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Evaluation of Lipid Profile of High Salt fed Rats treated with L-Arginine

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Abstract

There is a global concern on salt consumption above the dietary guideline; salt consumption evokes physiological responses with cardiovascular risks associated with dyslipidemia other than increased blood pressure as numerous studies have pointed out. This study aimed at evaluating the effect of L-Arginine on lipid profile of rats fed high salt diet. Forty Male Albino Wister rats weighing between 70-120g were randomly selected assigned into four groups of 10 rats each. Group 1 served as the control and was given distilled water and normal rat chow. Group 2 was fed with high salt diet (8% Nacl in feed, and 1% Nacl in drinking water) Group 3 was treated as group 2 with the introduction of L-Arginine on the 43rd day of the experiment. Group 4 was treated as group 2 with the introduction of losartan administration on the 43rd day of the experiment. Administration of L-Arginine and losartan lasted for 14 days, making a total duration of feeding and drugs administration 56 days. At the end of the 56th day, the rats were fasted overnight for 12 hours and sacrificed under anaesthesia using sodium pentobarbitone. Blood samples were then collected from each animal via cardiac puncture into heparinized tubes and centrifuged at 3500rpm for a period of 15 min, and the clear supernatant plasma were collected and stored at -20°C for biochemical analyses of lipid profile. The results showed a significant increase in TG, LDL-C, TC, VLDL-C and a reduction of HDL-C in the salt fed group. Conversely, a significant reduction in TG, LDL-C, TC, VLDL-C and an increase in HDL-C was shown in the salt + L-Arginine treated group when compared to the control. The changes observed in the L-Arginine treated groups reversed the hyperlipidemia in the salt treated group which indicates L-Arginine is beneficial in treatment of salt induced dyslipidemia and cardiovascular diseases.

Keywords: cardiovascular risks, dyslipidemia, L-Arginine, rats fed high salt diet

INTRODUCTION

Not until the Neolithic period, our ancestors consumed minimal or no salt until farming practices developed its widespread use as a taste enhancer, preservative and more recently a key ingredient in processed foods[1]. During the past decades, the evidence for the risks imposed on human health by excess salt consumption has become compelling. Health concerns related to excess dietary salt have traditionally focused on its relationship with hypertension and the increased risk of stroke and cardiovascular diseases[2]. The relation between habitual dietary salt intake and blood pressure has been established through epidemiological, experimental, migration, and intervention studies[3]. However, some evidence suggests that high salt intake can also lead to higher cardiovascular disease risk independent of its effect on blood pressure[4]. Some studies have suggested that low sodium intake can have a deleterious effect on cardiovascular diseases because of adverse effects on blood lipids[5,6].

Cholesterol and triglycerides are blood lipids, which is an important component of every cell structure in the body. It is necessary for repairs and formation of existing and new cells[7]. It is also utilized during the synthesis of testosterone and cortisol by the testicles and adrenal gland respectively[7]. Several studies have correlated the relationship between cholesterol and sodium intake. For instance, it has been reported that a low sodium intake increases the levels of total cholesterol and triglycerides, leading to cardiovascular and endocrine complications[8,9], but the exact mechanism by which salt intake affects these blood lipids is not clearly understood[10].

L-Arginine is a chemical building block called an amino-acid. It is obtained from the diet and is necessary for the body to make proteins. L-Arginine is found in red meat, poultry, fish and daily product. It can also be made in the laboratory and used as medicine[11]. It is suggested that L-Arginine can be useful in improving lipid profile, due to its potency to increase NO production. Hence, L-Arginine has been investigated in several studies as a potential cardio-protective compound[12] and sighted the potential of L-Arginine supplementation for the treatment of abnormal lipid profile. However, the these results are inconsistent[13,14].

The present study aimed to evaluate the relationship between lipid profile and high salt fed rats treated with L-Arginine.

