Original Article

Lipid profile and oxidative stress status in vegetarians

Anyasor Godswill Nduka[1], Adeseye Lanisa Opeyemi[2], Ogunnowo Ayodele Adewale[3], Erukainure Ochuko Lucky[4]

ABSTRACT

Objectives: To investigate the lipid profile and oxidative stress status in vegetarians and non-vegetarians.

Methodology: Fifty healthy volunteered adults, 25 vegetarians and 25 non-vegetarians (meat eaters) ages 20-50 Years from Babcock University community were recruited for this study. Venous blood sample was collected pre meal and two hours post-prandial for biochemical assay. We assayed for the plasma levels of total cholesterol, triglyceride, LDL-cholesterol, HDL-cholesterol, protein content, superoxide dismutase (SOD), catalase (CAT), glutathione *s*-transferase (GST), reduced glutathione (GSH). Data collected were subjected to statistical analysis using the Student's t-test and One way ANOVA with the aid of SPSS for windows version 14.0. $P<0.05$ was considered statistically significant.

Results: Lipid profile analysis showed non-vegetarians to be significantly higher ($P<0.05$) in total cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol than vegetarians respectively. Plasma protein concentration was significantly higher ($P<0.05$) in vegetarians (1.23 ± 0.29; 1.22 ± 0.18) than non-vegetarians (0.83 ± 0.09; 0.84 ± 0.17) in pre and post meal respectively. Furthermore, plasma superoxide dismutase (0.25 ± 0.72; 0.35 ± 1.60) and catalase activities (0.04 ± 0.00; 0.01 ± 0.27) were significantly reduced ($P<0.05$) in vegetarians than SOD (0.93 ± 1.80; 0.63 ± 1.52) and CAT (0.08 ± 0.24; 0.02 ± 0.05) in non-vegetarians in pre and post meal respectively. More so, non-vegetarians expressed a higher level of reduced glutathione (0.05 ± 0.00) post meal than vegetarians (0.02 ± 0.00). Glutathione *S*-transferase activity was found to be higher in vegetarians (460.28 ± 44.77) than non-vegetarians (100.61 ± 79.28) after meal.

Conclusion: Vegetarians may have lower lipid and oxidative stress status than non-vegetarians.

KEY WORDS: Vegetarians, Non-vegetarians, Lipid profile, Antioxidant.

Pak J Med Sci April - June 2011 Vol. 27 No. 2 409-413

How to cite this article:

Nduka AG, Opeyemi AL, Adewale OA, Lucky EO. Lipid profile and oxidative stress status in vegetarians. Pak J Med Sci 2011;27(2):409-413

Correspondence:

Anyasor Godswill Nduka,
Dept. of Chemical and Environmental Sciences,
School of Sciences and Technology,
Babcock University,
P. M. B. 21244. Ikeja,
Lagos 100 000,
Nigeria.
E-mail: gencc144@yahoo.com
anyasorg@babcockuni.edu.ng

* Received for Publication: December 28, 2009

* Revision Received: April 14, 2010

* Revision Accepted: April 19, 2010

INTRODUCTION

Oxidative stress is a condition caused by an imbalance between the production of free radicals and/or reactive oxygen species (ROS) and antioxidants in favour of the ROS, potentially leading to tissue damage.[1,2] ROS are involved in various related physiological processes and diseases such as aging,[3] cancer[4] and atherosclerosis.[5]

Free radicals are highly reactive molecules that are capable of independent existence possessing an extra unpaired electron(s) in its outermost orbital.[6] It is often produced in the body as product of oxidation

during normal metabolic functions, inflammatory processes or introduced from the environment. For example, when cells use oxygen to generate energy, free radicals are produced as a consequence of adenosine triphosphate (ATP) production by the mitochondria. They are inherently unstable since they contain an extra energy in their molecular structure which causes them to react instantly with key organic substrates such as lipids, proteins, and DNA. Oxidation of these biomolecules can damage them, disturbing normal functions and may contribute to a variety of disease states.[7] There are numerous types of free radicals that can be formed within the biological system, but the most common types are: reactive oxygen species (ROS) and reactive nitrogen species (RNS) and examples are: superoxide anion, hydroperoxyl radical, peroxyl radical, alkoxyl radical, hydroxyl radical, hydrogen peroxide, hypochlorite, hypochlorous acid, Ozone, Singlet oxygen and Nitric oxide, Nitrogen dioxide, peroxynitrite, peroxynitrous acid, dinitrogen trioxide, nitryl chloride, nitronium ion, alkylperoxynitrite, nitrosothiols respectively.[8]

