ASSESSMENT OF THE POSSIBLE LIPID LOWERING EFFECTS OF VITAMIN B6 SUPPLEMENTATION IN RATS

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ABSTRACT

Hyperlipidaemia is the major known cause of cardiovascular diseases. Literatures has established the links between vitamin B complex supplementation and reduction in oxidative stress and inflammation which are two events associated with cardiovascular diseases. This study is therefore designed to assess the effects of vitamin B6 in the regulation of serum lipids such as total cholesterol, phospholipids, triglycerides, and high density lipoprotein cholesterol (HDL-C). Results showed that serum total cholesterol and triglyceride were significantly reduced ($p \leq 0.05$) in rats placed on vitamin B6 supplements compared with control group. Serum phospholipids and high density lipoprotein cholesterol were significantly elevated ($p \leq 0.05$) in rats placed on vitamin B6 supplements when compared with control group. The data showed anti-hyperlipidaemic effects of vitamin B6 supplementation in rats.
Key words: Hyperlipidaemia, cardiovascular diseases, vitamin B6, serum lipids.

INTRODUCTION

Heart disease or cardiovascular diseases are the class of diseases that involve the heart or blood vessels (arteries and veins). While the term technically refers to any disease that affects the cardiovascular system , it is usually used to refer to those related to atherosclerosis (arterial disease). These conditions usually have similar causes, mechanisms, and treatments (Maton *et al*., 1993).

Lipid abnormalities (high cholesterol), hypertension, smoking and metabolic disorders such as hypercholesterolemia, hyperhomocysteinemia and diabetes are risk factors held responsible for the development of cardiovascular disease (Niessen *et al*., 2003). In addition to these ‘traditional’ risk factors, inflammatory reactions considerably take part in both development and outcome of atherosclerosis e.t.c (Ross, 1999).

Cardiovascular disease is one of the main health problems concerning health care services in western societies (Ureland *et al.*, 1989). Despite the established significance of the classical risk factors, there continues to be a large number of cardiovascular disease patients who have no relation to any of them (Ureland *et al.*, 1992). It has recently been discovered that elevated plasma levels of the amino acid homocysteine are associated with a greater risk and increased mortality from cardiovascular disease in the general population (Boushey *et al.*, 1995).

Homocysteine is produced from methionine as a product of a large number of trans methylation reactions dependent on S-adenosylmethionine (Finkelstein 1990). Three enzymes contribute to homocysteine metabolism. When there is an excess of methionine, homocysteine follows the transulphydryliosis pathway, through which homocysteine is converted automatically to cysteine. The first reaction in this pathway is catalysed by an enzyme dependent on vitamin B6, cystathionine synthase (Finkelstein, 1990).

Elevation in plasma homocysteine levels promote oxidative damage, inflammation and endothelial dysfunction which are independent risk factors for cardiovascular diseases.

Lipid profile is the collective term given to the estimation of, typically, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides. An extended lipid profile may include very low-density lipoprotein. This is used to identify hyperlipidemia (various disturbances of cholesterol and triglyceride levels), many forms of which are recognized risk factors for cardiovascular disease (William, 2003).

This study was aimed at studying the anti-atherogenic effects of vitamin B6 supplementation in albino rats.

METHODOLOGY

EXPERIMENTAL ANIMALS AND ADMINISTRATION OF VITAMIN

Ten albino rats with an average weight of 136g were purchased from commercial breeders in Ilorin, Kwara state. They were kept in a well-ventilated cage in the animal house of the department of Anatomy, LAUTECH with conducive atmospheric pressure and temperature range between 25^0-30^0C. The animals do not suffer any observable disorder and have unrestricted access to clean water.

The ten rats were separated into two groups consisting of five rats per group. Group 1 were not given vitamin B6 supplement, while group 2 were administered a therapeutic dose of 0.2mg/Kg body weight of vitamin B6.

SAMPLE COLLECTION

The rats were sacrificed on the 15^{th} day, using chloroform as an anesthesia. After which each rat was opened up, and 5mL of blood was collected directly from the heart into appropriately labeled samples bottles. Organs such as the liver was quickly excised and kept in appropriate buffer solution.

Non-heparinized bottles containing the dispensed serum were correspondingly labeled according to the groups and identity of each animal respectively. 5mL serum bottle of blood from the collections were arranged in the centrifuge and spun at 4,000rpm for 10 mins. After the centrifuge had finally revolved, serum bottles were brought out and decanted from the lysed erythrocytes seated at the bottom of each bottle into new sets of labeled non-heparinised bottles.

All samples were kept in a refrigerator alongside the reagent kit at a temperature range of 2-6^0C, for analysis of biochemical parameters.

DETERMINATION OF BIOCHEMICAL PARAMETERS

The biochemical parameters determined are total cholesterol, triglyceride, high density lipoprotein cholesterol (HDL-C), and phospholipid.

