Yatoo GN. Association between copper

excess, zinc deficiency, and TP53 mutations in esophageal squamous cell carcinoma from Kashmir Valley, India—a high risk area. Nutr Cancer 2008;60:585–91.
15. Davies S, McLaren Howard J, Hunnisett A, Howard M. Age-related decreases in chromium levels in 51,665 hair, sweat, and serum samples from 40,872 patients implications for the prevention of cardiovascular disease and type II diabetes mellitus. Metabolism 1997;46:469–73.
16. Fleming RE, Migas MC, Zhou X et al. Mechanism of increased iron absorption in murine model of hereditary hemochromatosis: increase duodenal expression of the iron transport DMTI.Proc Natl Acad Sci USA 1999; 96: 3143-48.
17. Giannoglou GD, Konstantinou DM, Kovatsi L, Chatzizisis YS, Mikhailidis DP. Association of reduced zinc status with angiographically severe coronary atherosclerosis: a pilot study. Angiology 2010; 61:449–55.
18. Gupta R: Serum copper in patients with acute myocardial infarction. JAPI 1981; 29: 287.
19. Ikeda M, Ohashi F, Fukui Y, Sakuragi S, Moriguchi J. Cadmium, chromium, lead, manganese and nickel concentrations in blood of women in non-polluted areas in Japan, as determined by inductively coupled plasma-sector field-mass spectrometry. Int Arch Occup Environ Health 2011;84:139–50.
20. Issa N, Nasser N, Abduvahab EZ, Anahita M. Serum levels of Zn, Cu, Cr, and Ni in Iranian subjects with atherosclerosis. ARCH Irn Med 2001; 4(1): 21-24.
21. Jenner A, Ren M, Rajendran R, Ning P, Tan BK-H, Watt F, Halliwell B: Zinc supplementation inhibits lipid peroxidation and the development of atherosclerosis in rabbits fed a high cholesterol diet. Free Rad Biol Med 2007, 42:559-566.
22. Kazi TG, Afridi HIN, Kazi Jamali MK, Arain MB, Jalbani N, Baig JA. Distribution of zinc, copper and iron in biological samples of Pakistani myocardial infarction (1st, 2nd and 3rd heart attack) patients and controls. Clin Chim Acta 2008;389:114–9.
23. Khoo KL, Tan H, Liew YM, Deslypere JP, Janus E. Lipids and coronary heart diseasesin Asia Atherosclerosis. Asia Ather J. 2003;169(1):1-10.
24. Kodavanti UP, Moyer CF, Ledbetter AD, Schladweiler MC, Costa DL, Hauser R et al. Inhaled environmental combustion particles cause myocardial injury in the Wistar Kyoto rat. Toxicological Sciences 2003; 71: 237-245.
25. Koropatnick DJ, Zalups R (eds). Molecular biology and toxicology of metals. London: Taylor & Friends 2000; p 414-455.
26. Kromhout D. Blood lead and coronary heart disease risk among elderly men in Zutphen the Netherlands. Environ Health Per-spect. 1988; 78: 43-46
27. Lamb DJ, Reeves GL, Taylor A, Ferns GAA: Dietary copper supplementation reduces atherosclerosis in the cholesterol-fed rabbit. Atherosclerosis 1999, 146(1):33-43.
28. Lee TS, Shiao MS, Pan CC: Dietary Iron Restriction Increases Plaque Stability in Apolipoprotein-E-Deficient Mice. J Biomed Sci 2003, 10:510- 517.
29. Lee, AJ, Mowbray, PI, Lowe, GD, Rumley, A, Fowkes, FG, Allan, PL, 1998. Blood viscosity and elevated carotid intima-media thickness in men and women. The Edinburgh Artery Study. Circulation 97, 1467-1473.
30. Liao Y, Cooper RS, McGee DL: Iron status and coronary heart disease: negative findings from the NHANES I epidemiologic follow-up study. Am J Epidemiol 1994, 39(7):704-712.
31. Lustberg M, Silbergeld E. Blood lead levels and mortality. Arch Intern Med. 2002; 162: 2443-2449.
32. Martin AC. Clinical chemistry and metabolic medicine. 7th ed. London. Hodder Arnold; 2006.
33. Messner B, Knoflach M, Seubert A, Ritsch A, Pfaller K, Henderson B, et al. Cadmium is a novel and independent risk factor for early atherosclerosis mechanisms and in vivo relevance Arterioscler. Arterioscler Thromb Vasc Biol 2009; 29:1392–8.
34. Metwalli O, AL-Okabi S, Motawi T, EL-Ahmady O, Abdul-Hafeez S, EL-Said E. Study of serum metals and lipids profile in patients with acute myocardial infarction. J Islamic Acad Scien 1998; 11(1): 5-12.
35. Minqin R, Watt F, Huat BTK, Halliwell B: Iron and copper can theoretically both induce free radical mediated damage and thus promote atherogenesis. Free Radic Biol Med 2003, 34(6):746-752.
36. Navas-Acien A, Guallar E, Silbergeld EL, et al. Lead Exposure and Cardiovascular Disease—A Systematic Review. Environ Health Perspect. 2007; 115: 472-482
37. Nissen SE, Nicholls SJ, Sipahi I. Effects of very high-intensity statin therapy on regression of coronary atherosclerosis. The ASTEROID Trial. 2006;295(13):1556 1565