Antioxidants are molecules that can neutralize free radicals by accepting or donating an electron(s) to eliminate the unpaired condition. Consequently, an antioxidant molecule becomes a free radical in the process of neutralizing a free radical molecule to a non free radical molecule. However, antioxidant molecule will usually be a much less reactive free radical than the free radical neutralized. An antioxidant molecule may be very large (allowing it to "dilute" the unpaired electron), it may be readily neutralized by another antioxidant or it may have another mechanism for terminating its free radical condition.[6] The majority of the antioxidant activity is due to the presence of flavones, isoflavones, flavonoids, anthocyanin, coumarin lignans, catechins and isocatechins in plant and plant products.[9]

The human diet contains many compounds of oxidant and antioxidant nature.[10] Among these diets, vegetarian diet is the richest in dietary antioxidants. Studies over the years have revealed that plants are a rich source of phyto-nutrients,[11] vitamins C & E and carotenes[11,12] as well as micronutrient such as selenium, iron, copper, zinc, and manganese which are co-factors for optimum catalytic activity of enzymes.[11] It is important to note that there are some plant compounds which act as potential oxidants, including a variety of quinones, capable of redox cycling, and substrates for enzyme systems which generate oxidants.[13]

Vegetarian have been broadly classified into three diet types, these are: Restricted or total vegetarians with no animal product in their food (they are also called vegans); lacto vegetarians which include only milk and diary products in their diet and lacto-ovo vegetarians which also allows the inclusion of egg.[14]

Diet associated with non-vegetarians (meat-eaters) also offers some benefits such as protein-rich and calorie dense nutrients. It is also a rich source of vitamin B complex, especially B_{12} which is not available in plant foods. The occurrence of cardiovascular diseases, obesity, high blood pressure and high blood cholesterol levels is found to be greater among non-vegetarians owing to the high concentration of saturated fatty acids in animal food.[15]

Studies suggest that the association between cholesterol high serum levels and the prevalence of arterial diseases, especially atherosclerosis, may cause, among other problems myocardial infarction and cerebral vascular accidents.[16-17] Recent evidence has suggested that increased cholesterol levels have also been found to be a risk factor for Alzheimer disease.[18] Cholesterol is transported in the blood by lipoproteins. Among them are: very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL). Differently from VLDL and LDL, HDL does not contain the apolipoprotein B-100, which is recognized by the tissues. It is responsible for reverse transportation of cholesterol from tissues to the liver,[19] and therefore helps protect individuals against lipid related diseases. Therefore, if an individual reports high ratio between high density and low density lipoproteins, the probability of developing atherosclerosis is significantly reduced.[20] According to the Vahit Study (Veterans Affairs High Density Lipoprotein Cholesterol Intervention Trial), a 1 mg/dl HDL reduction results in 3-4% increase in coronary artery disease prevalence. High levels of triglycerides have also been associated with higher prevalence of coronary diseases from atherosclerosis.[20]

In the present study, attempt was made to test the hypothesis that vegetarians may experience longetivity and reduced health risk than meat eaters by evaluating the levels of the various lipid profile biomarkers and oxidative stress status in vegetarians and non- vegetarians.

