

Full Length Research Paper

Atherosclerosis can be strongly influenced by iron and zinc overload or deficiency in the lung and kidney tissues of rabbits

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The present study was conducted to investigate the changes in iron (Fe) and zinc (Zn) levels in lung and kidney tissues of rabbits fed high fat diet supplemented with cholesterol (HFD+CHO) for 12 weeks. Twenty male rabbits (New Zealand White) were individually caged and divided into two groups. The control group was fed a normal rabbit diet (NOR). The HFD+CHO group received normal diet supplemented with 1.0% of olive oil and 1.0% of cholesterol. The lung and kidney tissue samples from the control and HFD+CHO rabbits were analysed for Fe and Zn concentrations using atomic absorption spectroscopy (AAS). Compared with the control animals the Fe concentration in HFD+CHO rabbits was significantly ($p < 0.05$) larger in both types of tissue, with percentage normalized changes of 95.29% for lungs and 7.08% for kidneys. The concentration of Zn in lung tissue was decreased, with percentage normalized change of 3.61%, and was significantly ($p < 0.05$) lower, with percentage normalized change of 71.40% in kidneys of HFD+CHO rabbits in comparison with the control (NOR) animals. The results showed that the high concentration of Fe in lungs was accompanied by the low concentration of Zn, while the high concentration of Zn in kidneys was accompanied by the low concentration of Fe. This suggests that the increase in iron concentrations in lung and kidney tissues may accelerate atherosclerosis probably through the production of free radicals which promote the production of oxidative parameters and that inducing anemia in HFD rabbits may delay or inhibit the progression of atherosclerosis. This also implies that zinc may be highly excreted from kidneys, which can be seen as an important risk factor and that using Zn-supplemented diets may retard and/or prevent the progression of atherosclerosis probably by reducing lesion Fe content, intracellular and extracellular lipids in the intima, connective tissue formation and smooth muscle proliferation.

Key words: HFD+CHO, Zn-supplemented diets, connective tissue formation, smooth muscle proliferation.

INTRODUCTION

The role of Fe as a potential risk factor in coronary heart disease was investigated in several epidemiological studies, although research in this area has led to a conflicting conclusion. Fe may participate in diverse pathological processes by catalyzing the formation of reactive oxygen free radicals (Lynch and Frei, 1993). It has been hypothesized that iron-mediated oxidation is involved in this process. Some epidemiological studies have also shown that the level of body Fe stores positively correlated with the incidence of coronary heart disease in human populations (Liao et al., 1994). The

experiments with animals have further revealed that the severity of atherosclerosis can be markedly influenced by Fe overload or deficiency (Chau, 2000; Lum and Roebuck, 2001). Watt et al. (2006) indicated that inducing mild anemia in cholesterol-fed rabbits decreases the progression of atherosclerosis, in conjunction with decreases in lesion Fe content and that Fe is enhanced in the lesion compared with artery wall.

The animal model studies reveal that Zn is vital to vascular endothelial cell integrity and Zn deficiency causes severe impairment of the endothelial barrier

function (Reiterer et al., 2004). Zn supplementation decreased the elevated levels of cholesterol oxidation products in the aorta and plasma caused by a high-cholesterol diet intake. Several experiments have also shown that Zn reduced oxidative damage and the risk of cardiovascular disease. Some authors have suggested that because Zn supplementation lowered both the formation of atheromas and lipid peroxidation rate it may have an antioxidant activity. Since Zn is not redox active, it may not act directly as a scavenging antioxidant but instead may act as an indirect antioxidant by competing with pro-oxidant metals (e.g. Fe) for strategic binding sites (Reiterer et al., 2004; Jenner et al., 2007). The influence of a high-fat diet feeding on the concentrations of such trace elements as iron and zinc in lung and kidney tissues has not been extensively studied in rabbits. Thus, the aim of this experiment with rabbits was to evaluate the effects of high fat diet supplemented with cholesterol on levels of Fe and Zn in these tissues using atomic absorption spectroscopy.

