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PJBS

ISSN 1028-8880

# Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 8 (5): 771-775, 2005
ISSN 1028-8880
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# Effects of Hyperoxia and Aging on Hepatocyte, Cholesterol and Triglyceride Levels in Pigeon

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**Abstract:** This study was carried out to examine the effects of intermittent hyperoxia and aging on the ultrastructure of hepatosyte, blood cholesterol and triglycerides levels in pigeon. Young and old pigeons were exposed to hyperoxia (100% $O_2$), for 2 h daily for 6 weeks period. Ultrastructure of hepatocytes showed irregular clump of chromatin margination, large lipid accumulation, lipdec derbis and micropinocytosis vermiform. Baseline cholesterol of the young and the old groups were 288.30 and 441.83 mg/100 mL, respectively, which were significantly ($p< 0.05$) different. Following exposure to hyperoxia neither cholesterol for the young (288.30 vs 281.75 mg/100 mL) nor for the old (441.83 vs 443.37 mg/100 mL) changed significantly ($p > 0.05$) but the difference between the two groups remained significant ($p<0.05$). Thus hypercholesterolemia induced by aging remained independent of hyperoxia exposure. Baseline TG were 187.49 and 215.04 mg/100 mL for the young and old, respectively which were not significantly ($p>0.05$) different. Following exposure to hyperoxia TG decreased significantly ($p<0.05$) in the young group. Thus young pigeon were able to compensate for oxidized lipoprotein and the associated hepatocyte injury induced by oxidative stress.

**Key words:** Aging, cholesterol, hyeroxia, hepatocyte, pigeon, triglyceride

## INTRODUCTION

Hyperbaric oxygen (HBO) has been used in variety of medical treatment for respiratory insufficiency, hypothyroidism, liver disease, pancreatitis, myocardial infarction, metabolic disorders, toxemia and nephritic syndrome, long term therapy for intoxication, soft tissue infections, the treatment of carbon monoxide poisoning and diabetic patients[1,2]. Patients diagnosed with these disease conditions are already at increased risk of atherosclerosis[3] and suffer from hypoxemia. Glycerol stored in adipose issues, fatty acids and monoglyceroids, are reconverted to triglycerides (TG) by the liver. Almost, 90% of the dietary intake and 95% of the fat stored in tissues are TG. We hypnotized that repeated exposure to hyperoxia may be expected to increase the production of hydrogen peroxide, a major reactant in the myeloperoxidase-catalyzed formation of hypoclorous acid[4-6] which, in turn, may interfere with hepatocyte TG uptake.

TG and cholesterol (CHOL) are major components in low density lipoprotein that is used as marker for athreoscholorosis. It have been well known that high cholesterol levels induce apoptosis in vascular cells[7,8] and the induction of oxidative stress has been shown to increases the progression of atherosclerosis[6,9-12]. In other words the liver's ability to protect against reverse cholesterol transport and protection against peripheral cholesterol is impaired.

Because birds live on elevation and are physiologically adapted to moderate hypoxia, we wondered whether the additional periodic exposure to hyperoxia (HBO) would increase blood TG and CHOL levels. In the present study, the hypothesis tested that hyperoxia elevate CHOL and, TG in peripheral circulation. Surprisingly, we found that repeated exposure to hyperoxia for short periods of time does not significantly increases cholesterol but it does decrease TG in young pigeon. We also report ultrastructure changes of hepatocyte induced by HBO which showed variety of pathological changes in adult pigeon. These observations should extend our understanding of the etiology of oxygen therapy in the treatment during hospitalization period.

## MATERIALS AND METHODS

**Birds and hyperoxia treatment:** Twelve pigeons male, six adult and six young served as experimental groups. The adult group has a mean age and weight of 2.5 years and 258 g, respectively as compared with 7 weeks and 267 g in the younger group. The two groups were matched with their control groups for age and weight.

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**Hyperoxia exposure:** Experimental groups underwent hyperxia exposure for 2 h daily for six weeks period. Birds were placed in a closed box that has an inlet flow which was connected to 100% $O_2$ tank, medical grade, in which the flow was maintained at 5 L/min. The regulator of the tank was provided with a humidifier in order to saturate the inspired air with $H_2O$. The outlet of the box was adjusted at 3 L/min to ensure that the concentration of oxygen in the box would be approximately equal to the concentration of oxygen in the tank.

**Blood and tissue samples collection:** Birds were sacrificed and blood samples were collected into heparinized chilled glass viles that were centrifuged immediately at 3,000 rpm for 10 min then serum was analyzed for CHOL and TG. Liver tissue samples were selected by random and prepared as described below.