METHODOLOGY

A total of fifty healthy volunteered adults, 25 vegetarians and 25 non-vegetarians (meat eaters) ages 20-50 years from Babcock University community were recruited for this study. Whole blood samples (5 ml) from subjects were drawn with syringe through venipunture pre meal or fasting (at least 8-12 hours from the last meal) and two hours post meal (postprandial). Blood samples were stored in lithium hep-

arin (LiH) bottles to avoid clotting and immediately centrifuged at 4000 rpm for five minutes to obtain plasma and stored at 4°C to maintain optimum enzyme activity. The plasma protein content was determined by Lowry's method using Bovine Serum Albumin (BSA) as standard.[21] Plasma total cholesterol, triglyceride and high density lipoprotein (HDL) were measured by enzymatic colorimetric method using Randox kits. The concentration of low density lipoprotein (LDL) cholesterol was calculated by the formula of Friedwald et al.[22] Superoxide dismutase (SOD) activity in plasma was determined by the method of Mistra and Fridovich.[23] 0.5 ml plasma was diluted in 4.5 ml of distilled water (1:10) dilution factor. An aliquot of 0.2 ml of diluted plasma sample was added to 2.5 ml of 0.05 M carbonate buffer (pH 10.2) to equilibrate in a spectrophotometric curvette and the reaction was started by addition of 0.3 ml of freshly prepared 0.3 mM epinephrine. The reference curvette contained 2.5 ml of carbonate buffer, 0.3 ml of substrate (adrenaline) and 0.2 ml of distilled water. The increase in absorbance at 480 nm was monitored every 30 seconds for 150 seconds.

$$\text{Increase in absorbance per minute} = \frac{A_1 - A_0}{2.5}$$

Where A_0 = absorbance after 30 seconds

A_1 = absorbance after 150 seconds

$$\% \text{ inhibition} = 100 - 100 \times \frac{\text{Increase in absorbance for substrate}}{\text{Increase in absorbance of blank}}$$

One unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline and SOD levels were expressed as units/mg protein.

Catalase (CAT) activity was determined according to the method of Sinha.[24] The assay mixture contained 4 ml of H_2O_2 solution and five ml of phosphate buffer, pH 7.0 in a 10 ml flat bottom flask. One ml of diluted plasma sample was rapidly mixed with the reaction mixture by a gently swirling motion at room temperature. Immediately, 1 ml portion of the reaction mixture was withdrawn and blown into two ml dichromate/acetic acid reagent at 60 s interval and optical density was measured using SpectrumLab 752S uv-visible spectrophotometer at 570 nm. CAT activity was expressed as moles of H_2O_2 consumed/min/mg protein. Plasma samples were also assessed for the level of reduced glutathione (GSH) Sedlak and Lindsay;[25] Jollow et al.,[26] and glutathione *S*-transferase (GST). Data collected were subjected to statistical analysis using the Student's t-test and One way ANOVA with the aid of SPSS for windows version 14.0. $P<0.05$ was considered significant.

RESULTS

The experimental analysis showed that the total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol were significantly reduced ($P<0.05$) among vegetarians than non-vegetarians in pre and post meals (Table-I). Plasma protein concentration was significantly elevated ($P<0.05$) in vegetarians (1.23 ± 0.29; 1.22 ± 0.18) than non-vegetarians (0.83 ± 0.09; 0.84 ± 0.17) in pre and post meal respectively. Superoxide dismutase (0.25 ± 0.72; 0.35 ± 1.60) and catalase (0.004 ± 0.00; 0.01 ± 0.27) were significantly reduced ($P<0.05$) in vegetarians than SOD (0.93 ± 1.80; 0.63 ± 1.52) and CAT (0.08 ± 0.24; 0.02 ± 0.05) non-vegetarians in pre and post meal respectively. Furthermore, non-vegetarians (993.00 ± 52.50) showed a higher significant ($P<0.05$) level of GST pre meal than vegetarians (132.15 ± 92.48), although post meal GST level was higher ($P<0.05$) among vegetarians (460.28 ±44.77) than non-vegetarians. GSH level was significantly higher ($P<0.05$) in non-vegetarians (0.05 ± 0.00) post meal than vegetarians (0.02 ± 0.00) (Table-II).