MATERIAL AND METHODS

Animals and feeding

The atherosclerotic model used in this study was the 12-week-old New Zealand White male rabbit, obtained from the Laboratory Animal Center (College of Pharmacy, King Saud University). A total of 25 animals were individually caged and divided into two groups. The control group NOR ($n = 10$) was fed 100 g/day of a normal diet (Purina Certified Rabbit Chow # 5321; Research Diet Inc., New Jersey, USA) for 12 weeks. For the same period of time the HFD+CHO group ($n = 15$) received the normal diet (100 g/day) supplemented with 1.0% of olive oil and 1.0% of pure cholesterol (Abdelhalim et al., 2010).

Tissue sampling and preparing

The animals were sacrificed by intravenous injection of Hypnorm (0.3 ml/kg body mass) in accordance with the guidelines approved by King Saud University Local Animal Care and Use Committee. To obtain protoplasm representative of the *in vivo* situation and to avoid autolysis changes and bacterial proliferation, the lungs and kidneys were carefully removed in a manner which avoided any damage to the tissues. Each segment was immediately flushed with deionized water to remove residual blood. All samples were flash-frozen in liquid nitrogen and stored at -85°C until analysis.

The tissue samples were wet digested with nitric acid and converted into acidic digest solutions for analysis by AAS method. Each 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 polyethylene bottles for later analysis by instrumental techniques.

Atomic absorption spectroscopy (AAS) measurements

The 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. A calibration curve was constructed by running standards of various concentrations: 10, 15 and 20 ppm. The concentration of both the Fe and Zn elements in each tissue sample was calculated by comparing the absorbance produced by the sample with that produced by standards (Abdelhalim et al., 2010).

Atherosclerotic changes assessment

To clarify the degree of atherosclerotic lesions, specimens from the thoracic aorta of NOR and HFD+CHO dietary treatment animals were evaluated by a standard microphotography analysis.

Statistical analysis

The results were expressed as mean \pm 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 significance assessed at 5% confidence level.

RESULTS

Figure 1 shows the Fe concentrations in the examined tissues. The Fe concentration was significantly ($p < 0.05$) higher in HFD+CHO rabbits (mean \pm SE: 19.90 ± 2.41 in lung and $14.06 \text{ mg/dl} \pm 0.51$ in kidney; $n = 15$) compared with the control rabbits (lung: 10.19 ± 1.09 and kidney: $13.13 \text{ mg/dl} \pm 0.81$; $n = 10$ specimens). In HFD+CHO rabbits the Fe levels were higher with percentage normalized changes of 95.29 and 7.08%, for lung and kidney respectively, compared with the control animals.

The concentrations of Zn in lung and kidney tissues are shown in Figure 2. The concentration was lower in lung tissue (mean \pm SE: 10.42 ± 1.42 ; $n = 15$) and significantly ($p < 0.01$) decreased in kidney tissue of HFD+CHO rabbits (15.94 ± 2.12 ; $n = 15$) in comparison with the mean value of control animals (lung: 10.81 ± 2.49 and kidney: $55.74 \text{ mg/dl} \pm 1.58$; $n = 10$ specimens). In the HFD+CHO rabbits Zn levels were lower with percentage normalized changes of 3.61% for lung and 71.40% for kidney compared with the control animals.

Figure 3 is a photomicrograph of thoracic aorta of rabbits fed normal and HFD+CHO diets (in this figure the abbreviation CHO means HFD+CHO rabbits). 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 show a marked disruption with loss of elastin and collagen, less condensed and fragmented elastin and collagen was observed near the innermost and the outermost boundary of the media. The intima contains intracellular and extracellular lipids, connective tissue formation, and smooth muscle proliferation.

