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Calcium-rich Diet and Vitamin D Supplementation Improve Lipid Profiles and Reduce Atherogenic Index in High Salt fed Male Wistar Rat

Ofem O.E.*[1], Okon U.E.[1], Ujong G.O.[2], Ekam O.S [1]

[1]Department of Physiology, College of Medical Sciences, University of Calabar, Nigeria. [2]Department of Physiology, Faculty of Medical Sciences, Cross River University of Technology, Okuku, Ogoja, Nigeria

Summary: To ascertain the effect of calcium rich diet and/ or vitamin D supplementation on atherogenic parameters in high salt loaded rats. Thirty male rats were randomly assigned into five groups of six rats each, namely; control; salt only; salt + Calcium; salt + Vit. D and salt + Vit. D + Calcium. High salt diet constituted 8% NaCl diet + 1% NaCl drinking water, while calcium diet was made from 2.5% $CaCl_2$ diet. Serum lipids and atherogenic indices were estimated using standard laboratory procedures. The control rats took normal rodent chow, the feeding lasted 6 weeks. Rats fed high salt diet only had significantly ($p<0.05$) reduced high density lipoprotein cholesterol levels, however this was significantly ($p<0.05$) increased upon treatment with calcium rich diet and vitamin D supplementation. The high salt groups placed on Vit. D and/or calcium diet supplementation had a significant ($p<0.05$) decrease in low density lipoproteins, total cholesterol and atherogenic indices (cardiac risk ratio, atherogenic coefficient and atherogenic index of plasma) compared to the group fed on high salt only. These results suggest the ameliorative potentials of calcium rich diets and vitamin D supplementation against atherogenic tendencies and possibly cardiovascular diseases.

Keywords*:* Calcium rich diet, vitamin D, serum lipids, atherogenic indices, high salt loading.

**Address for correspondence*: ofemo2003@yahoo.com; ofemofeme@unical.edu.ng

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INTRODUCTION

Dietary salt forms an essential part of our meal (Ha, 2004), it is composed of sodium chloride (40% sodium and 60% chloride), (Alderson, 2010). Dietary or table salt serves as food preservative, it is also a component of oral rehydration therapies and used as wound disinfectant, (Ha, 2004).

However, intake of dietary salt in excess of the body's requirement (5g/day) could be deleterious in some humans and animals. With the recently large increase in the consumption of highly salted processed food, salt intake is increasing astronomically in most countries around the world (Alderson, 2010). High salt intake is a predisposing factor to high blood pressure and mortality. High salt has been reported to cause increase in oxidative stress by induction of the release of free radicals in tissues of the kidneys, and in the arteriolar and venular walls of skeletal muscles in rats (Lenda and Boegehold, 2002). Excessive amount of salt consumption has also been shown to adversely affect the pattern and duration of sleep in experimental animals (Heydapour and Heydarpour, 2014). Previous reported has also revealed that high salt damages and destroys cardiac tissues and promotes cardiac hypertrophy and cardiovascular diseases (Conrad et al., 1996).

High salt diet is also associated with the activation of adipokines that may stimulate hepatic triacylglycerol synthesis, which in turn promote assembly and secretion of low density lipoprotein cholesterol (LDL-c), very low density lipoprotein cholesterol (VLDL-c) and reduction of high density lipoprotein cholesterol (HDL-c), (Gorter et al., 2004). In addition, high salt intake causes leptin resistance, insulin resistance and the development of obesity by stimulating endogenous fructose production and fructose metabolism into cholesterol (Lanaspa et al., 2018).

Calcium, an essential food nutrient plays vital role in signal transduction pathways. Calcium rich diet reduces incidence of hypertension (Singh et al., 1987) and high serum cholesterol (Olatunji et al., 2008). In rats, the recommended daily allowance (RDA) is 5.0 mg per kg feed (National Research Council, 1995).

Vitamin D is a secosteroids which cannot be produced by human metabolism, the source of vitamin D in human is from plant and animal products like fatty fish (tuna, mackerel, and salmon), orange, soy milk, cereals, beef liver, cheese and egg yolks. Vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol) are the most important forms of vitamin D, (Holick, 2006; Holick et al., 1971). Vitamin D is required for the intestinal absorption of calcium, magnesium and

phosphate and it enhances other biological effects in the body (Holick, 2004). Vitamin D plays a significant role in calcium homeostasis and metabolism thereby enhancing availability of calcium ions in the blood, (Wolf, 2004).

