Full Length Research Paper

The changes of iron and zinc concentrations in heart and aortic tissues of rabbits fed on high fat diet during the progression of atherosclerosis

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High level of serum triacylglycerol and cholesterol is an important risk factor for the progression of atherosclerosis. The severity of atherosclerosis can be markedly influenced by iron (Fe) and zinc (Zn) overload or deficiency in aorta tissues of the rabbits. The changes of Fe and Zn in heart and aortic tissues of rabbits during the progression of atherosclerosis have not been well documented. Thus, the aim of the present study was to evaluate the changes of Fe and Zn in heart and aortic tissues of rabbits fed on high fat diet (HFD) for 12 weeks. The HFD group was fed a normal rabbit chow supplemented with 1.0% cholesterol plus 1.0% olive oil for a feeding period of 12 weeks. Fe and Zn concentrations were measured in two types of tissue from control and HFD rabbits using atomic absorption spectroscopy (AAS). The Fe concentration was significantly ($p < 0.05$) increased in HFD rabbits (Mean ± SE; heart: 10.52 ± 1.04 and aorta: 3.01 ± 2.61; n = 25 specimens) compared with control rabbits (Mean ± SE; heart: 8.41 ± 0.01 and aorta: 2.25 ± 0.37; n = 20 specimens). The Fe concentration was increased with percentage normalized changes of 25.09% in heart and 33.78% in aortic tissues of HFD rabbits compared with control rabbits while the Zn concentration was significantly ($p < 0.05$) decreased with percentage normalized changes of 14.39% in heart and 18.37% in aortic tissues of HFD rabbits compared with control rabbits. AAS was used to elucidate the changes of Fe and Zn in heart and aortic tissues of HFD rabbits compared with control rabbits. The findings of this study can be summarized as follows; percentage normalized change of increase of Fe was 25.09% in heart tissue accompanied by percentage normalized change of decrease of Zn 14.39% in heart tissue while percentage normalized change of increase of Fe was 33.78% in heart tissue accompanied by percentage normalized change of decrease of Zn 18.37% in aortic tissue. This study suggests that the increase in Fe concentrations in heart and aortic tissues may accelerate atherosclerosis through the production of free radicals while the decrease in Zn concentrations may act as a protective factor against atherosclerosis perhaps by reducing lesion Fe content. These results suggest that the changes in Fe and Zn concentrations in heart and aortic tissues of rabbits are closely related to the progression of atherosclerosis.

Key words: High cholesterol diet, iron, zinc, atherosclerosis, heart and aortic tissues, atomic absorption spectroscopy.

INTRODUCTION

Fe may participate in diverse pathological processes by catalyzing the formation of reactive oxygen free radicals. It has been hypothesized that iron-mediated oxidation is involved in this process. Several epidemiological studies have shown that the level of body Fe stores is positively correlated with the incidence of coronary heart disease in humans. Additional experiments on animals have further revealed that the severity of atherosclerosis can be markedly influenced by Fe overload or deficiency (Chau, 2000; Lum and Roebuck, 2001; Lynch and Frei, 1993; Jenner et al., 2007; Berger et al., 2004; Reiterer et al., 2004). Zn supplementation decreased the elevated levels of cholesterol oxidation products in the aorta and plasma caused by eating a high-cholesterol diet. Several studies have shown that Zn reduces oxidative damage and the risk of cardiovascular disease (Jenner et al., 2007; Berger et al., 2004; Ren et al., 2005). Scientists have suggested that because Zn supplementation reduces the
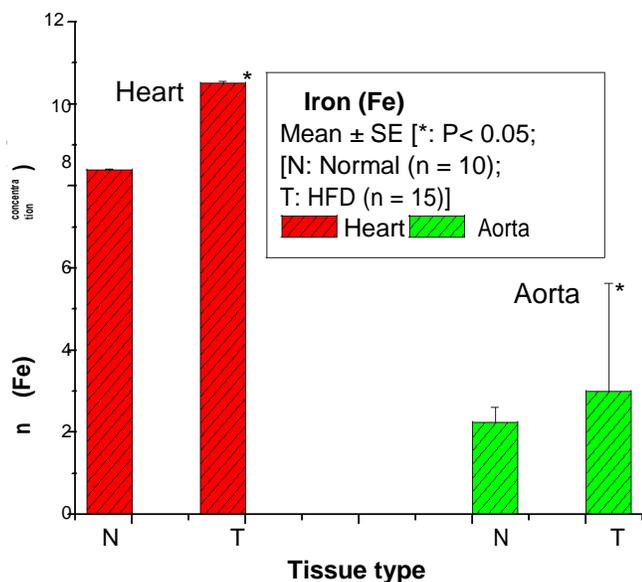
Figure 1. Fe concentrations in heart and aortic tissues of control and HFD rabbits.

formation of atheromas and lowers lipid peroxidation, it may have antioxidant activity (Watt et al., 2006; Ren et al., 2005; Beattie and Kwun, 2004). Since Zn is not redox active, it may not act directly as a scavenging antioxidant but instead it may act as an indirect antioxidant by competing with pro-oxidant metals such as Fe for strategic binding sites (Sullivan, 1981; Rice-Evans and Burdon, 1993; Lee et al., 2003). The role of a high fat diet (HFD) on Fe and Zn in heart and aortic tissues of rabbits has, in general, not been studied. Thus, the aim of this study was to evaluate the effects of an HFD on Fe and Zn in heart and aortic tissues of rabbits using atomic absorption spectroscopy (AAS).