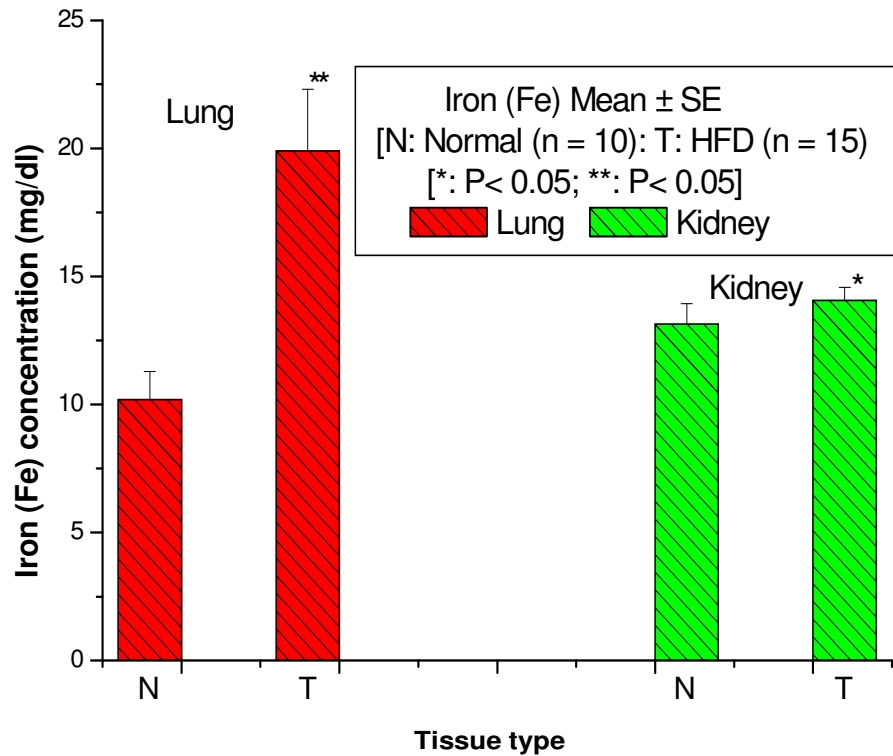


Figure 1. The iron (Fe) concentrations in lung and kidney tissues of the control (NOR) and HFD+CHO rabbits.

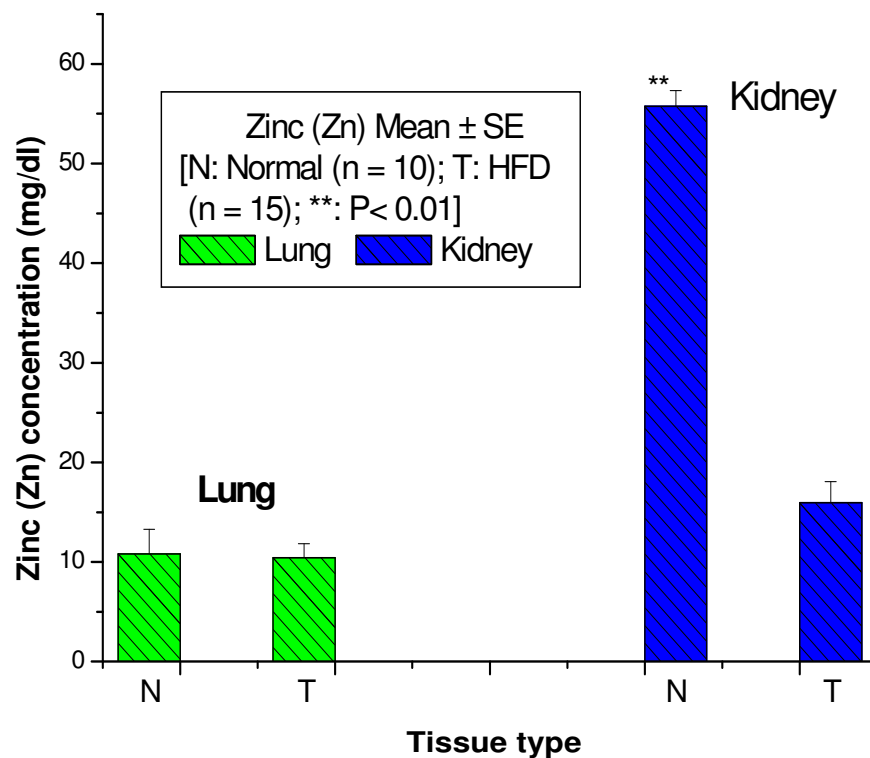


Figure 2. The zinc (Zn) concentrations in lung and kidney tissues of the control (NOR) and HFD+CHO rabbits