High salt intake alters atherogenic indices, which is a predisposing factor to hypertension. There is paucity in scientific literature on the role of calcium rich diet or vitamin D supplementation in ameliorating the menace of high salt loading in atherogenic indices in rats. Hence, this present study investigated the effects of calcium rich diet and / or vitamin D on atherogenic indices in salt loaded rats.

MATERIALS AND METHODS

Preparation of the experimental diets: The salt used for this study was a product of Sigma Aldrich (USA). High salt diet containing 8% sodium chloride was prepared using a standard diet containing 0.3% sodium chloride. The salt drinking water was prepared as 1% NaCl solution according to the method of Obiefuna and Obiefuna, (2001)

Calcium rich diet containing 2.5% of calcium chloride was prepared from the rat chow composed of 0.3% calcium chloride. The $CaCl_2$ used in this study was purchased from Sigma Aldrich (USA). The 2.5% calcium rich diet for the high salt fed group was prepared using the already prepared 8% sodium chloride diet (which also contained 0.3% calcium chloride) (Ladipo *et al.*, 2006). In rats, the recommended daily allowance (RDA) is 5.0 mg per kg feed (National Research Council, 1995).

Vitamin D administered was calculated thus; Vitamin D dose – 200 IU/kg i.e. 20 IU/ 100g (rat weight). 400 IU was dissolved in 5ml of olive oil (yielding 80 IU per 1ml of olive oil. It was administered orally to the rats using orogastric cannula, (Ghaly *et al*., 2019).

The standard rat chow used in this study was produced by Pfizer feeds Aba, it was purchased from a local dealer in Calabar, Nigeria.

Experimental animals: In total, 30 male albino Wistar rats weighing between 90-120g were used for this study. They were obtained from the Animal Science Department of the University of Calabar, Nigeria. They had access to their feed and water *ad-libitum.* Approval for the use of the animals was obtained from the College Ethical Committee on the use of experimental animals, the animals were housed and handled in accordance with internationally accepted principles for laboratory animal use and care as found in the European Community guidelines (EEC, 1986).

Experimental design: The 30 rats were randomly assigned into five groups of 6 rats, namely; control group that received normal rodent chow, group 2 were fed high salt diet (8% NaCl diet + 1% NaCl drinking water), group 3 took high salt diet + calcium rich diet, group 4 received high salt diet + Vitamin D and group 5 took high salt diet + calcium rich diet + Vitamin D. The feeding was done orally *ad libitum* for 6 weeks.

Thereafter, rats were sedated with 5% chloroform (Goodies *et* al., 2015) after an overnight fast. The thoracic cage was dissected to expose the heart and blood samples were collected via cardiac puncture, a modified method of Ohwada (1986). The sera obtained were used to assay for the different serum lipids.

Atherogenic indices: Atherogenic index (AI) was calculated using the formula: log (TG / HDL-c) (Takasaki, 2005; Onat *et al.,* 2010). The cardiac risk ratio (CRR) was calculated using the formula: TC / HDL-c (Ikewuchi and Ikewuchi, 2009) while the atherogenic coefficient (AC) was calculated using the formula: (TC – HDL-c) / HDL-c (Ajiboye *et al.,* 2015).

Lipid profile Analysis: Serum and tissues TC, TG and HDL-c were determined by enzymatic colorimetric method using Dialab kit, while LDL-c and VLDL-c were calculated using the formula of Friedwald *et al.,* (1972), thus:

VLDL-c in mmol/L = TG / 2.2

LDL-c in mmol/L = TC - (HDL-c + VLDL-c)

Statistical Analysis: All data were analyzed by one-way analysis of variance (ANOVA) followed by post hoc student's Newman–Keuls test done with SPSS (17.0) for Windows, (SPSS Inc., Chicago, IL). Results obtained were presented as mean + SEM and p-value ≤ 0.05 was considered statistically significant.

