Journal of Applied Sciences

ISSN 1812-5654

science alert

ANSInet
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http://ansinet.com

Journal of Applied Sciences 9 (11): 2185-2189, 2009
ISSN 1812-5654

Evaluation of Electrical Conductivity of Hemoglobin and Oxidative Stress in High Fat Diet Rabbits

[1]Sherif Abdelmottaleb Moussa, [2]Mohamed Anwar K. Abdelhalim and [2]Hisham A. Alhadlaq
[1]Department of Science, King Khalid Millitary College, P.O. Box 22140, Riyadh, 11495, Saudi Arabia
[2]Department of Physics and Astronomy, College of Science, King Saud University,
P.O. Box 2455, Riyadh, 11451, Saudi Arabia

Abstract: The aim of this study was to evaluate the effects of feeding high cholesterol and saturated fat diet for a period of 10 weeks on the antioxidant status and the electrical conductivity of hemoglobin in rabbits. Thus, twenty of 12 weeks old male New Zealand white rabbits obtained from the Laboratory Animal Centre (College of Pharmacy, King Saud University, Saudi Arabia) were used. The rabbits were individually caged and divided into control group and high fat diet group. Serum lipids were measured using standard techniques. The electrical conductivity of hemoglobin and oxidative stress were evaluated in both groups of rabbits. We found that the levels of Total Cholesterol (TC), Triglycerides (TG), Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL) and Malondialdehyde (MDA) were significantly increased in the high fat diet rabbits compared with the control rabbits, and a significant decrease in the activities of plasma antioxidant enzymes, such as Superoxide Dismutase (SOD) and Glutathione Proxidase (GPx), was observed in the high fat diet rabbits compared with the control rabbits. The electrical conductivity of hemoglobin was significantly increased in the high fat diet rabbits compared with the control rabbits. Furthermore, a significant increase in the oxidative stress was observed in the high fat diet rabbits compared with the control rabbits, which was concomitant with the increase in the electrical conductivity of hemoglobin. Our results suggest that feeding rabbits a high cholesterol and saturated fat diet for a period of 10 weeks induces significant changes in TC, TG, LDL, HDL, MDA, SOD, GPx, the electrical conductivity of hemoglobin and the oxidative stress. Indeed, SOD, GPx, the electrical conductivity of hemoglobin and the oxidative stress may help in diagnosing and monitoring the progression of atherosclerosis.

Key words: Atherosclerosis, electrical conductivity of hemoglobin, oxidative stress, rabbits

INTRODUCTION

Atherosclerosis and heart disease are major causes of morbidity and mortality in adults in industrialized nations (Glass and Witztum, 2001). During the last decade, research work suggested that Low Density Lipoprotein (LDL) peroxidation within the arterial vessel wall plays a key role in atherogenesis. Atherosclerosis can generally be viewed as a form of chronic inflammation that is induced and perturbed by lipid accumulation (Glass and Witztum, 2001). One of the initial events in the development of atherosclerosis is the accumulation of cells containing excess lipids within the arterial wall. Hyperlipidemia or high levels of serum Triglyceride (TG) and cholesterol is a risk factor for premature atherosclerosis. A high fat diet has been reported to affect the health of humans and animal species (Ghosh *et al*., 2001). It has been reported that high levels of fat increase fat-mediated oxidative stress and decrease antioxidative enzyme activity. Thus, oxidative damage and its consequences may result in many chronic health problems that are attributed to high fat diet. The liver plays a central role in the maintenance of systemic lipid homeostasis and is especially susceptible to Reactive Oxygen Species (ROS) damage (Hamelet *et al*., 2007). This organ supplies energy substrates to peripheral tissues by the Cori cycle and glycogen catabolism and is important for detoxification.

Oxidative stress-related factors could be implicated in the functional impairment of the liver. ROS have detrimental effects on hepatocytes by damaging DNA, lipids and proteins, leading to a disruption in cellular homeostasis and aggravating metabolic syndrome features (Raval *et al*., 2006; Kohen and Nyska, 2002). Oxidative stress occurs when there is an excessive production of free radicals in the face of defective anti-

Corresponding Author: Mohammad Anwar K. Abdelhalim, Department of Physics and Astronomy, College of Science, King Saud University, P.O. Box 2455, Riyadh, 11451, Saudi Arabia
Tel: 009660509649633 Fax: 009331-4675791

oxidant defenses. Oxidative stress produces profound alterations to cellular membrane lipids, proteins and nucleic acids, impairing cell metabolism and viability and has been considered to be involved in diseases such as diabetes mellitus (Son *et al*., 2004), uremia (Vaziri, 2004), atherosclerosis (Stocker and Keaney, 2004), hypercholesterolemia (Warnholtz *et al*., 2001), rheumatoid arthritis (Hitchon and El-Gabalaw, 2004), adult respiratory distress syndrome, human immunodeficiency virus infection (Bautista, 2001), cystic fibrosis (Van der Vliet and Cross, 2000) and Friedreich's ataxia (Cooper and Schapira, 2003). Oxidative stress corresponds to an imbalance between the production of ROS, mainly the superoxide anion, hydroxyl radical, peroxyl radicals and hydrogen peroxide and protective mechanisms.

