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Research Article

Short-term Manifestations of High-cholesterol Feed and Effect of Remediation with Antioxidants - The Ocular Implications

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Abstract

Cholesterol is a waxy, white, slightly shiny fat-like substance, found in a variety of food. This substance is essential for life as it is ubiquitous in every cell in the body; vital for maintenance of cell membrane structure including the structure of organelles in the cytoplasm. It is important for insulation of nerve cells and a major component of the brain. Also, cholesterol assists skin cells to retain moisture and is the major building block for production of vitamin D and the essential hormones. The aim of this study was to investigate the effect of some antioxidant vitamins (A, B, C and E) on high feed of cholesterol to understand possible ocular implications.

Twenty (20) New Zealand rabbits were divided into four (4) groups: Group A (CTRL) was control and fed daily with standard chow; Group B (CHOL), was fed daily with normal diet and with high cholesterol (200mg/kg body weight), Group C (ANTX), was fed daily with a standard diet supplemented with vitamins A, B, C and E and Group D (CHOL+ANTX) was fed daily with normal diet and 200 mg/kg body weight cholesterol combined with supplements of vitamins A, B, C and E. Levels of total plasma cholesterol were monitored before and at four (4) weeks of treatment. After four (4) weeks of treatment, group B (CHOL) had significantly high total cholesterol value ($p < 0.05$) and group C (ANTX) had significantly reduced level of cholesterol ($p < 0.05$) while group D (CHOL+ANTX) had the same levels of cholesterol ($p > 0.05$) as on day 0. The results show that the of high cholesterol feed and consequent ocular implications could be mitigated by the intake of a combination of vitamins A, B, C and E whose action may be attributed to their antioxidant potentials against oxidative mechanisms in the rabbit's eyes specifically.

Keywords: *Cholesterol; Hypercholesterolemia; Antioxidant Vitamins; Ocular Tissues*

Abbreviations

CTRL: Group 1 which was the control and received normal feed daily; CHOL: Group 2 which was fed daily with 200 mg/kg body weight of cholesterol for 28 days without any interventional agent; ANTX: Group 3 which was fed daily with the normal diet and supplemented with vitamins A, B, C and E; CHOL+ANTX: Group 4 which was fed daily with the normal diet, high cholesterol (200 mg/kg body weight) and supplemented with a combination of vitamins A, B, C and E; TPC: Total Plasma Cholesterol; EDTA: Ethylene Diamine Tetra-acetate; HDL: High Density Lipoproteins

Introduction

Lipid metabolism is very vital biologically because it serves important metabolic functions in tissues. It forms substrates for lipid oxidation which provides tissues about 50% of energy utilized for basal metabolism. Lipids are all integral parts of cellular membranes and function as cellular thermal blankets due to their low conductivity. Lipids also function as protective cushion for the skeletal system at all levels of organisation of an organism [1].

Subcutaneous lipid deposits make up the vital sexual characteristics of most eukaryotic organisms. Sterols, which are referred to as monohydric alcohol are induced complex alcohols. They are important components of lipids of both plants and animals, in fact, all living tissues. These monohydric alcohols are phenanthrene derivatives. Phytosterols (sitosterol, campesterol and stigmasterol) are characteristic of plant components [2] while cholesterol is uniquely, a major component of animal lipids. Because cholesterol and triglycerides are insoluble in plasma, they are encapsulated by special fat carrying proteins known as lipoproteins for transportation in the blood. Moreover, about 70% of the cholesterol in the lipoproteins of the plasma is in the form of cholesterol esters. Cholesterol absorbed from food

and digested in the gastro-intestinal tract is called exogenous cholesterol while those produced internally are endogenous cholesterol. The following factors affect plasma cholesterol levels in the body: amount of cholesterol ingested daily, highly saturated fat diet, highly unsaturated fat diet, deficiency of insulin and/or thyroid hormone and genetic disorders [3]. To enhance homeostasis, the liver synthesizes cholesterol, triglycerides, phospholipids and lipoproteins. It produces both the low density lipid and high density varieties of cholesterol, releases them to the blood stream and withdraws them when necessary. The substrate for this activity is the presence of body fat which when elevated results to dyslipidemia which is an indicator of abnormal metabolism of cholesterol. Hyperlipidemia arises from elevation of plasma cholesterol and/or triglycerides or low high density lipid level that contributes to the occurrence of atherosclerosis [4]. There is a linear relationship between plasma lipid levels and some specific diseases due to this increase.

Hinging on amount of cholesterol ingested each day, the purpose of this study was to investigate the effect of short term high cholesterol feed on total plasma cholesterol and to find out the effect of antioxidant vitamin supplementation on the high cholesterol fed animals to understand possible ocular implications.

Rabbits were used in this experiment because they normally have reduced plasma cholesterol level due to their vegetarian dietary pattern. Increase in cholesterol based diet showcases a disruption of their vascular systems [3].

About 28 to 30% of Americans manifest hyperlipidemia (high cholesterol) while 31 to 32% of Americans have high blood pressure or hypertension [5]. Both hyperlipidemia and hypertension have a common route - increase in plasma cholesterol. Atherosclerosis is the major cause of death in developed countries.