MATERIALS AND METHODS

Experimental Animals

Forty Male Albino Wister rats weighing between 70-120g were randomly selected from the colony raised in the Animal Holding of College of Medical sciences, University of Calabar, Calabar, Cross River state. The rats were housed in polycarbonate cages with stainless wire lids, at a temperature of 22 ± 1°C, under a 12-hour dark/light cycle with free access to water and standard rat pellets. All research was carried out in compliance with the Institutional Animal Research Ethical guidelines and all animals were handled according to the Animals in Research: Reporting in Vivo Experiments (ARRIVE) guidelines[15].

Experimental Design

The rats were randomly assigned into four groups (1, 2, 3 and 4) of ten rats each and allowed to acclimatize for a period of one week before commencement of experiment. Group 1 served as the control and was given distilled water and normal rat chow. Group 2 was fed with high salt diet (8% Nacl in feed, and 1% $Nacl^-$ in drinking water) Group 3 was treated as group 2 with the introduction of L-Arginine on the 43rd day of the experiment. Group 4 was treated as group 2 with the introduction of losartan administration on the 43rd day of the experiment. Administration of L-Arginine and losartan lasted for 14 days, making a total duration of feeding and drugs administration 56 days.

Formulation of high salt feed

Eight percent of common salt (8% NaCl) was added to grower's mash feed (i.e. 8 g of salt was thoroughly mixed with 92 g of feed) which were then formed to pellets, while 1g of NaCl was mixed with 100ml of water.

Blood sampling and biochemical analysis

At the end of the 56th day, the rats were fasted overnight for 12 hours and sacrificed under anaesthesia using sodium pentobarbitone. Blood samples were then collected from each animal via cardiac puncture into heparinized tubes and centrifuged at 3500rpm for a period of 15 min, and the clear supernatant plasma were collected and stored at -20°C for biochemical analyses of lipid profile.

The levels of plasma triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) were evaluated according standard methods[16,17] respectively, using the enzymatic colorimetric method while very low density lipoprotein-cholesterol (VLDL-C) was estimated using to the formula below[18].

VLDL - c (mg/dL) = Triglycerides concentration / 5

Statistical analysis

The data obtained from the result was subjected to statistical testing using one-way (ANOVA) followed by Tukey test using GraphPad Prisms software 6.0. Data were expressed as Mean ±Standard error of the mean (SEM) and $p<0.001$ was considered significant.

RESULTS

Total cholesterol:

The result for total cholesterol is presented in fig.1.The total cholesterol in the control, salt fed, salt + L-Arginine, and salt + losartan groups was 1.98 ± 0.08, 1.48 ± 0.05, 2.36 ± 0.05, 1.8 ± 0.04 (mmol/L) respectively. The result showed a significant ($p<0.001$) increased total cholesterol in the salt fed group when compared with the control. Treatment with both L-Arginine and resulted in a significant ($p<0.001$) decreased total cholesterol.

Triglyceride concentration:

The result for triglyceride concentration is presented in fig.2. Triglyceride (TG) concentration in the control, salt fed, salt + L-Arginine, and salt + losartan groups was 0.97 ± 0.01, 1.09 ± 0.01, 0.87 ± 0.00, 0.84 ± 0.01(mmol/L) respectively. Triglycerides was significantly ($p<0.001$) increased in the salt fed group compared with the control. Treatment with L-Arginine and losartan reversed the increase towards normal.

Low density lipoprotein (LDL-c) concentration:

The result for Low density lipoprotein (LDL-c) concentration is presented in fig. 3. Very Low Density Liproprotein (LDL-c) concentration in control, salt fed, salt + L-Arginine, and salt + Losartan groups was 1.02 ± 0.04, 0.61 ± 0.04, 1.37 ± 0.03, and 0.95 ± 0.04(mmol/L). There was a significant ($p<0.001$) increase in the serum concentration of LDL-c in the high salt fed group compared with the control. Administration of L-Arginine and losartan caused a marked ($p<0.001$) decrease in the concentration of LDL-c compared with the salt fed group and the control.