**Ultrastructure procedure:** Tissue samples were immediately fixed in 3% buffered glutaraldhyde (0.1 M cacoddylate buffer at pH 7.4 for 4 h at 2 to 4°C. The tissue samples were washed then were post fixed in 1% osmium peroxide in the buffer for 2 h at 2-4°C, then washed and kept overnight. Fixed tissue samples were dehydrated in graded concentrations of ethyl alcohol, (30, 50, 70 and 90%) for 30 min each and finally in absolute ethanol (100%) for 40 min. Tissues were infiltrated gradually in resin and embedded in plastic capsules in fresh full strength agar 100 epoxy resins before being cured at 60-70°C for 2 days. Dehydrated tissue samples were embedded in open and araldite mixture. Polymerized resin blocks containing tissue samples were prepared for sectioning, first semi-thin sections which were stained with touluidine blue for purpose of orientation. Accordingly ultra sections (70 nm) were made and double stained with uranyl acetate and lead citrate. Ultra sections were mounted on carbon-coated grids, then examined and photographed by Transmission Electron microscope (JEOL- 100 CX) at 80 KV[13].

**Kinetic determination of TG and LDL:** LDL and TG measurements were determined by estimating the total and free cholesterol levels by an enzymatic calorimetric (hydrolysis and oxidation). Lipoprotein fractions were also assessed by the determination of conjugated quinoneimine, which is formed by hydrogen peroxide and 4-aminoantipyrine, after absorption at 546 nm. Esterified cholesterol was calculated as the difference between total and free cholesterol as follows:

CHOL (mg/dL) = 200 * [ nm Sample / nm Standard],
LDL-Cholesterol = Total Cholesterol-Cholesterol in the supernatant,
TG-Cholesterol = Total Cholesterol-TG in the supernatant.

## RESULTS

**Cholesterol and triglyceride:** Group means comparison, using independent t-test, showed significant ($p<0.05$) difference between the means of the two groups for both triglyceride and cholesterol (Table 1). Following exposure to hyperoxia, the differences in both TG and CHOL remained significant ($p<0.05$ ). Table 1 and 2 showed that TG for the old group had increased following exposure to hyperoxia but the difference was not significant ($p>0.05$). However, if sample size was large enough, the long run mean value could have increased significantly. Even though there were no significant ($p>0.05$) changes in CHOL induced by hyperoxia, the trend of the changes followed similar pattern (Table 1 and 2) to those changes in TG. Thus the effects of exogenous hyperoxia had on TG and CHOL dependent on age factor, being positive in the adult and negative in the young.

Figure 1 showed liver cell from the control group in which normal mitochondria size and distribution. In contrast, variety of pathological changes induced by hyperoxia that included mitochondria hyperplasia, cristae dislocation and abnormal cytoplasmic contents were

Table 1: Serum cholesterol and triglycerides before hyperoxia for the young and old

| | Cholesterol (mL/100 mL) | | Triglyceride (mL/100 mL) | |
|---|---|---|---|---|
| Birds | Adult | Young | Adult | Young |
| 1 | 465.99 | 318.52 | 153.38 | 253.38 |
| 2 | 212.79 | 250.51 | 160.15 | 236.84 |
| 3 | 437.04 | 281.48 | 172.18 | 129.32 |
| 4 | 578.45 | 355.56 | 242.86 | 199.25 |
| 5 | 585.19 | 233.67 | 190.23 | 117.29 |
| 6 | 448.48 | 263.30 | 228.57 | 159.40 |
| 7 | 423.57 | 266.67 | 311.28 | 133.83 |
| 8 | 383.16 | 336.70 | 261.65 | 270.68 |
| Average | 441.83 | 288.30 | 215.04 | 187.50 |
| SEM | 41.42 | 15.43 | 19.67 | 21.57 |

Table 2: Serum cholesterol and after hyperoxia exposure

| | Cholesterol (mL/100 mL) | | Triglyceride (mL/100 mL) | |
|---|---|---|---|---|
| Birds | Adult | Young | Adult | Young |
| 1 | 642.42 | 332.66 | 241.35 | 109.77 |
| 2 | 505.05 | 314.48 | 163.16 | 168.42 |
| 3 | 346.13 | 173.74 | 288.72 | 166.92 |
| 4 | 347.47 | 342.08 | 132.32 | 99.25 |
| 5 | 375.76 | 245.79 | 300.00 | 59.40 |
| Average | 443.37 | 281.75 | 225.26 | 120.75 |
| SEM | 57.72 | 31.83 | 33.52 | 20.92 |

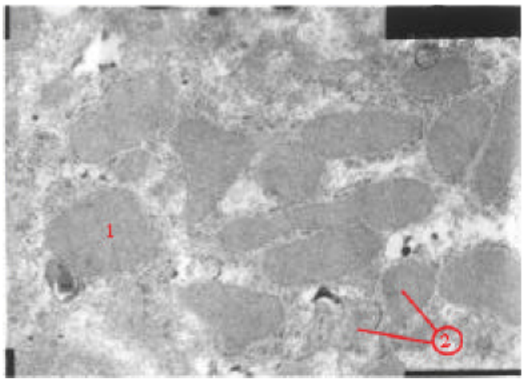


Fig. 1: Hyperplasia (2) resulted in giant mitochondria that could be due to fusion associated with large metrical dense golf-club-shape (1)