38. Price DJ, Joshi JG. Ferritin. Binding of beryllium and other divalent metal ions. J Biol Chem 1983;258:10873–80
39. Price Evans DA, Tariq M, Dafterdar R, Al Hussaini H, Sobki SH. Chromium chloride administration causes a substantial reduction of coronary lipid deposits, aortic lipid deposits, and serum cholesterol concentration in rabbits. Biol Trace Elem Res 2009;130:262–72.
40. Reiterer G, Toborek M, Hennig B: Peroxisome proliferator activated receptors alpha and gamma require zinc for their anti-inflammatory properties in porcine vascular endothelial cells. J Nutr 2004, 134(7):1711- 1715
41. Ren MQ, Rajendran R, Pan N, Huat BTK, Halliwell B, Watt F: The protective role of iron chelation and zinc supplements in atherosclerosis induced in New Zealand white rabbits: A nuclear microscopy study. Nucl Instr and Meth B 2005, 231:251-256
42. Rice-Evans C, Burdon R: Free radical-lipid peroxidation interactions and their pathological consequences. Prog Lipid Res 1993, 32:71-110
43. Samal KK, Kar CR, Sinha AK, et al. Serum zinc and copper levels in acute myocardial infarction. Recent Adv Nutr. 1990; 2:177-9
44. Skoczyńska A, Poreba R, Steinmentz-Beck A, Martynowicz H, Affelska-Jercha A, Turczyn B, et al. The dependence between urinary mercury concentration and carotid arterial intima-media thickness in workers occupationally exposed to mercury vapour. Int J Occup Med Environ Health 2009; 22:135–42.
45. Stadler N, Lindner RA, Davies MJ: Direct detection and quantification of transition metal Ions in human atherosclerotic plaques: Evidence for the presence of elevated levels of iron and copper. Arteriosclerosis Thrombosis Vascular Biol 2004, 24(5):949-954.
46. Suciu A, Chirulescu Z, Zeana C, Pirvulescu R. Study of serum ceruloplasmin and of the copper/zinc ratio in cardiovascular diseases. Rom J Intern Med 1992; 30 (3): 193-200.
47. Tan IK, Chua KS, Toh AK. Serum magnesium, copper and zinc concentrations in acute myocardial infarction. J Clin Lab Anal 1992; 6(5): 324-328
48. Tan LK, Chua KS, Toh AK : Serum magnesium, copper and zinc concentrations in acute myocardial infarction. J Clin Lab Anal 1992; 6: 324.
49. Waalkes MP, Perez-Olle R. Role of metallothionein transport and toxicity of metals. In Koropatnick DJ, Zalups R (eds). Molecular biology and toxicology of metals. London: Taylor & Friends 2000; p 414-455.
50. Waalkes MP. Cadmium carcinogenesis in review. J Inorg Biochem 2000;79:241–4.
51. Waisberg M, Joseph P, Hale B, Beyersmann D. Molecular and cellular mechanisms of cadmium carcinogenesis. Toxicology 2003;192:95-117.
52. Waisberg M, Joseph P, Hale B, Beyersmann D. Molecular and cellular mechanisms of cadmium carcinogenesis. Toxicology 2003;192:95-117.
53. Walsh CT, Sandstead H, Prasad A et al. Zinc: health effects and research priorities for the 199s. Environ Health Perspect 102 (Suppl 2) 1994; 5-46.
54. Watjen W, Beyersmann D. Cadmium-induced apoptosis in C6 glioma cells: influence of oxidative stress. Biometals 2004;17:65–78
55. Watt F, Rajendran R, Ren MQ, Tan BKH, Halliwell B: A nuclear microscopy study of trace elements Ca, Fe, Zn and Cu in atherosclerosis. Nuclear Instruments and Methods in Physics Research section B: beam Interactions with Materials and Atoms 2006, 249(1-2):646-652.
56. Yuan XM, Li W: The iron hypothesis of atherosclerosis and its clinical impact. Annals of Medicine 2003, 35:578-591.

10/7/2013