High-Density lipoprotein (HDL-c) concentration:

The result for High-Density lipoprotein (HDL-c) concentration is presented in fig.4. High Density Lipoprotein (HDL-c) concentration in the control, salt fed, salt + L-Arginine, and salt + Losartan groups was 0.51 ± 0.02, 0.36± 0.01, 0.58 ± 0.01, 0.45 ± 0.00(mmol/L) respectively. HDL concentration in the salt fed group was significantly ($p<0.001$) decreased compared with the control group. Treatment with L-Arginine and losartan significantly ($p<0.001$) increased the HDL concentration above the salt fed group, an increase and decrease in salt + L-arginine, and salt + Losartan groups when compared to the control respectively. While the salt + Losartan group was significantly lower than the salt + L-Arginine group.

Very Low Density Lipoprotein Cholesterol (VLDL-c):

Very Low Density Lipoprotein cholesterol (VLDL-c) concentration in the control, salt fed, salt + L-arginine, and salt + Losartan groups was 0.44 ± 0.00, 0.5 ± 0.0, 0.4 ± 0.0, and 0.38 ± 0.00(mmol/L) respectively. There was a significant ($p<0.001$) increased of VLDL-c concentration in the salt fed group compared with the control. Treatment with both l-arginine and losartan reversed the effect of high salt with a significant ($p<0.001$) decrease in VLDL-c towards normal control group. This is presented in fig. 5.

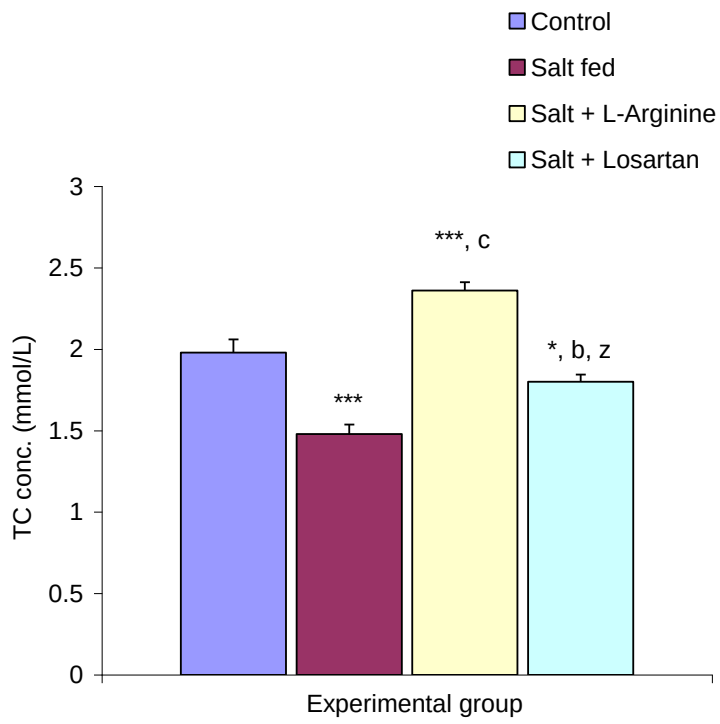

Figure 1: Total cholesterol concentration in the control and test groups.

Values are expressed as mean +SEM, n = 5.
*** = p<0.001 vs control
b = p<0.01, c = p<0.001 salt fed
z = p<0.001 salt + L-arginine