DISCUSSION

Key et al.[27] reported that vegetarian diets contain a variety of proven health benefits. Vegetarian diets significantly reduces the rates of obesity, coronary heart disease, hypertension, type II diabetes, diet related cancers, diverticular disease, constipation and gall stones.

Table-I: Mean and standard deviation levels of plasma lipid profile in vegetarians and non-vegetarians.

Parameters	*Before meal non-vegetarians‡*	*After meal non-vegetarians‡*	*Before meal vegetarians*	*After meal vegetarians*
Total cholesterol (mg/dl)	209.76±20.54†	247± 20.10	125.65±49.04	163.3±20.55
Triglyceride (mg/dl)	86.60±35.88	107.06±44.70	68.35± 44.15	83.80±30.66
HDL-cholesterol (mg/dl)	52.23± 04.91	57.86±02.80	45.55± 16.00	36.95±14.10
LDL-cholesterol(mg/dl)	140.13±20.38	167±60.20	66.50 ± 39.68	109.60±20.58

† indicates mean ± standard deviation ‡ indicates significant difference ($p<0.05$)

Table-II: Mean values and standard deviation of plasma protein and antioxidant level in vegetarians and non-vegetarians.

Parameters	*Before meal non-vegetarians‡*	*After meal non-vegetarians‡*	*Before meal vegetarians*	*After meal vegetarians*
Protein concentration (mg/ml)	0.83 ± 0.09†	0.84 ± 0.17	1.23 ± 0.29‡	1.22 ± 0.18‡
SOD (unity of enzyme activity	0.93 ± 1.80‡	0.63 ± 1.52‡	0.25 ± 0.72	0.35 ± 1.60
Catalase (Katf)	0.08 ± 0.24‡	0.02 ± 0.05‡	0.04 ± 0.00	0.01 ± 0.27‡
GST (µmol/min/mg protein)	993.00± 52.50‡	100.61± 79.28	132.15± 92.48	460.28 ±44.77‡
GSH (µg/ml)	0.03 ± 0.00	0.05 ± 0.00‡	0.03 ± 0.00	0.02 ± 0.00

† indicates mean ± standard deviation ‡ indicates significant difference ($p < 0.05$)

In this present study, lipid profile analysis result showed the level total cholesterol, triglyceride (TG), HDL-cholesterol, LDL-cholesterol were significantly higher among non-vegetarians in pre and post meals. This is in agreement with other lipid profile studies that concluded that individuals under vegetarian diets have lower lipid blood levels, especially total cholesterol, LDL, as well as triglycerides, as compared to individuals who eat meat.[28-30] Thus, high TG observed among the non-vegetarian might be as a result of the high concentration of saturated fatty acid in animal fat ingested.

Furthermore, free radicals and oxidative stress have often been implicated to contribute towards the pathology of lipid related diseases causing an alteration in normal metabolic processes. The high plasma protein concentration in vegetarians pre and post meal compared with those of non vegetarians indicates a possible reduction in the generation of ROS that might interfere with protein synthesis and induction.[31] The elevated activities of superoxide dismutase and catalase in non-vegetarians pre and post meal could be attributed to their increased synthesis due to the induction. These enzymes are often induced in response to signals of oxidative stress,[31] which may have resulted from the high concentration of saturated fatty acid from animal fat consumed by non-vegetarians. Glutathione directly quenches ROS such as lipid peroxides, and also plays a major role in xenobiotic metabolism,[6] this may have been responsible for the increased level of GSH in plasma of the non-vegetarians post meal in response to the elevation of free radicals or reactive species from. The increase GST level observed among the vegetarians correlates with the increase in the level of GSH as GSH serves as substrates for GST. GST also protects against ROS, and is induced in response to increased oxidative stress.[32]

CONCLUSION

Results obtained from this study indicate that there were reduced lipid content and oxidative stress status in vegetarians compared with non-vegetarians.

ACKNOWLEDGEMENT

We thank Mr. K. Adetoyi, for his invaluable technical assistance. Our gratitude is due to Prof. Edward B. Esan for his critical review of the manuscript. Authors declare no competing interest.