RESULTS

As shown in Fig. 1, the mean values of total cholesterol (TC) in the control group was 3.91 ±0.06 mmol/L, in the group fed with high salt diet only (HS) it was 3.60 ±0.08 mmol/L, TC levels in the group fed with high salt + calcium rich diet (HSCR) was 3.29 ±0.03 mmol/L, while in the group fed with high salt + Vit. D supplementation (HSVD) it was 2.66 ±0.06 mmol/L and in the group fed with high salt + calcium rich diet + Vit. Supplementation (HSCRVD) it was 2.60 ±0.06 mmol/L. TC concentrations of HS and HSCR groups decreased significantly ($p<0.05$) compared with the control group. It was in turn significantly lower in the HSVD and HSCRVD groups were compared to HS and HSCR.

In Fig. 2, the mean values of triglyceride (TG) in the control group was 0.31 ±0.02 mmol/L, in the group fed with high salt diet only (HS) it was 0.20 ±0.01 mmol/L, TC levels in the group fed with high salt + calcium rich diet (HSCR) was 0.22 ±0.01 mmol/L, while in the group fed with high salt + Vit. D supplementation (HSVD) it was 0.15 ±0.01 mmol/L and in the group fed with high salt + calcium rich diet + Vit. Supplementation (HSCRVD) it was 0.11 ±0.01 mmol/L. TC concentrations of HS and HSCR groups decreased significantly ($p<0.05$) compared with the

control group. It was in turn significantly lower in the HSVD and HSCRVD groups were compared to HS and HSCR.

High density lipoprotein cholesterol (HDL-c) concentration of the control group was 0.98 ±0.03 mmol/L. It was significantly ($p<0.05$) lower in the HS, 0.55 ±0.04 mmol/L and HSCR, 0.77 ±0.01 mmol/L groups compared with the control group. HDL-c concentration in the HSVD, 0.79 ±0.02 mmol/L and HSCRDV, 0.83 ±0.02 mmol/L were in turn significantly ($p<0.05$) higher compared to control and HS groups (Fig. 3).

Low density lipoprotein cholesterol (LDL-c) in control, HS, HSCR, HSVD a HSCRVD groups were 2.49 ±0.03 mmol/L, 2.77 ±0.06, 2.20 ±0.01, 1.65 ±0.06 and 1.61 ±0.04 mmol/L respectively. LDL-c concentration of the HS group was significantly higher ($p<0.05$) compared to the control group but was significantly ($p<0.05$) lower in HSCR, HSVD and HSCRVD groups compared with control and HS groups, (Fig. 4).

Mean values of the very low density lipoprotein cholesterol (VLDL-c) reduced significantly ($p<0.05$) in all the test groups compared to the control (Fig. 5). The mean values of VLDL-c in control group was 0.14 ±0.01 mmol/L, in HS group it was 0.09 ±0.001, the

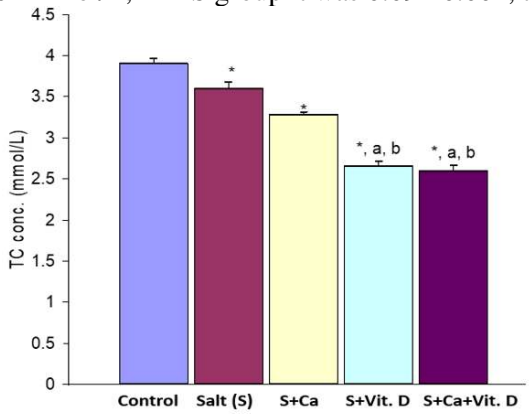

Fig. 1: Comparison of total cholesterol concentration in the different experimental groups. *=p<0.05 when compared with control, a=p<0.05 when compared with salt, b=p<0.05 when compared with Salt + Ca.

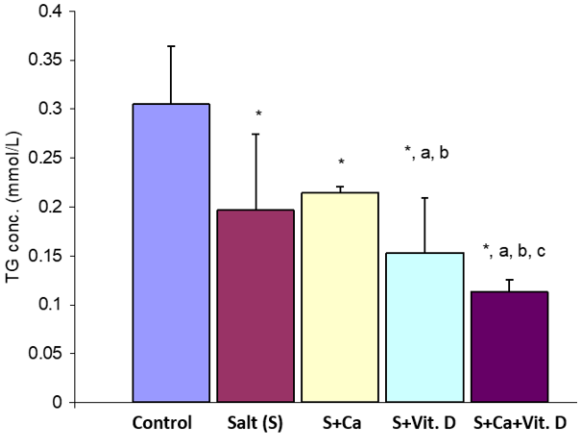

Fig. 2: Comparison of triglyceride concentrations in the different experimental groups. *=p<0.05 when compared with control, a=p<0.05 when compared with salt, b=p<0.05 when compared with Salt + Ca.