Determination of Total Cholesterol

Total cholesterol was determined using enzymatic method described by Allain *et al*. (1974). Cholesterol esterase hydrolyses cholesterol esters to free cholesterol. The free cholesterol produced is oxidized by cholesterol oxidase to cholesten-4-ene-3-one with simultaneous production of hydrogen peroxide which couples with 4-aminoantipyrine and phenol in the presence of peroxidase to yield chromogen with maximum absorption at wavelength 510 nm, The colour intensity is proportional to the cholesterol concentration.

Determination of triglycerides

Triglyceride was determined using enzymatic method described by Buccolo and David (1973). Triglycerides are hydrolyzed by lipases to yield glycerol and fatty acids. The glycerol produced is oxidized to dihydroxylacetone phosphate with the production of hydrogen peroxide which couples with 4-aminophenazone and 4- chlorophenol to produce a chromogen referred to as quinoneimine. The reaction is catalyzed by peroxidase. The degree of absorbance of the chromogen is directly proportional to the concentration of triglyceride measured at 505 nm.

Determination of Phospholipid

Phospholipids are hydrolysed by phospholipase D and the liberated choline is subsequently oxidized by choline oxidase (CHO) to betaine with the simultaneous production of hydrogen peroxide. In the presence of peroxidase (POD) the hydrogen peroxide couples oxidatively the 4 – aminophenazone (4-AP) and dichlorophenol to form a quinoimine dye.

Determination of High Density Lipoprotein-Cholesterol (HDL-cholesterol)

The precipitation method by Assmann *et al*. (1983) was used to determine HDL-cholesterol. The addition of phosphotungistic acid in the presence of magnesium ions precipitates quantitatively low density lipoprotein, very low density lipoprotein and chylomicron fractions from whole plasma, leaving the HDL fraction in the supernate. The cholesterol in the HDL which remains in the supernatant after centrifugation is estimated using the enzymatic method of Allain *et al*. (1974).

STATISTICAL ANALYSIS

Quantitative data were presented as mean ± SD. Triglyceride, total cholesterol, phospholipid and high density lipoprotein-cholesterol (HDL-cholesterol) between the two groups were compared using student's 't' test. A value of $p<0.05$ was considered statistically significant.

RESULT

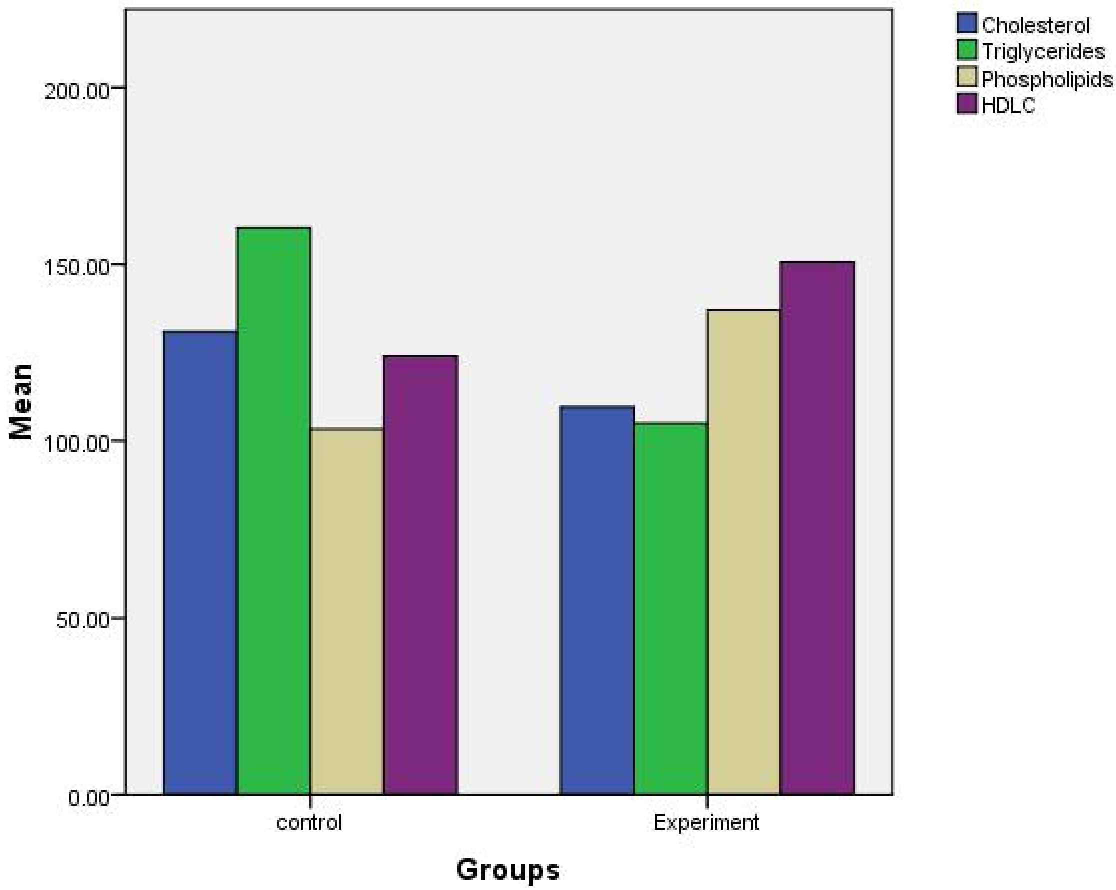
Table 1 shows the mean plasma concentrations of selected biochemical parameters (total cholesterol, triglyceride, phospholipids, HDL-cholesterol) in the different experimental groups: the control group and group given vitamin B6.