Thus, the aim of this study was to evaluate the effects of feeding high cholesterol and saturated fat diet for a period of 10 weeks on the antioxidant status and the electrical conductivity of hemoglobin in rabbits.

MATERIALS AND METHODS

Animal protocol and atherosclerosis samples: The atherosclerosis models used in this study were twenty (12-weeks) New Zealand white male rabbits obtained from the Laboratory Animal Centre (College of Pharmacy, King Saud University, Saudi Arabia). This study was obtained ethical clearance e.g., the approval given by the appropriate Ethical Committee. The rabbits were individually caged and divided into control group and high fat diet group. The control group (n = 8) was fed 100 g day^{-1} of normal diet (Purina Certified Rabbit Chow No. 5321; Research Diet Inc., New Brunswick, NJ 08901, USA) for a period of 10 weeks. The high fat diet group (n = 12) was fed a normal Purina Certified Rabbit Chow No. 5321 with 1.0% added cholesterol plus 1.0% olive oil (100 g day^{-1}) for the same period of time. The rabbits were sacrificed following intravenous injection of heparin (400 U kg^{-1} b. wt.).

Collection of blood and preparation of serum: Blood samples of 2 mL were obtained from the rabbits via venepuncture of an antecubital vein. Blood was collected into two polypropylene tubes, 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 37°C and to centrifuge at 3000 rpm for 10 min.

Determination of Total Cholesterol (TC), Triglyceride (TG), Low-Density Lipoprotein (LDL) and High-Density Lipoprotein (HDL): Serum TC and TG levels were analyzed by the clinical laboratory centre of King Khaled University Hospital. LDL and HDL concentrations were determined by the previously reported method (Lee *et al*., 1998; Koenig *et al*., 1992).

Antioxidant parameters

Superoxide Dismutase (SOD): The plasma activity of SOD was measured at 500 nm with a commercially available kit (Randox Laboratories, kit Ransod superoxide dismutase) by testing the inhibition degree of a tetrazolium salt oxidation reaction. The coefficient of variability between assays was 4.2% (Sun *et al*., 1988).

Glutathione peroxidase (GPx): The palsma activity of GPx was measured with a commercially available kit (Ransel glutathione peroxidase, Randox Laboratories) at 340 nm by measuring the decrease of NADPH absorbance. This method is based on that of Paglia and Valentine (1967). The coefficient of variability between assays was 4%.

Oxidative parameter

Malondialdehyde (MDA): Plasma MDA concentration was determined by using the method described by Draper and Hadley (1990) based on TBA reactivity. Briefly, 2.5 mL of 10% trichloracetic acid and 0.5 mL of plasma were added into tubes and mixed. After incubating for 15 min at 90°C and cooling with cold water, the mixture was centrifuged at 3000 rpm for 10 min. Two milliliters of supernatant was taken and 1 mL of 0.675 % TBA was added. The tubes were sealed and incubated at 90°C for 15 min and then cooled to room temperature. The optical density was measured at 532 nm by a spectrophotometer.

Electrical conductivity of hemoglobin: Electrical conductivity was measured by a conductivity meter (Digimeter L21; Conductivity Meter, Machwiss-Techn, D812 Walhuim, Germany) in the range 0-200 μS cm^{-1} coupled with automatic temperature compensator.

Statistical analysis: Data were analyzed using SPSS statistical software (SPSS/11 for windows). All the measurements were done in triplicates. A student's paired t-test was used to estimate the differences between the groups. All parameters were given as Mean±SE. The criterion for significance was $p<0.05$.

RESULTS

Table 1 shows the levels of TC, TG, LDL and HDL concentrations in control and high fat diet rabbits. Table 1 indicates significant increases in the levels of TC, TG, LDL and HDL in high fat diet rabbits compared with control rabbits.