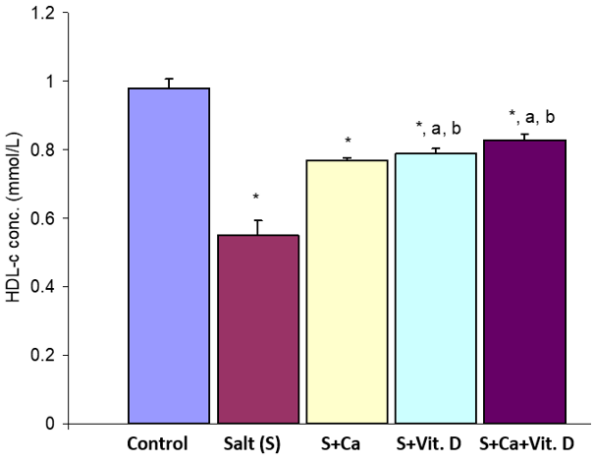

Fig. 3: Comparison of high density lipoprotein cholesterol concentration in the different experimental groups. *=p<0.05 when compared with control, a=p<0.05 when compared with salt, b=p<0.05 when compared with Salt + Ca.

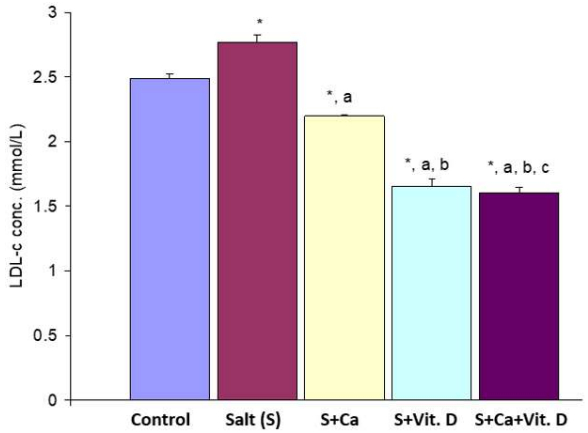

Fig. 4: Comparison of low density lipoprotein cholesterol concentration in the different experimental groups. *=p<0.05 when compared with control, a=p<0.05 when compared with salt, b=p<0.05 when compared with Salt + Ca.

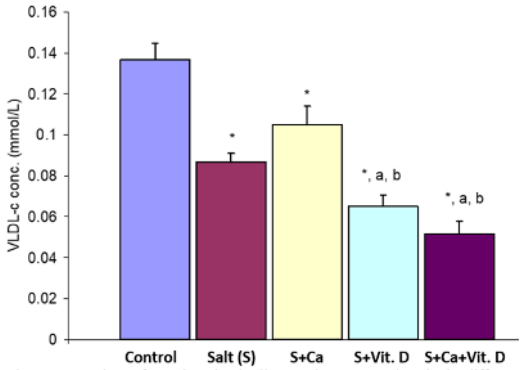

Fig. 5: Comparison of very low density lipoprotein concentrations in the different experimental groups. *=p<0.05 when compared with control, a=p<0.05 when compared with salt, b=p<0.05 when compared with Salt + Ca.

Table 1: Atherogenic indices of the different experimental groups

Groups	Cardiac risk ratio	Atherogenic coefficient	Atherogenic index
Control	4.00 ±0.07	3.00 ± 0.07	2.55 ± 0.07
Salt only	6.71 ±0.44*	5.71 ± 0.43*	5.15 ±0.37*
Salt + Ca	4.28 ±0.05*	3.28 ± 0.05*	2.86 ± 0.04*
Salt + Vit. D	3.38 ±0.09*, a, b	2.38±0.09*, a, b	2.10±0.09*, a, b
Salt + Ca + Vit D	3.14±0.06*, a, b	2.14±0.06*, a, b	1.94±0.06*, a, b

Values are presented as mean + SEM, n = 6. * = significantly different from control at $p<0.05$; a = significantly different from salt only at $p<0.05$; b = significantly different from salt + Calcium at $p<0.05$.