METHODS

Animal treatment

The atherosclerotic model used in this study was the New Zealand white rabbit (male, 12 weeks old) (2.45 ± 0.25 kg), obtained from the Laboratory Animal Center (College of Pharmacy, King Saud University). Twenty rabbits were individually caged and divided into control group and HFD group. The control group (n = 8) was fed on 100 g/day of NOR diet (Purina Certified Rabbit Chow # 5321; Research Diet Inc., New Jersey, USA) for 12 weeks. The HFD group (n = 12) 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 animals were sacrificed after 12 weeks 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 (Abdelhalim, 2010). To obtain protoplasm representative of the *in vivo* situation and to avoid autolysis changes and bacterial growth, the aortas and hearts were carefully removed in a manner which avoided any damage to

the tissues. Each segment was rapidly flushed with deionized water to remove any residual blood.

Digestion of rabbit tissue samples

Various rabbit tissue samples were wet digested with nitric acid and converted into acidic digest solutions for analysis by AAS 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. Samples were then diluted to a final volume of 20 ml with quartz distilled water and stored in 1 oz. polyethylene bottles for later analysis by instrumental techniques.

Atomic absorption spectroscopy measurements

AAS measurements were carried out at the Research Center for Girls, King Saud University. Fe and Zn were measured using a Specter AA-220 series double-beam digital atomic absorption spectrophotometer. AAS was used to determine the presence and concentration of Fe and Zn in different tissues of rabbits. The sample of interest was aspirated into the flame. If that metal is present in the sample, it absorbs some of the light and this, reduces its intensity. A calibration curve was constructed by running standards of various concentrations (10, 15 and 20 PPM) on the AAS and observing the corresponding absorbance. A calibration curve was made and then samples were tested and measured against this curve. The concentration of Fe and Zn elements in each tissue sample was calculated by comparing the absorbance produced by the sample with that produced by a series of standards as follows:

Conc. of Sample = [(Absorbance of Sample/Absorbance of Standard) × (Conc. of Standard)].

Assessment of aortas atherosclerotic changes

According to the routine procedures, thoracic aortic tissue specimens were stained by Masson trichrome staining to examine fatty streaks, fibrous plaques, and any degenerative changes in collagen and elastin of the aortic tissues.

Statistical analysis

The results were expressed as mean ± standard error (SE). To assess the significance of the differences between the control group and HFD group of rabbits, statistical analysis was performed using one-way analysis of variance (ANOVA) for repeated measurements, with an assumed 5% error level (confidence interval) and a confidence level of 95%).

RESULTS

Figure 1 shows the Fe concentrations in heart and aortic tissues of control and HFD rabbits. The Fe concentration was significantly ($p < 0.05$) increased in HFD rabbits (Mean ± SE; heart: 10.52 ± 1.04 and aorta: 3.01 ± 2.61; n = 25 specimens) compared with control rabbits
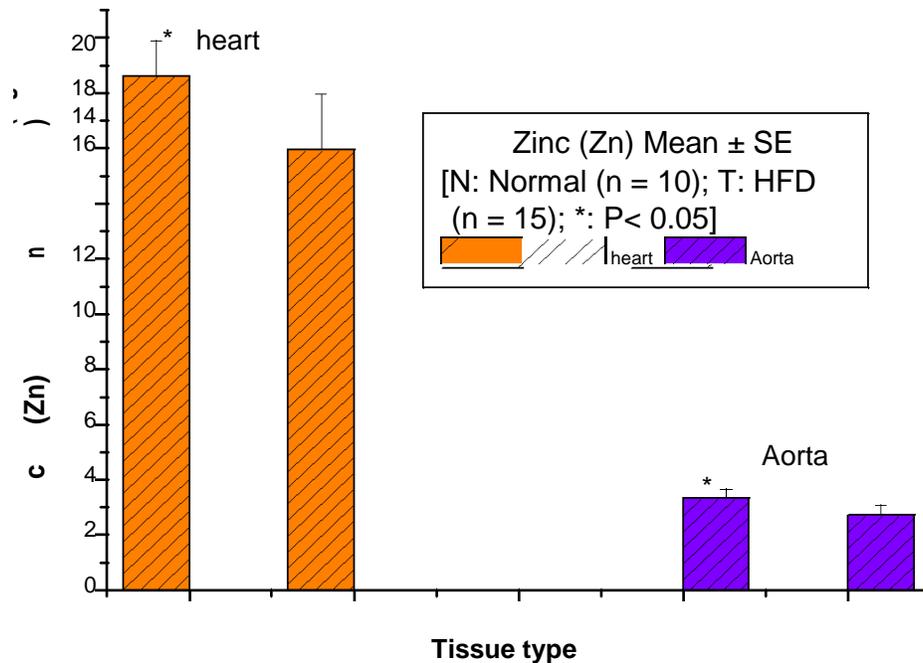
Figure 2. Zn concentrations in heart and aortic tissues of control and HFD rabbits.