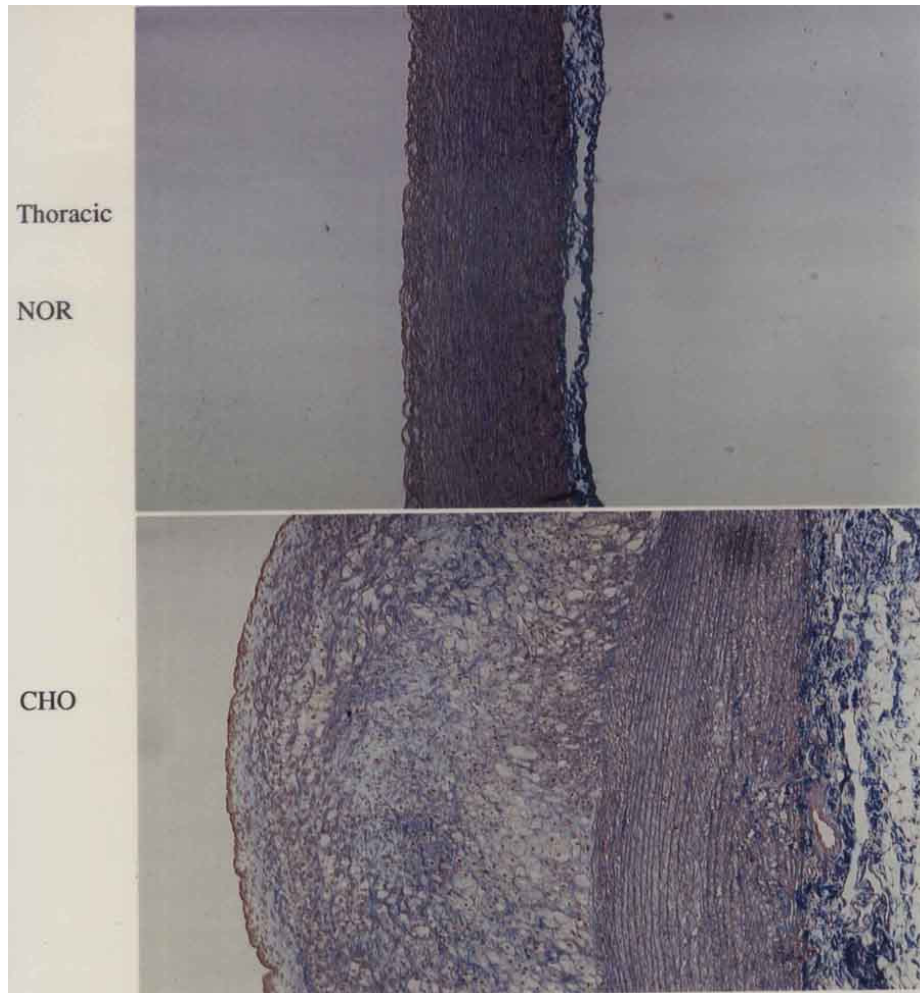


Figure 3. Photomicrographs of the Masson trichrome stained thoracic aorta obtained from normal (NOR) and HFD+CHO (CHO) rabbits.

DISCUSSION

In the current experiment rabbits were fed fat enriched-cholesterol-supplemented diet for 12 weeks. Comparing data from the control and HFD+CHO animals, we found that Fe concentration was significantly larger in HFD+CHO rabbits, with percentage normalized changes of 95.29% for lung and 7.08% for kidney. These results suggest that Fe plays an important role in atherogenesis, probably through the production of free radicals which promote the production of oxidative parameters, and that inducing anemia in HFD rabbits may delay or inhibit the progression of atherosclerosis (Alissa et al., 2004; Stadler et al., 2004).