Table 1: Plasma lipid status in control and high fat diet rabbits

Parameters (mg dL^{-1})	Control rabbits	High fat diet rabbits
Total cholesterol	58±22	708±14*
Triglycerides	48±2.3	406±120*
LDL	23±7	608±24*
HDL	11±2	26±4*

High fat diet rabbits (1.0% added cholesterol plus 1.0% olive oil); *Values are expressed as Mean±SE. *$p<0.05$ high fat diet vs. control; LDL: Low density lipoprotein, HDL: High density lipoprotein

Table 2: Plasma antioxidant and oxidative stress parameters in control and high fat diet rabbits

Plasma parameters	Control rabbits	High fat diet rabbits
SOD (U mL^{-1}) Superoxide dismutase	220±23	195±20*
GPx (U mL^{-1}) Glutathione peroxidase	38±15	24±10*
MDA (μmol mL^{-1}) Malondialdehyde	2.10±0.18	3.27±0.13*

*$p<0.05$ high fat diet vs. control; *Values are expressed as Mean±SE

Table 3: Electrical conductivity of Hb in control and high fat diet rabbits

Parameters	Control rabbits	High fat diet rabbits
Electrical conductivity (μS cm^{-1})	44±0.58	64±0.57*

*$p<0.05$ High fat diet vs. control; *Values are expressed as Mean±SE

Table 2 shows lipid peroxidation marker level, MDA and antioxidant defense system enzymes in control and high fat diet rabbits. MDA is significantly increased in high fat diet rabbits compared with control rabbits. While, SOD and GPx were significantly decreased in high fat diet rabbits compared with control rabbits.

Table 3 shows the electrical conductivity of hemoglobin in control and high fat diet rabbits. Table 3 indicates a significant increase in the electrical conductivity of hemoglobin in high fat diet rabbits compared with control rabbits.

DISCUSSION

In the present study, group of rabbits was fed on high cholesterol and saturated fat diet for a feeding period of 10 weeks. The serum TC, TG, LDL and HDL concentrations were significantly increased in high fat diet rabbits compared with control rabbits. The elevations in serum TC, TG and LDL levels observed in this study were in agreement with those reported in several studies (Abdelhalim and Alhadlaq, 2008; Augusti *et al*., 2001; Tanaka *et al*., 2001). It has shown that high serum abnormally levels of LDL and HDL are associated with an increased risk for atherosclerosis (Abdelhalim and Alhadlaq, 2008; Korhonen *et al*., 1996; Duverger *et al*., 1996). Hypercholesterolemia and hypertriglyceridemia are independent risk factors that alone or together can accelerate the development of coronary artery disease and the progression of atherosclerotic lesions (McKenney, 2001). Increased cholesterol concentration in high fat diet rabbits may be due to an increase in biosynthesis and/or diminished clearance from the blood. Normally circulating LDL can undergo reuptake in the liver via specific receptors and get cleared from the circulation (Aldons, 2000). This increased LDL concentration in plasma may be due to defect in LDL receptor either through failure in its production or function.

HDL concentration may play a protective role through reversing cholesterol transport, inhibiting the oxidation of LDL and by neutralizing the atherogenesis effects of oxidized LDL. Oxidative stress is considered as one of the causative factors that links hypercholesterolemia with the pathogenesis of atherosclerosis (Young and McEneng, 2001). An imbalance between free radical production and antioxidant level leads to oxidative stress, which is obvious from the depressed antioxidant defense parameters in the high fat diet rabbits of this study. A fat-enriched diet is regarded as an important factor in the development of cardiac diseases because it leads to the development of hyperlipidemia, atherosclerosis and abnormal lipid metabolism (Abdelhalim and Alhadlaq, 2008).

Present data clearly showed that feeding rabbits a high fat diet for a feeding period of 10 weeks significantly increased concentrations of plasma TC, TG, LDL and HDL. These high fat diet rabbits showed diminished concentrations of these antioxidants SOD and GPx. The diminished antioxidant defense system in high fat diet rabbits leads to lipid peroxidation. We have observed increased concentration of MDA and lipid peroxidation indices in the serum of high fat diet rabbits. The high fat diet results in a significant plasma oxidative damage as characterized by an increased MDA concentration and decreased SOD and GPx activities in high fat diet rabbits. MDA is a major reactive aldehyde that appears during the peroxidation of biological membrane polyunsaturated fatty acid (Fumelli *et al*., 1996). SOD and glutathione peroxidase have been shown to play a protective role against the oxidative damage in the various tissues by neutralizing ROS (Fumelli *et al*., 1996). The removal of ROS by SOD and of hydrogen peroxide by glutathione peroxidase prevents the formation of the reactive hydroxyl radical which is postulated to be responsible for the cellular damage. In this study, the electrical conductivity of hemoglobin was more prominent in high fat diet rabbits compared with control rabbits which is attributed to an increase in the free radical production. Consequently, hypercholesterolemia influences the electrical charge distribution on the surface of cell membrane (Daniel *et al*., 2008).

In summary, it became evident from the results of the present study that a significant increase in oxidative stress was observed in high fat diet rabbits compared with control rabbits which is attributed to an increase in the electrical conductivity of hemoglobin of high fat diet rabbits.

ACKNOWLEDGMENTS

The authors are very grateful for Research Centre of College of Science, King Saud University. This study was financially supported by College of Science, Research Centre Project No. Phy2007/60, King Saud University, Saudi Arabia.

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