(Mean ± SE; heart: 8.41 ± 0.01 and aorta: 2.25 ± 0.37; n = 20 specimens). The Fe concentration was increased with percentage normalized changes of 25.09% in heart and 33.78% in aortic tissues of HFD rabbits compared with control rabbits.

Figure 2 shows the Zn concentrations in heart and aortic tissues of control and HFD rabbits. The Zn concentration was significantly (p < 0.05) decreased in HFD rabbits (Mean ± SE; heart: 15.94 ± 2.02 and aorta: 2.71 ± 0.37; n = 25 specimens) compared with control rabbits (Mean ± SE; heart: 18.62 ± 1.28 and aorta: 3.32 ± 0.30; n = 20 specimens). The Zn concentration was decreased with percentage normalized changes of 14.39% in heart and 18.37% in aortic tissues of HFD rabbits compared with control rabbits.

To clarify the degree of atherosclerotic lesions, specimens from the aorta of NOR and CHO were stained with Masson trichrome (Figure 3). Figure 3 is a photomicrograph of Masson trichrome-stained thoracic aorta from an NOR and a CHO. The upper panel (NOR) illustrates normal arterial wall morphology. The lower panel (CHO) shows marked intimal thickening as well as a significant focal loss of medial architecture compared with the NOR specimen. In the CHO specimen, tunica media underlying plaques shows a marked disruption, with loss of elastin, less condensed and fragmented elastin was observed near the innermost and the outermost boundary of the media, and the intima contains intracellular and extracellular lipids, connective tissue formation, and smooth muscle proliferation.

DISCUSSION

In this study, rabbits were fed with HFD for 12 weeks. On comparing HFD rabbits to control rabbits, we found that Fe concentration was significantly increased in HFD rabbits compared with control rabbits. The Fe concentration was increased with percentage normalized changes of 25.09% in heart and 33.78% in aortic tissue of HFD rabbits 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 HFD rabbits may delay or inhibit the progression of atherosclerosis. Abdelhalim (2010) has reported that when rabbits were fed with high fat diet for 12 weeks, the plasma antioxidant enzyme activity of SOD and glutathioneperoxidase GPx (U/ml) was significantly deceased while oxidative parameter MDA (mol/ml) was significantly increased compared with control rabbits.

It has been reported that premenopausal women suffered a lower incidence of coronary heart disease compared with men of the same age because of their lower body Fe storage (Watt et al., 2006). Any unregulated Fe has the potential to catalyze and generate hydroxyl radicals from superoxide and hydrogen peroxide through the Fenton reaction. The highly reactive hydroxyl radicals subsequently cause lipid peroxidation, degradation of other macromolecules, leading to cell damage or death (Minqin et al., 2003). In the study by Lee et al. (2003) using apo E-deficient mice, vascular Fe deposition has shown to be closely related to the
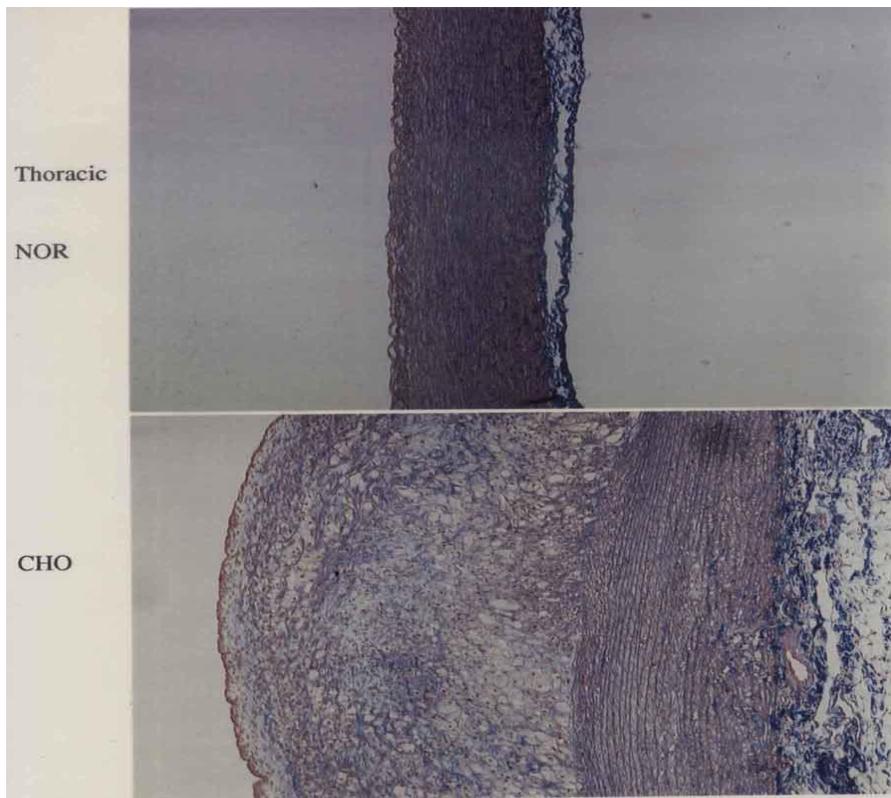
Figure 3. Photomicrographs of the Masson trichrome stained thoracic aorta obtained from a normal-fed rabbit (NOR) and a cholesterol-fed rabbit (CHO).