The mean concentrations of total cholesterol and triglyceride were decreased significantly in group that was administered vitamin B6 compared with the values in the control group. Also, there was increase in the mean concentrations of phospholipids and HDL-cholesterol when compared with the values obtained in the control group.

Parameters	Control	Group administered vit B_6
Total cholesterol	130.96 ± 19.84	109.62 ± 19.64

Triglyceride	160.32 ± 50.15	104.93 ± 13.04
Phospholipids	103.33 ± 28.45	137.04 ± 47.12
HDL-C	124.03 ± 62.79	150.60 ± 84.05

Table 1.0: Effect of vitamin B_6 supplement on Total cholesterol, Triglyceride, Phospholipid and HDL-cholesterol.



Barchart showing the comparism of the mean concentrations of total Cholesterol, triglyceride, phospholipids, and HDL-cholesterol in different experimental group of animals.

DISCUSSION

Cholesterol plays a significant role in the body. High blood cholesterol is very serious because it is one of the major risk factors for heart disease. People with high blood cholesterol have a greater chance of getting heart disease.

It was observed that there was a significant decrease in the level of cholesterol in animals that were administered with vitamin B6 compared to the control animals. Studies have proved that low cholesterol is desirable for normal and proper body function (Zuhani *et al*., 2010). This results suggests that vitamin B6 might be used to reduce the level of cholesterol in the body. High plasma cholesterol has been linked with hyperlipidemia which is associated with increased risk of cardiovascular disease (Naito *et al*., 1984)

High Density Lipoprotein-Cholesterol (HDL-C) is commonly referred to as the "good cholesterol" because of its relevance to the cardiovascular system as it helps to remove extra cholesterol from the body. When HDL-C is high, there is lower chance of heart disease hence it helps in preventing the body against heart attacks and strokes (Hongbao, 2006). It was observed that there was a significant increase in the levels of HDL-C in animals that were administered with vitamin B6 compared to the control animals. These results suggests that vitamin B6 might be used to increase levels of HDL-C. This results corroborate the findings of Eun-Yong and Youn-ok (2009) that HDL-cholesterol was significantly low in vitamin B6 deficient rats. The observation is also synonymous to the findings of Acar *et al*, (2006), that serum HDL-cholesterol was significantly higher in vitamin B6 administered rats.

Triglycerides are fats in the blood stream and high level of triglycerides has been linked to atherosclerosis (hardening of arteries) and by extension the risk of heart disease and stroke. It was observed that there was a significant decrease in the level of triglycerides in animals that were administered with vitamin B6 as compared to control animals. Studies have shown that low concentration of triglyceride is normal for the body (Zuhani *et al*., 2010). These results suggests that vitamin B6 might be used to reduce the levels of triglycerides in the body. Triglycerides play an important role in metabolism as energy sources and also serve as transporters of dietary fat.

High triglyceride level does indicate a defect in the system and recent evidence strongly suggests that high serum concentration is significantly associated with cardiovascular disease (Altan *et al*., 2006).

The reduced plasma level of triglyceride causes a condition known as hypotriglyceridemia, which may be associated with some metabolic and abnormalities such as impaired fat transportation.

Phospholipids are compound lipids that participate in the lipoprotein complexes which are thought to constitute the matrix of cell walls, membranes, the myelin sheath, and the mitochondria. It was observed that there was a significant increase in the level of phospholipids in animals that were administered with vitamin B6 compared to the control animals.

CONCLUSION AND RECOMMENDATION

From this study, it was observed that the supplementation with vitamin B6 resulted in an increased serum concentration of HDL-cholesterol and phospholipids and a reduction in the serum level of total cholesterol and triglyceride. High levels of HDL-cholesterol and low levels of total cholesterol and triglyceride are indications of a good cardiovascular health. It may be suggested that administration of vitamin B6 should be encouraged as this may help in reducing the rate of accumulation of total cholesterol and triglyceride, which is the two major factors of atherogenicity.

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