Authors Contribution: Anyasor GN and Ogunnowo AA developed the concept, planned the research design and co-supervised as well as participated in execution the work. Anyasor GN carried out the statistical analysis and interpretation of data. Blood sample collections were done by Adeseye Lanisa O. and Erukainure O. and both executed the bench work and also assisted in preparation of the final manuscript. Anyasor GN prepared the initial manuscript that was critically evaluated by Ogunnowo A.A. before the final manuscript draft was agreed for submission.

REFERENCES

1. Ravindra PS, Shash S, Suman K. Free Radicals and Oxidatives stress in Neurodegenerative Diseases: Relevance of Dietary Antioxidants. J Indian Acad Clin Med 2004;5(3):218-225.
2. Sies H. Oxidative Stress: Oxidants and Antioxidants. In Physiological Society Symposium: Impaired Endothelial and Smooth Muscle Cell Function in Oxidative Stress. Exp Phys 1997;82:291-295.
3. Finkel T, Holbrook NJ. Oxidant, oxidative stress and the biology of aging. Nature 2000; 408:239-347.

4. Senthil K, Aranganathan S, Nalini N. Evidence of oxidative stress in the circulation of ovarian cancer patients. Clin Chim Acta 2004;339:27-32.
5. Upston JM, Kritharides L, Stocker R. The role of vitamin E in atherosclerosis. Prog Lipid Res 2003;42:405-422.
6. Wang SY, Jiao H. Scavenging capacity of berry crops on superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen. J Agri Food Chem 2000;48:677-684.
7. Hertog ML, Feskens EJ. Dietary antioxidant flavonoids and risk of coronary heart disease. The Zutphen Elderly study. Lancet 1993;342:1007-1011.
8. Stocker R, Keany JF. Role of oxidative modifications in atherosclerosis. Phy Rev 2003;84:1381-1478.
9. Aqil F, Ahmed I, Mehmood Z. Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. Turk J Biol 2006;30:177-183.
10. Ames BN. Dietary carcinogens and anticarcinogens. Science 1983;221:1256-1263.
11. Lim Y, Murtijaya J. Antioxidants protperties of phyllanthus amarus as affected by different drying methods. Lebensin Wiss Technol 2007;40:1614-1660.
12. Padayatty SJ, Katz A, Wang Y, Eck P, Kwon O, Lee J, et al. Vitamin C as an Antioxidant: Evaluation of Its Role in Disease Prevention. J Am Coll Nutr 2003;22:(1)18–35.
13. Sies H. Oxidative stress: introduction. In Oxidative Stress: Oxidants and Antioxidants, ed. Sies, H., Academic Press, London; 1991;15-22.
14. De Biase SG, Fernandes SF, Gianni RJ, Duate JL. Vegetarian diet and Cholesterol and Triglyceride. Arq Bra Cardiol 2007;88(1)32-36.
15. Duyff R, Larson M, Minneapolis MN. Diets. Ame D Ass Com Food Nutr 1996;22:123-134.
16. Steimberg D, Gotto AM Jr. Preventing coronary artery disease by lowering cholesterol levels- fifty years from bench to beside. JAMA 1999;282:2043-2050.
17. Rizos E, Mikhailidis DP. Are high density lipoprotein (HDL) and triglyceride levels relevant in stroke prevention. Cardiovasc Res 2001;52:199-207.
18. Kivipelto MP, Helkala EL, Laakso, MP, Hanninen T, Hallikainen M, Alhainen K, et al. Apolipoprotein E epsilon 4 allele, elevated midlife total cholesterol level, and high midlife systolic blood pressure are independent risks factors for late-life Alzheimer disease. Ann Intern Med 2002;137:149-155.
19. Rizos E, Mikhailidis DP. Are high density lipoprotein (HDL) and triglyceride levels relevant in stroke prevention. Cardiovasc Res 2001;52:199-207.