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 studies, rabbits fed with 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 of HFD controls (Minqin et al., 2003; Halliwell and Gutteridge, 2006). Watt et al. (2006) have reported that Fe is enhanced in the lesion compared with artery wall at levels at 90 ppm. 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. 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.

Zn is a co-factor of many enzymes and has been shown to have anti-inflammatory and anti-proliferatory properties. Studies have also indicated that Zn is vital to vascular endothelial cell integrity and Zn deficiency causes severe impairment of the endothelial barrier function (Lee et al., 2003). Zn is believed to have specific anti-atherogenic properties by inhibiting oxidative stress-responsive transcription factors which are activated during an inflammatory response in atherosclerosis (Lamb et al., 1999). In other works, lesion area analyses have shown that the average lesion area was significantly reduced for the rabbits on the Zn-supplement diet (Beattie and Kwun, 2004). Several studies (Beattie and Kwun, 2004; Ren et al., 2005; Alissa et al., 2004) 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 aortic 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. It has been proposed that Zn displaces Fe from oxidation-vulnerable sites, thereby protects against damage (Sullivan, 1981; Ren et al., 2005; Alissa et al., 2004; Xi-Ming and Li, 2003). This study proposes that the increase in Fe concentrations and the decrease in Zn concentrations in heart and aortic tissues of rabbits may enhance the deposition of intracellular and extracellular lipids in the intima, promote the connective tissue form-ation and smooth muscle proliferation, and increase fatty streaks and fibrous plaque stability. The Zn concentration

was decreased with percentage normalized changes of 14.39% in heart and 18.37% in aortic tissues of HFD rabbits compared with control rabbits. This study also suggests that Zn displaces Fe from oxidation-vulnerable sites, thereby protects against damage. 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 normalized change of Fe in heart and aortic tissues of HFD rabbits. The 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 (Rice-Evans and Burdon, 1993). From this study, it has become evident that the changes in Fe and Zn would alter initiation and progression of atherosclerosis in HFD rabbits. The evidence for the same can be found only in aortic tissue (Watt et al., 2006; Ren et al., 2005; Alissa et al., 2004), but not in heart and aortic tissues as in our study. 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 (Watt et al., 2006). Xi- Ming and Li (2003) have reported that published data from 11 countries clearly indicate that the mortality from cardiovascular diseases is correlated with liver iron. Their study proposes that redox active iron in tissue is the atherogenic portion of total iron stores. Further studies are required to clarify the concentration of Fe and Zn in several tissues of rabbits (lung and kidney tissues) and the excretion of Fe and Zn in stool or urine. Finally, a correlation between the Fe and Zn concentrations and the degree of atherosclerosis can be proved.

Conclusions

We used AAS to elucidate the changes of Fe and Zn in heart and aortic tissues of HFD rabbits compared with control rabbits. The results of the study showed that the percentage normalized change of increase of Fe was 25.09% in heart tissue accompanied by percentage normalized change of decrease of Zn 14.39% in heart tissue while percentage normalized change of increase of Fe was 33.78% in heart tissue accompanied by percentage normalized change of decrease of Zn 18.37% in aortic tissue. This study suggests that the increase in

Fe concentrations in heart and aortic tissues may accelerate atherosclerosis through the production of free radicals while the decrease in Zn concentrations may act as a protective factor against atherosclerosis perhaps by reducing lesion Fe content. These results suggest that the changes in Fe and Zn concentrations in heart and aortic tissues of rabbits are closely related to the progression of atherosclerosis.

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