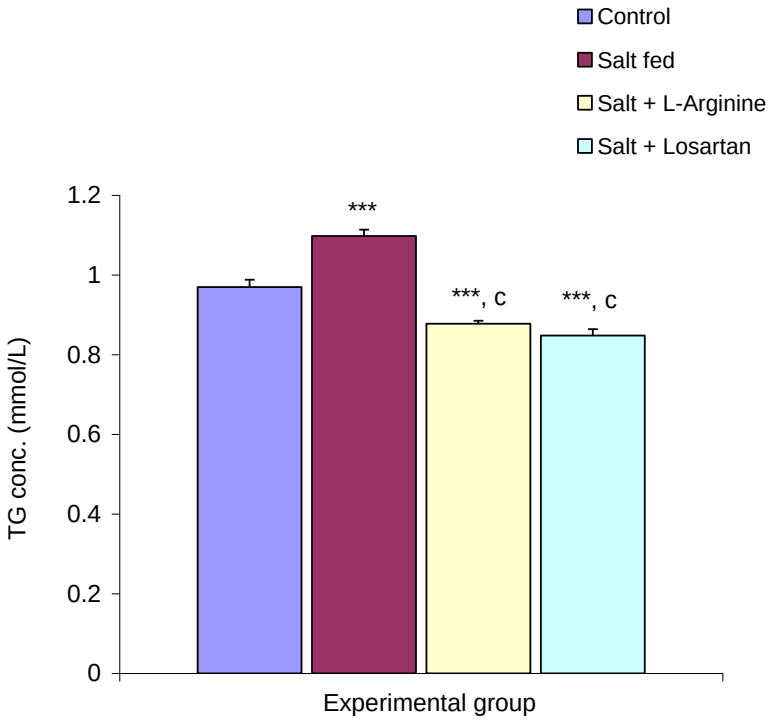

Figure 2: Triglyceride concentration in the control and test groups.

Values are expressed as mean +SEM, n = 5.
*** = p<0.001 vs control
c = p<0.001 salt fed

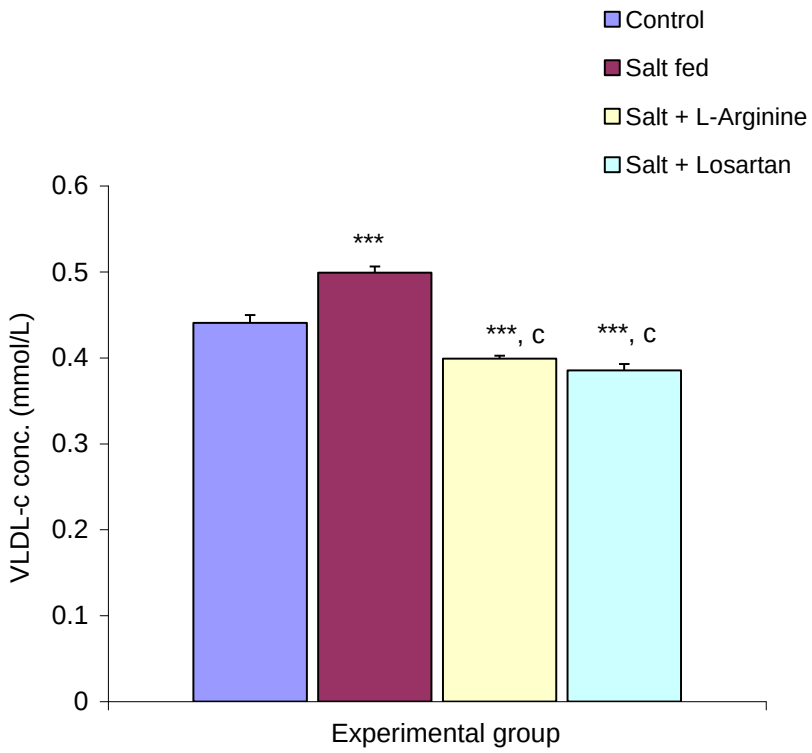

Figure 3: Very low density lipoprotein cholesterol concentration in the control and tests groups.

Values are expressed as mean +SEM, n = 5.
*** = p<0.001 vs control
c = p<0.001 salt fed