20. Rubins HB, Robins SJ, Collins D, Fye CL, Anderson JW, Elam MB, et al. Gemfibrozil for the secondary prevention of coronary heart disease in men with low levels of high-density lipoprotein cholesterol. N Engl J Med 1999;341:410-417.
21. Lowry OH, Rosebrough NJ, Farr AL, Randal RJ. Protein measurement with Folin phenol reagent. JBC 1951;193:265-275.
22. Friedwald WT, Levy RT, Frederickson DS. Estimation of the concentration of low lipoprotein cholesterol in plasma without use of preparative ultracentrifuge. Clin Chem 1972;18:499-802.
23. Misra HP, Fridovich I. The role of Superoxide anion in the autoxidation of epinephrine and a simple assay of Superoxide Dismutase. J Bio Chem 1972;247:3170-3175.
24. Sinha AK. Colorimetric assay of Catalase. Anal Bioc 1972;47:389-394.
25. Sedlak J, Lindsay RH. Estimation of total protein bound and non protein sulphydryl groups in tissues with Ellman's reagent. Anal Bioc 1968;25(1):192-205.
26. Jollow DJ, Mitchell JR, Zampaglione N, Gillete J. Bromobenzene induced liver necrosis: Protective role of glutathione and evidence for 3,4 bromobenzene oxide as the hepatotoxic metabolite. Pharm 1974;11:151-169.
27. Key TJ, Appleby PN, Rosell MS. Healthy benefits of a vegetarian diet: In Proc Nutri Soc 1999;58:209-210.
28. Mancilha-Carvalho JJ, Crews DE. Lipid profiles of Yanomano Indians of Brazil. Prev Med 1990;19:66-75.
29. Melby LC, Toohey ML, Cebrik J. Blood pressure and blood lipids among vegetarian, semivegetarian, and nonvegetarian, African Americans. Am J Clin Nutr 1994;59:103-109.
30. Harman SK, Parnell WR. The nutritional health of New Zealand vegetarian and non-vegetarian Seventh-day Adventists: selected vitamin, mineral and lipid levels. N Z Med J 1998;111:91-4.
31. Cliffton PM, Nestel PJ. Influence of gender, body mass index, and age response of plasma lipids to dietary fat plus cholesterol. Ateroscler Thromb 1992;12:955-962.
32. Tricopoulou A, Vasipoulou E. Mediterran diet and longevity. Br J Nutr 2000;84:205-209.
33. Root C, Chari D, Smith CD, Sundseth SS, Pink HM, Wilson JG, et al. Ileal bile acid transporter inhibition, CYP7A1 induction, and antilipemic action of 264W94. J Lipid Res 2002;43:1320–1330.
34. Moghadasian MH, Mancini GB, Frohlich JJ. Pharmacotherapy of hypercholesterolaemia: statins in clinical practice. Expert Opin Pharmacother 2000;1:683–695.
35. Probstfield JL, Rifkind BM. The lipid clinics coronary primary prevention trial: Design, results, and implications. Eur J Clin Pharmacol 1991;40(Suppl 1):S69–S75.
36. Hennig B, Toborek M, McClain CJ. High-energy diet, fatty acids and endothelial cell function: implications for atherosclerosis. J Am Coll Nutr 2001;20:97–105.
37. Limaye PV, Raghuram N, Sivakami S. Oxidative stress and gene expression of antioxidant enzymes in the renal cortex of streptozotocin-induced diabetic rats. Mol Cell Biochem 2003;243:147–152.
38. Onyema OO, Farombi EO, Emerole GO, Ukoha AI, Onyeze GO. Effect of Vitamin E on Monosodium Glutamate induced hepatoxicity and oxidative stress in rats. In J Bio Biop 2005;43:20-24.

Authors:

1. Anyasor Godswill Nduka,
2. Adeseye Lanisa Opeyemi,
3. Ogunnowo Ayodele Adewale,
4. Erukainure Ochuko Lucky,

1-3: Dept. of Chemical and Environmental Sciences, School of Science and Technology, Babcock University, Ilisan-Remo, Nigeria.

4: Food Technology Division, Federal Institute of Industrial Research, Oshodi, Nigeria.