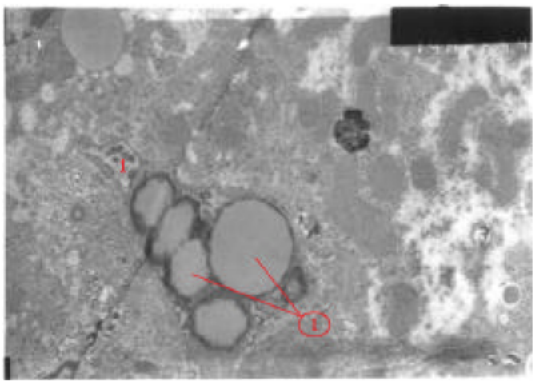


Fig. 3: Lipofuscin which is lipofuscin granules close to the nucleus associated with microbodies and microvilli

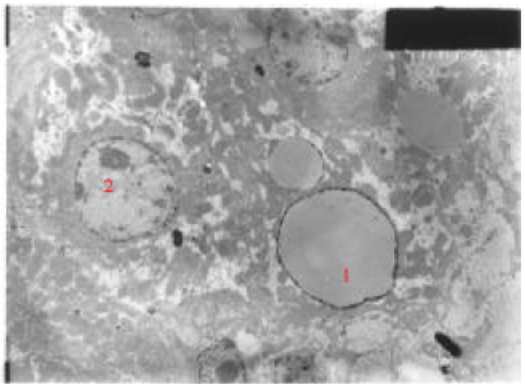


Fig. 2: Lipid bounded formation associated with increase in lipid droplet size (1) and liposomes (2).

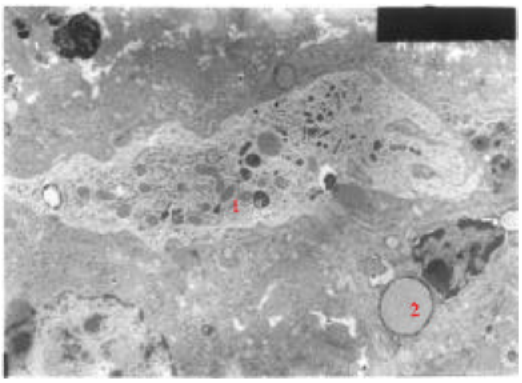


Fig. 4: Kupher cell-resident hepatic macrophages (1) and phagocyte activity (2).

clearly observed in the experimental group (Fig. 2), which related to oxidative damage. It is obvious and clear that damaged mitochondria and associated metabolic defect can cause excess blood clotting that is involved in the development of hypercholesterolemia atherosclerosis. From metabolic stand point it can be said that hyperoxia had caused mitochondria damage and delay in mitochondrial turnover and hence an accumulation of oxidative modified- proteins and lipids in the cytoplasm awaiting degradation which aggravates cytosolic lipid peroxidation. Furthermore hyperoxia resulted in accumulation of lipofuscin-bound iron in a redox-active form which might promote further intralysosomal lipid peroxidation (Fig. 3) as well as formation of lysosomal secondary structure that was followed by disruption of lysosomal membranes and increased activities of resident hepatic macrophages-Kupher (Fig. 4).

## DISCUSSION

In the present study TG and CHOL of young and old pigeon kept under intermittent hyperoxic conditions in order to correlate the age-change response. TG is important in the process of energy supply and as a modulator and mediator of several cellular biological functions. The results showed a decrease in TG and CHOL after hyperoxic exposure in the young pigeon. In contrast, the adult pigeons were less responsive to hyperoxia. As metabolic rate varies with age, the alterations in TG and CHOL occurring in the blood in response to hyperoxic are necessary protective role against alteration from oxidative metabolism and help to maintain the body in homeostatic ranges for energy requirements[5,14]. It was reported that hyperoxia may stimulate the production of antioxidant enzymes by tissues[15] and hence it may reduce the extent of

formation of oxidized lipoproteins and atherosclerosis. In contrast, other investigators concluded that hyperoxia affects the normal feedback-regulated cellular cholesterol uptake by peripheral cells and lead to foam cell production in the liver[16,17]. It have been well known that high cholesterol levels induce apoptosis in vascular cells[7,8,]. In addition, it was reported that hyperoxia caused atherosclerotic lesions[18,19] as oxidized LDLs potentiate monocyte-endothelial cell interaction[20-23].

Pathological changes in liver cells were indicative of cellular necrosis. These changes associated with increased levels of blood TG suggest a role of oxygen free radicals in the pathogenesis of hypercholesterolemia-induced atherosclerosis, which supports the hypothesis that oxygen free radicals are involved in the development of hypercholesterolemia atherosclerosis. Induction of oxidative stress has been shown to increases the progression of atherosclerososis[6,9,11,12]. Thus oxidative stress appears to make HDLs lose their ability to lower circulating cholesterol and reduces the body's ability to protect against atherosclerosis.

## ACKNOLEDGEMENTS

This project was supported by, College of Science, Research Center, grant No. ZOO/24-25/03. The authors thank S. Bashandi, Ph.D and F. Al-Shahrany for their technical assistant during data collection.

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