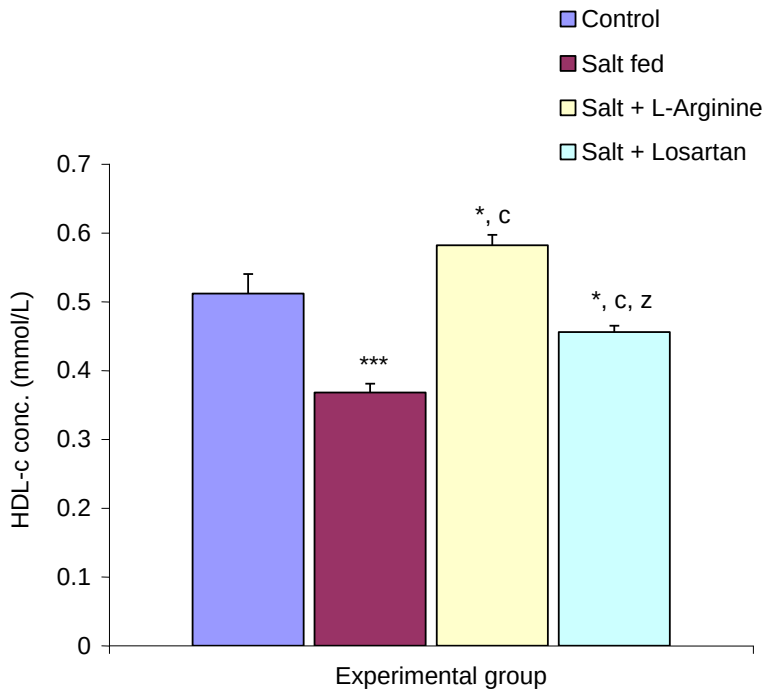

Figure 4: High density lipoprotein cholesterol concentration in the control and tests groups.

Values are expressed as mean +SEM, n = 5.
* = p<0.05, *** = p<0.001 vs control
c = p<0.001 salt fed
z = p<0.001 salt + L-arginine

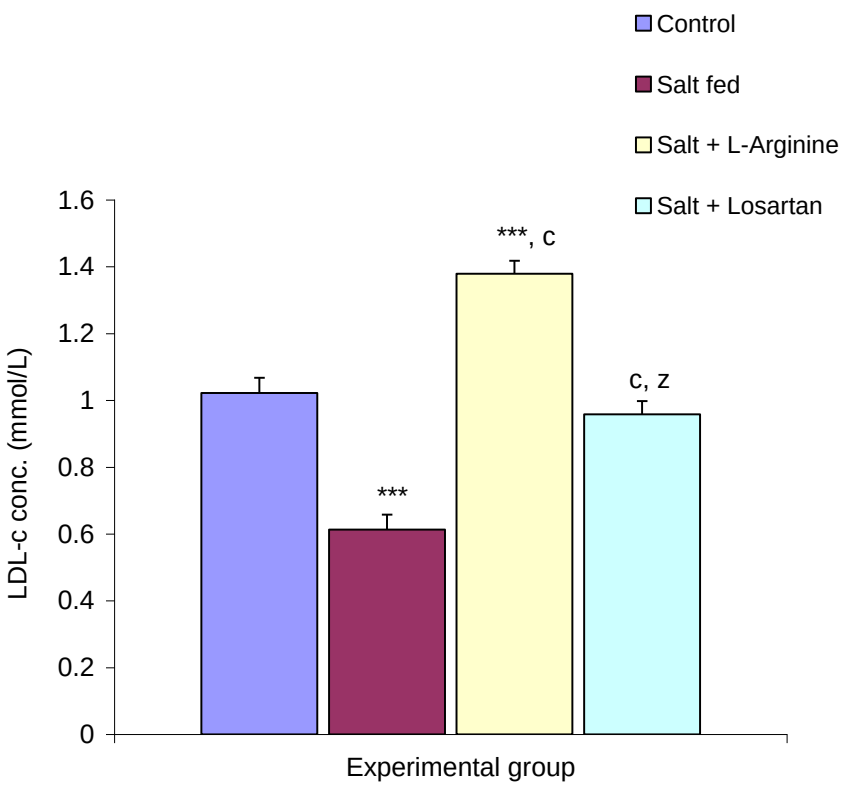

Figure 5: Low density lipoprotein cholesterol concentration in the control and test groups.

Values are expressed as mean +SEM, n = 5.
*** = p<0.001 vs control
c = p<0.001 salt fed
z = p<0.001 salt + L-arginine

DISCUSSION

This study evaluated the effect of lipid profile in high salt fed rats treated with L-Arginine and losortan. It is undisputed that dietary lifestyles affect coronary risk factors and hence the risk of developing a coronary event[19].

Blood lipids are important structural component of the cell, in addition to it being the precursor for synthesis of some hormones, like cortisol, testosterone and estrogen/progesterone[20]. Nonetheless, hyperlipidemia (abnormally increased levels of blood cholesterols) occurs when there is an abnormal increase in the total plasma concentrations of triglycerides (TG), cholesterol and low density lipoproteins cholesterol (LDL-C), with a reduction in the level of high density lipoprotein cholesterol (HDL-C). These abnormal levels of the different fractions of cholesterol form the basis of the development of coronary diseases, which by themselves, constitute a major health problem[21].