The degree of atherosclerotic lesions was supported by histological study. It has been reported previously that, when aortic specimens of NOR and high-fat-diet rabbits were stained with Sudan, the aortas of NOR rabbits were completely free of fatty streaks and fibrous plaques and were characterized by a barely visible intima. In contrast,

the aortic specimens from rabbits fed cholesterol-supplemented diets exhibited lesions which comprised of fatty streaks and fibrous plaques (Abdelhalim et al., 2010).

Some authors reported that premenopausal women suffered a lower incidence of coronary heart disease compared with men at the same age because of their lower body Fe storage (Stocker and Keaney, 2004; Sullivan, 1981). 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-Evans and Burdon, 1993). In the study by Lee et al. (2003) with apo E-deficient mice, vascular Fe deposition was shown to be closely related to the progression of atherosclerosis and LDL oxidation. Stadler et al. (2004) 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 decreased the extent of lesion lipid oxidation and attenuates atherosclerotic burden, despite insignificant changes in lesion Zn.

Compared with the control rabbits Zn concentration was lower in the examined tissues of HFD+CHO animals, with percentage normalized changes of 3.61% for lung and 71.40% for kidney. This may suggest that Zn displaces Fe from oxidation-vulnerable sites, thereby protect against damage. This study also suggests that Zn may act as an endogenous protective factor against atherosclerosis, probably by reducing lesion Fe content, intracellular and extracellular lipids in the intima, connective tissue formation, and smooth muscle proliferation. It seems that Zn supplements may inhibit the progression of atherogenesis, perhaps by reducing the Fe in lung and kidney tissues of HFD+CHO rabbits. The study of Liao et al. (2010) showed 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. Zn is a co-factor of many enzymes and has been shown to have anti-inflammatory and anti-proliferatory properties. 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 (Beattie and kwun, 2004). In other work, lesion area analyses showed that the average lesion area was significantly reduced for the rabbits on the Zn-supplement diet (Ren et al., 2005). Several studies 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-supplemented diet were significantly decreased by zinc supplementation. It has been proposed that Zn displaces Fe from oxidation-vulnerable sites, thereby protect against damage (Ren et al., 2005).

The present results show that the changes in Fe and Zn in lung and kidney tissues of rabbits fed high-fat diet can be considered as important risk factors during the progression of atherosclerosis, as well as the factor that would alter the initiation and progression of atherosclerosis. The evidence for this phenomenon can be found only in aortic tissue (Beattie and Kun, 2004; Ren et al., 2005; Alissa et al., 2004), but not in lung and kidney 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 it is also observed to be anti-correlated with local lesion development (Watt et al., 2006). Xi-Ming and Li (2003) reported that published data from 11 countries clearly indicate that the mortality from cardiovascular diseases is correlated with liver iron. This suggests that redox active iron in the tissue is the atherogenic portion of total iron stores.

CONCLUSIONS AND APPLICATIONS

The results of this experiment indicate that high concentration of iron in lung is accompanied by low concentration of zinc, while high concentration of zinc in kidney is accompanied by low concentration of iron. These observations are important arguments that the increase in Fe concentrations in lung and kidney tissues may accelerate atherosclerosis through the production of free radicals; while the decrease in Zn concentration may delay or prevent atherosclerosis through reducing lesion Fe content. Thus Zn acts as a protective factor against atherosclerosis. The present study also suggests that zinc may be highly excreted from the kidney which can be used as an important risk factor during the progression of atherosclerosis. Zinc may replace iron in the aortic tissue of rabbits, therefore it seems that supplementation of diet with Zn may retard and/or prevent the progression of atherosclerosis. Overall changes of Fe and Zn concentrations in lung and kidney tissues of rabbits may be considered as closely related to the progression of atherosclerosis.

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