HSCR group recorded a mean value of 0.11 ±0.01 mmol/L, HSVD group had 0.07 ±0.01 mmol/L as their mean value while 0.05 ±0.01 mmol/L was recorded for HSCRVD group.

In Table 1, the cardiac risk ratio (CRR), atherogenic coefficient (AC) and atherogenic index (AI) increased significantly ($p<0.05$) in the HS group compared to control, whereas the values for the other treated groups (HSCR, HSVD and HSCRVD) were significantly lower compared to the HS group. HSVD and HSCRVD had significantly ($p<0.05$) lower values compared with the control groups.

DISCUSSION

In this study, results obtained indicate that calcium rich diet and vitamin D supplementation reduced the elevated triglycerides, LDL-c, and VLDL-c concentrations, but increased the low HDL-c concentration caused by high salt loading in rats. This agrees with previous studies which indicate that increased calcium from 0.9% to 2.5% abrogated altered plasma lipoprotein-cholesterol levels (Olatunji *et al.*, 2008), although not in high salt loaded rats. It has been noted that the cholesterol lowering effect of dietary calcium could be attributed to the ability of calcium to bind bile acids and saturated fatty acid in the gut and form inabsorbable chelates, therefore resulting in impaired lipid absorption (Vaskonen *et al.*, 2002).

The salt-induced increase in LDL-c (bad cholesterol), TG and VLDL-c levels as observed in this study has been reported as one of the predisposing factors to the development of atherosclerosis a major cause of hypertension and other cardiovascular diseases (Messerli *et al.*, 1997). Earlier report shows that high salt diet is associated with the activation of adipokines that may stimulate hepatic triacylglycerol synthesis, which in turn promote assembly and secretion of low density lipoprotein (LDL), very low density lipoprotein (VLDL) and reduction of high density lipoprotein (Gorter et al., 2004). In addition, high salt intake causes leptin resistance, insulin resistance and development of obesity by stimulating endogenous fructose production and fructose metabolism into cholesterol (Lanaspa et al., 2018).

On the contrary, HDL-c counters the adverse effect of the bad cholesterol by sequentially mopping up LDL-c from the blood, hence HDL-c it is regarded or called the "good cholesterol", this property of HDL-c is physiologically significant because it enhances good cardiovascular health (Nerses *et al.*, 2007). HDL-c also plays a vital role in 'reverse transport' of cholesterol from extra-hepatic tissues to the liver for onward excretion in the bile (Rader *et al.*, 2009). Cholesterol is also removed from macrophages in the sub-intima of vessel wall by the interaction of HDL with ABCA-1, SR-B1, or by passive diffusion process, (Ohashi *et al*., 2005). HDL-c plays another vital role preventing oxidative damage of the vascular system and other parts of the body by acting as a carrier of lipid hydro-peroxides and paraoxynase, these enzymes are involved in preventing and reversing oxidative damage (Shao *et al*., 2009).

The increase in cardiac risk ratio (CRR), atherogenic coefficient (AC) and atherogenic index (AI) observed in HS group were reversed by calcium rich diet and vitamin D supplementation. These atherogenic indices (CRR, AC and AI) are strong markers or indicators of cardiovascular risk (Emul *et al*., 2016). The ability of calcium rich diet and vitamin D supplementation to lower the levels of these indices clearly shows the hypocholesterolemic and hypolipidemic effect of dietary calcium. Vitamin D may have played a complementary role to cause this effect because one of the functions of vitamin D is the enhancement of calcium absorption in the gut.

In conclusion, calcium rich diet and vitamin D supplementation reverses increases in TG, LDL-c, VLDL-c and atherogenic indices occasioned by high salt loading. Calcium rich diet and vitamin D supplementation also improve HDL-c levels in high salt loaded rats. These show the ability of the calcium rich diet and vitamin D supplementation to minimize cardiovascular risk in the face of high salt loading in rats.

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