In this study, we observed an increase in total cholesterol was significantly increased in the salt fed group compared to the control, while the salt + L-Arginine group and salt + Losartan group was significantly reduced compared with control. This is suggestive of the fact that, L-Arginine and losartan reversed the increase observed in the salt fed group towards a desirable direction, with L-Arginine showing the most desirable reduction in TC.

Evaluation of Triglyceride (TG) concentration showed a similar pattern as TC, the salt fed group was significantly increased when compared to the control, while the salt + L-Arginine and salt + losartan showed a significant reduction compared to compared to the control. Several studies have explored the effects of L-Arginine supplementation on triglyceride levels. A study conducted earlier found that L-Arginine significantly reduced the level of TG in diabetic subjects when compared to control groups[22]. Another study evaluate the effects of L-Arginine supplementation in individuals with hypertriglyceridemia and observed a beneficial effect in TG reduction, suggesting that L-Arginine supplement may be beneficial in treatment of hypertriglyceridemia[23].

High density lipoprotein (HDL-C) referred as "good cholesterol" was decreased in the all the experimental groups except for L-Arginine treated group which showed a significant increase when compared to the control group. Conversely, low density lipoprotein (LDL-C) referred to as "bad cholesterol" showed an inverse relationship in L-Arginine treated group when compared to the control group; having a significant reduction in LDL-C. However, very low density lipoprotein cholesterol (VLDL-C) was significantly increased in the salt fed group while the L-Arginine and lorsatan treated group had a significant decrease compared to the control group. These L-Arginine induced effects are desirable because HDL-C is responsible for reversed cholesterol transport which involves movement of cholesterol from peripheral tissues to the liver for elimination or recycling thereby reducing the risk of atherosclerosis and cardiovascular diseases[24,25]. As opposed to HDL-C, LDL-C carries cholesterol from the liver to

peripheral tissues and increases the risk of cardiovascular diseases[26,27]. This is suggestive of the fact that consumption of L-Arginine may be beneficial since it reversed the dyslipidemia observed in the salt fed group. These observations are in consonant with a study which revealed that L-Arginine supplementation significantly reduced LDL-C levels and increased HDL-C levels compared to the control group[28]. Furthermore, a study on L-Arginine concluded that L-Arginine supplementation significantly reduced total cholesterol, LDL-C, and triglyceride levels, while increasing HDL-C levels compared to control groups[29]. These variables are in tandem with our study but the exact mechanism by which L-Arginine affects these lipid parameters in unclear.

CONCLUSION

The present study shows that L-Arginine administration plays a significant role in reducing the modified serum cholesterol levels in high salt fed rats as it promotes the elevation of HDL-C and reduction of LDL-C, TC and VLDL-C which is beneficial in treatment of dyslipidemia, hence, it can be employed in management of salt induced hyperlipidemia and cardiovascular diseases.

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Authors Contribution

Justin Atiang Beshel: Conceived and designed the study

Justina Andornimye Ashipu: Carried out the bench work and wrote the first graft

Paulicarp Umim Adie: Participated in the bench work and writing of the first graft

Favour Nyoh Beshel: Read and edited the first graft

Gabriel Otu Ujong: Read and edited the final draft

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Competing Interest/Conflicts of Interest

No competing interest / no conflicts of interest

Ethical Approval

Ethical approval was obtained from the faculty of Basic Medical Sciences ethical committee.