About 2 million Americans suffer from age-related macular degeneration. This trend is increasing in developed countries where the life span of the elderly is increasing. In underdeveloped countries incidences of cholesterol related ocular manifestations are on the increase [5].

The ocular manifestations of hypercholesterolemia include arcus senilis, arcus juvenilis, xanthelasma palpebrarum, cataract, glaucoma, retinal anomalies such as retinal pigment epithelium detachment, retinal detachment, age-related macular degeneration, retinal vessel occlusion, lipemia retinalis and Hollenhorst plaque or cholesterol emboli. Others include choroidal anomalies and synchysis scintillans or cholesterolosis bulbi.

Materials and Methods

A total of twenty (20) New Zealand rabbits weighing between 1.40 - 2.15 kg (mean weight 1.67 ± 0.05 kg) consisting of ten (10) males and ten (10) females were used for the research. The rabbits were of same colony and purchased at Aduwawa Animal market, Benin City. They were transported in pet carriers of appropriate sizes and were well ventilated. They were kept in the animal house of the Department of Pharmacology in the Faculty of Pharmacy, University of Benin, where they were fed and acclimatized for two (2) weeks before commencement of the experiments. They were divided into four groups, each group consisting of five (5) rabbits. Group A (CTRL) was the control and fed daily with standard feed. Group B (CHOL) was fed daily with normal diet and high cholesterol (200 mg/kg body weight). Group C (ANTX) was fed daily with standard diet and combination of supplements of antioxidant vitamins such as vitamin A, B, C and E. Group D (CHOL+ANTX) was fed daily with high cholesterol and a combination of antioxidant vitamins - A, B, C and E and the normal diet.

Approval for consent for the animal research was gotten from the departmental research committee. The following parameters were measured: weights (kg) of the animals and Total Plasma Cholesterol (TPC) count.

The cholesterol powder used was Sigma Life Sciences Cholesterol, obtained from Sigma Aldrich Company, U.S.A. Lot No ST8C0068V, manufactured 2010, Expiry 2014.

The antioxidant vitamins used were:

- Vitamin A (Retinol) 200,000 IU (USP) Banner Pharmacaps Ltd, Canada. Batch No 30120262, manufactured 11, 2010, Expiry 11, 2013;
- Vitamin C (Natural Ascorbic acid) 500 mg (USP), mason vitamins inc. USA Lot/Exp 9524E/2 - 15;
- Vitamin E (Archy's Vitamin E 1000 IU) by Future biotics LLC, U.S.A. Lot/Exp1427/6 - 2015;
- Vitamin B complex (High Potency) vitamin B1, 5 mg (BP), vitamin B2, 2 mg (BP), vitamin B6, 2 mg (BP) and Nicotinamide-B3, 20 mg (BP) by Vitabiotics Nig. Ltd., Lagos, Nigeria. Batch No T1931, manufactured 2 - 2013, Expiry 2 - 2015, NAFDAC No 04-0076; and
- Meyer's Folic acid and B12. Folic acid 5 mg (BP) and B12, 5 mcg, Batch No T101212 manufactured 12 - 2012, Expires 11 - 2014, Vitabiotics Nig. Ltd, Lagos, Nigeria.

Weights were measured with galaxy balance, GT4800, Cat. NO Z16, 012-1.

Random Cholesterol Kits were used by the laboratory scientist to determine the Total Plasma Cholesterol (TPC) count.

5 ml syringes and blood disodium Ethylene Diamine Tetra-acetate (EDTA) tubes were used to collect the blood samples.

Mode of antioxidant vitamins and cholesterol administration

All vitamins (A, B, C and E) and cholesterol were administered orally. The water soluble vitamins B and C were administered mixed with 5 ml of distilled water while cholesterol and vitamins A and E, which are lipid soluble, were administered with 5 ml olive oil [6].

These liquids (water and lipid soluble) were administered by syringing without needle into the mouth, with the rabbit in appropriate position, once daily.

Blood Sampling - Rabbits possess very unique and large ears which are enmeshed in a network of blood vessels, which are large and accessible. Blood was collected from the ear as described by Bivin and Timmons [7]. This was mixed with EDTA in the containers and centrifuged. Randox cholesterol kits were used for immediate analysis which allowed precision and accurate reading. Normal range of the cholesterol in rabbits is still controversial and would be explained. We fed 200 mg/kg daily and the mean weight of the rabbits was 1.67 ± 0.05 kg. That is, each animal got between 300 and 350 mg/day.

Results and Discussion

Results

	Group 1 (CTRL)	Group 2 (CHOL)	Group 3 (ANTX)	Group 4 (CHOL+ANTX)
Day 0	1.63	1.54	1.66	1.68
Day 28	1.90	1.66	1.88	1.76

Table 1: *Mean weights (kg) in all group on Days 0 and 28.*

The weights showed minimal and insignificant changes (p > 0.05).