REFERENCES

1. Cordain L, Eaton SB, Sebastian A, Mann N, Lindeberg S, Watkins BA, Brand-Miller J. Origins and evolution of the Western diet: health implications for the 21st century, The American journal of clinical nutrition, 2005; 81(2):341-354. https://doi.org/10.1093/ajcn.81.2.341 PMid:15699220

2. Liebson PR, Grandits GA, Dianzumba S, Prineas RJ, Grimm Jr. RH, Neaton JD, Stamler J. Comparison of five antihypertensive monotherapies and placebo for change in left ventricular mass in patients receiving nutritional-hygienic therapy in the Treatment of Mild Hypertension Study (TOMHS). Circulation, 1995; 91(3): 698-706. https://doi.org/10.1161/01.CIR.91.3.698 PMid:7828296

3. Strazzullo P, D'Elia L, Kandala NB, Cappuccio FP. Salt intake, stroke, and cardiovascular disease: meta-analysis of prospective studies. British Medical Journal; 2009:339. https://doi.org/10.1136/bmj.b4567 PMid:19934192 PMCid:PMC2782060

4. Richardson WJ, Clarke SA, Quinn TA, Holmes JW. Physiological Implications of Myocardial Scar Structure, Compr. Physiol, 2015; 5(4):1877-1909. https://doi.org/10.1002/cphy.c140067

5. Egan BM, Weder AB, Petrin J, Hoffman RG. Neurohumoral and metabolic effects of short-term dietary nacl restriction in men relationship to salt-sensitivity status. American Journal of hypertension, 1991; 4(5_Pt_1):416-421. https://doi.org/10.1093/ajh/4.5.416 PMid:1676891

6. Ruppert M, Overlack A, Kolloch R, Kraft K, Göbel B, Stumpe KO. (1993). Neurohormonal and metabolic effects of severe and moderate salt restriction in non-obese normotensive adults. Journal of hypertension, 1993; 11(7): 743-749. https://doi.org/10.1097/00004872-199307000-00010 PMid:8228194

7. Jefcoate CR, McNamara BC, Artemenko I, Yamazaki T. Regulation of cholesterol movement to mitochondrial cytochrome P450scc in steroid hormone synthesis. The Journal of Steroid Biochemistry and Molecular Biology, 1992; 43(8):751-767. https://doi.org/10.1016/0960-0760(92)90305-3 PMid:22217822

8. He FJ, Li J, MacGregor GA. Effect of longer term modest salt reduction on blood pressure: Cochrane systematic review and meta-analysis of randomised trials. British Medical Journal, 2013; 346. https://doi.org/10.1002/14651858.CD004937.pub2

9. Padilha BM, Ferreira RC, Bueno NB, Tassitano RM, Holanda LS, Vasconcelos SML, Cabral PC. Association between blood cholesterol and sodium intake in hypertensive women with excess weight. Medicine, 2018; 97(15): e0371. https://doi.org/10.1097/MD.0000000000010371 PMid:29642188 PMCid:PMC5908596

10. Harsha DW, Sacks FM, Obarzanek E, Svetkey LP, Lin PH, Bray GA, Appel LJ. Effect of dietary sodium intake on blood lipids: results from the DASH-sodium trial. Hypertension, 2004; 43(2):393-398. https://doi.org/10.1161/01.HYP.0000113046.83819.a2 PMid:14707154

11. Gulati K, Joshi JC, Ray A, Recent advances in stress research: Focus on nitric oxide. European Journal of Pharmacology, 2015; 765:406-414. https://doi.org/10.1016/j.ejphar.2015.08.055 PMid:26341014

12. Dashtabi A, Mazloom Z, Fararouei M, Hejazi N. Oral L-Arginine administration improves anthropometric and biochemical indices associated with cardiovascular diseases in obese patients: a randomized, single blind placebo controlled clinical trial. Research in Cardiovascular Medicine, 2016; 5(1). https://doi.org/10.5812/cardiovascmed.29419 PMid:26889456 PMCid:PMC4750008

13. Zhoa YX, Tong L, Zhang GM, Zhao XH, Sa YP, Liu Y, Lu DX, Ga Q, Wu PL. Arginine supplementation improves vascular endothelial dysfunction induced by high fat diet in rats exposed to hypoxia. Wilderness Environmental Medicine, 2020; 31:400-406. https://doi.org/10.1016/j.wem.2020.06.010 PMid:33132032

14. Bogdanski P, Suliburska J, Szulinka M, Sikora M, Walkowsiak J,Jakubowski HL. L-Arginine and vitamin C attenuate pro-atherogenic effects of high-fat diet on biomarkers of endothelial dysfunction in rats. Biomedical Pharmacotherapy, 2015:76:100-106. https://doi.org/10.1016/j.biopha.2015.10.001 PMid:26653556

15. Kilkenny C, Browne W, Cuthill IC, Emerson M, Altman DG. Animal research: reporting in vivo experiments-the ARRIVE guidelines. Journal of Cerebral Blood Flow & Metabolism, 2011; 31(4):991-993. https://doi.org/10.1038/jcbfm.2010.220 PMid:21206507 PMCid:PMC3070981

16. Fossati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clinical chemistry, 1982; 28(10):2077-2080. https://doi.org/10.1093/clinchem/28.10.2077 PMid:6812986

17. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical chemistry, 1972; 18(6): 499-502. https://doi.org/10.1093/clinchem/18.6.499 PMid:4337382

18. Berger, S. (1976). Fundamentals of Clinical Chemistry. Ed. Nobert W. Tietz. Philadelphia, W. B. Saunders, 2nd edition.

19. Mukuddem-Petersen J, Oosthuizen, W, Jerling JC. A systematic review of the effects of nuts on blood lipid profiles in humans. The Journal of Nutrition, 2005; 135(9):2082-2089. https://doi.org/10.1093/jn/135.9.2082 PMid:16140880

20. Valenzuela A, Sanhueza J, Nieto S. Cholesterol oxidation: Health hazard and the role of antioxidants in prevention, Biological Research; 2013; 36:3-6. https://doi.org/10.4067/S0716-97602003000300002

21. Mader SS. Inquiry into life. Septième edition. WMC Brown publishers. Dubique, lowa. Melbourne Australie. England, pp: 369-391, 1994.

22. Poirier P. Obesity and cardiovascular disease: patho-physiology, evaluation, and effect of weight loss. Circulation, 2006; 113: 898-918 https://doi.org/10.1161/CIRCULATIONAHA.106.171016 PMid:16380542

23. Lucotti P, Setola E, Monti LD, Galluccio E, Costa S, Sandoli EP, Comi GC. Beneficial effects of a long-term oral L-Arginine treatment added to a hypocaloric diet and exercise training program in obese, insulin-resistant type 2 diabetic patients. The American Journal of Physiology-Endocrinology and Metabolism, 2002; 283(2):E332-E339.

24. Mackness MI, Arrol S, Durrington PN, Paraoxonase prevents accumulation of lipoperoxides in low-density lipoprotein. (1991). FEBS Letters,1991; 286(1-2):152-154. https://doi.org/10.1016/0014-5793(91)80962-3 PMid:1650712

25. Gordon DJ, Probstfield JL, Garrison RJ, Neaton JD, Castelli WP, Knoke JD. High-density lipoprotein cholesterol and cardiovascular disease: Four prospective American studies. Circulation, 1989; 79(1): 8-15. https://doi.org/10.1161/01.CIR.79.1.8 PMid:2642759

26. Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal, R. S., Yancy CW 2018, AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA guideline on the management of blood cholesterol: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. J Am Coll Cardiol. 2019; 73:e285-e350 https://doi.org/10.1016/j.jacc.2018.11.003 PMid:30423393

27. Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, Landmesser U. 2019ESC/EAS Guidelines for the management of dyslipidaemias: Lipid modification to reduce cardiovascular risk. European Heart Journal, 2019; 41(1):111-188. https://doi.org/10.1093/eurheartj/ehz455 PMid:31504418

28. Monti LD, Setola E, Lucotti P, Marrocco-Trischitta MM, Comola M, Galluccio E, Pontiroli AE. Effect of a long-term oral L-Arginine supplementation on glucose metabolism: A randomized, double-blind, placebo-controlled trial. Diabetes, Obesity and Metabolism, 2002; 4(2):96-105.

29. Dong JY, Qin LQ, Zhang Z, Zhao YY, Wang J. Ar-ginine supplementation and metabolic diseases: A systematic review of randomized controlled trials. The Journal of Nutrition, 2016; 146(11): 2239S-2246S.