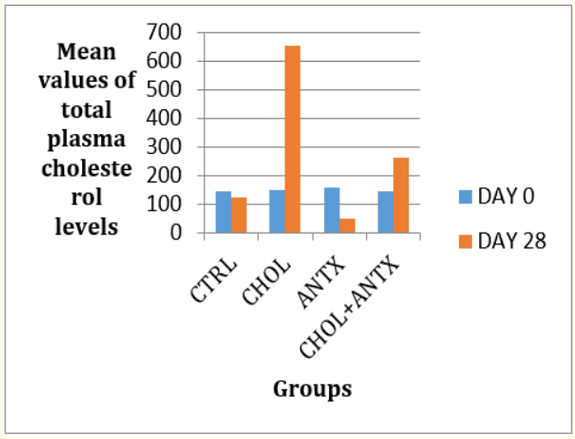

Figure 1: *Total plasma cholesterol levels (mg/dl).*

Discussion

Total plasma cholesterol value for rabbit is not distinct. Rather, there has been lots of differing result. Recent research still has showcased results that are different from other earlier results and quite a lot of reasons have been adduced to this discrepancy. Below are some of the reasons given regarding the differences.

Strain difference could manifest difference in values especially in cholesterol metabolism. Sex differences could also occur. There could be physiological differences among the breeding stock of a particular strain. Also it could be due to the method of chemical analysis, seasonal or circadian changes in blood constituents [8]. Another issue may be the method of bleeding.

From the result, Group A (CTRL), which was the control, had insignificant change between cholesterol value on day 0 and 28 days after ($p > 0.05$). However, Group B (CHOL), which was fed with high cholesterol feed showed very significant increase between day 0 and 28

days later ($p < 0.05$). As stated earlier, rabbits are non-ruminant herbivores. This implies that due to their dietary pattern, excess lipid would be reflected in their vascular system and in most of the organs. This hypercholesterolemia has a close correlation with a three times increase as observed by Javed and his associates [9]. Though Javed and colleagues used doses of between 300 and 500 kg/body weight, other studies have also used high cholesterol feed to induce hypercholesterolemia so as to assess hypercholesterolemia-metabolic disturbances [10]. Group C (ANTX), which was fed with the antioxidant vitamins, is indeed exciting as the Total Plasma Cholesterol count was reduced significantly after 28 days ($p < 0.05$). In Group D (CHOL+ANTX), which was fed with high cholesterol feed and antioxidant vitamins, there was no significant difference in cholesterol level between day 0 and 28 days after ($p > 0.05$).

From the results, antioxidant vitamins influence cholesterol content in the body. This is substantiated by the investigations by Cham., *et al.* [11] and Adams., *et al*. [12] and Braakhuis., *et a*l [13]. Though the appropriate mechanism has not been properly elucidated, there is the school of thought that postulates that these antioxidants reduce cholesterol in the body by enhancing healthy utilization of cholesterol through the control of oxidative mechanisms such as photo-oxidation. Also, this process reduces the Low Density Lipoproteins (LDLs) and increases the High Density Lipoproteins (HDLs) which is the good cholesterol. Zubay and his colleagues stated that HDLs function in the removal of cholesterol from the non-hepatic tissues and then return the cholesterol to the liver, where it is metabolized and excreted [14]. They also posited that lipoprotein lipase is an extracellular enzyme that is extremely active in the capillaries of adipose tissues, cardiac and skeletal muscles and lactating mammary gland. It is associated with HDL in this case, which thus supply the heart and adipose tissue with fatty acids. The fatty acid produced by lipoprotein lipase on circulating lipoprotein is taken to the heart, muscle and adipose tissues for energy production or stored as components of triglycerols, or the fatty acid may be bound to albumin and transported to other tissues, all in an effort to reduce the TPC level in the blood.

Elevated cholesterol can disturb the eyes and vision, and the consequence can be anything from harmless to devastating, irreversible blindness. The ocular manifestations of hypercholesterolemia include arcus senilis, arcus juvenilis, xanthelasma palpebrarum, cataract, glaucoma, retinal anomalies such as retinal pigment epithelium detachment, retinal detachment, age-related macular degeneration, retinal vessel occlusion, lipemia retinalis, choroidal anomalies and synchysis scintillans or cholesterolosis bulbi and Hollenhorst plaque or cholesterol emboli. Since our results show that the adverse effect of high cholesterol feed could be mitigated by the intake of a combination of vitamins A, B, C and E whose action may be attributed to their antioxidant potentials against oxidative mechanisms, it is therefore certain that consequent ocular implications from hypercholesterolemia may be lessened by the intake of the antioxidant vitamins in the rabbit's eyes.

Conclusions

Antioxidant vitamins enhance increased production of HDL and lipoprotein lipase which enhance appropriate metabolism of the cholesterol thereby reducing the plasma level. When the plasma cholesterol is high, antioxidants lower photo-oxidation synergistically with increased HDL production and lipoprotein lipase activities thereby lowering the high and risky cholesterol and its ocular implications in the rabbit's eyes specifically.

Conflict of Interest

The authors declare no conflicts of interest.

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