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The Effect Of Obesity On Oxidative Stress And Some Selected Antioxidants Among Nigerian Obese Diabetics And Hypertensive.

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Abstract

Objective: Co-existence of diabetes and hypertension is known to aggravate cardiovascular complications. Of interest is what impact obesity has considering its prevalence in the general population and the public health implication? Hence, this study investigated the effect of obesity on diabetes and hypertension vis-a-vis oxidative stress and some antioxidant status. **Methods:** The study population comprised of 264 subjects; obese hypertensive (OH; n=59), obese diabetes (OD; n=59), obese diabetes and hypertensive (ODH; n=41), obese non diabetes and non hypertensive (ONDH; 45) and non obese non diabetes and non hypertensive (NONDH; control; n=60). There ages, blood pressure, fasting blood glucose and body mass index were determined using standard techniques while Malondialdehyde (MDA) levels was used to determine oxidative stress status and Vitamins A and E with Nitric oxide (NO) were used for the assessment of the antioxidants status and these were analyzed via standard laboratory procedures. **Results:** The control were younger, however the age difference between groups were not significant. BMI was not significantly different when test groups were compared but with control, the difference was significant. The mean blood pressure for the control was significantly lower than those of the OH, ODH and OD but not significantly different from that of ONDH. Also, fasting blood glucose was significantly higher amongst the ODH and OD groups compared to the OH, NODH and control groups. Compared with the control, marker of oxidative stress (MDA) was lower although not significantly different from those of ODH, OD, OH and ONDH. Variations were observed with biomarkers of antioxidants status studied and these were observed to be significant in terms of vitamin A in the ODH, OD and OH groups compared to the ONDH and control groups. However, the vitamin E level for the control was not significantly different from those of ONDH and OD but significantly different from those of the OH and ODH groups. Also, nitric oxide levels among the groups were not significantly different. **Conclusion:** Obesity is an oxidatively stressed condition which worsens with diabetes and or hypertension complicating it but interestingly these complications increased the levels of antioxidants (vitamin A and NO) studied.

Keywords: Obesity, Oxidative stress, Antioxidant, Diabetes, Hypertension.

INTRODUCTION

Obesity is associated with increased incidence of hypertension and diabetes mellitus.[1] On the other hand, epidemiological studies as well as intervention studies,[2] indicated that weight loss is associated with reductions of blood pressure and glucose levels and with a decreased incidence of hypertension and non–insulin-dependent diabetes mellitus. Although the underlying mechanisms for the association of obesity with diabetes and/or hypertension are still not fully known,[3,4] it is known that diabetes and hypertension react favorably to weight reduction.[1,5,6]

It have been reported that in reality diabetes and hypertension are found in the same individual more often than would occur by chance, whereas the overlap between dysglycemia and raised blood pressure is even more substantial than that between diabetes and hypertension. This suggests that they either shared genetic or environmental factors in the etiology. Interesting, substantial overlap between diabetes and hypertension has previously been reported, reflecting substantial overlap in their etiology and disease mechanisms.

It is said that people with Obesity and type 2 diabetes mellitus (DM) have increased risk of death from CVD and that the possible mechanisms that relate obesity and diabetes to increased CVD risk include inflammation and oxidative damage.[7] There are data that indicate increased oxidative stress in human essential hypertension as well as in obese hypertensive patients.[8-10] Hence, could factors that reduce oxidative stress provide an important tool to reduce the burden associated with obesity and related chronic disease including diabetes and CVD?

We hypothesize that oxidative stress might be a common link that underlies both diabetes and hypertension induced by obesity. The aim of this study therefore, is to investigate the effect of obesity on markers of oxidative stress and antioxidants status in obese diabetic and hypertensive subjects.

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METHODS

Study Area This study was conducted in Ekpoma, Benin City, Kwale and Asaba, all in the south-south zone of Nigeria.

Study Design This study is a cross-sectional study involving simple random sampling and cohort sampling for subjects recruitment.

Ethical Consideration Ethical approval was sought and given by the research and Ethic Committee of AAU, Ekpoma. The intervention and control community gave their permission after the aims and objectives of the study were explained to them. Also, informed consent was sought and obtained from the respondents before enrollment into the study. At the end of the study the control group was also given the same intervention, for ethical reasons. Written informed consent/ questionnaire were administered to all

subjects.

Sampling Method and Sample Size A simple random sampling was done to recruit subjects for the study. The sample size was determined as follows:

$$N = \frac{Pq}{\left(\frac{\alpha}{1.96}\right)^2}$$

Where: N = sample size, P = Population or Prevalence rate (50% or 0.5), q = 1-0.2, Error margin = 0.05

Subjects and Grouping Subjects were classified into the following five (5) groups to meet the set goals of this research. Group 1: Obese Diabetic (OD); Group 2: Obese Hypertensive (OH); Group 3: Obese Diabetic/Hypertensive (ODH); Group 4: Obese Non Diabetic/Hypertensive (ONDH) and Group 5: Non Obese Non Diabetic/Hypertensive (NONDH) [normal control©]. They were classified as non-obese using BMI $\leqslant$24.5 kg/m2, diabetic using fasting plasma glucose (FBG) $\geqslant$7 mmol /l (126mg/dl), hypertensive using blood pressure of $\geqslant$ 140/90mmHg and A total of about two hundred and sixty-four subjects were recruited and was distributed as follow; OD=59; OH=59; ODH=41; ONDH=45; and NONDH=60

Inclusion Criteria All Subjects with BMI $\leqslant$24.5kg/m2 (control group) and $\geqslant$30kg/m2 (obese groups) and not on any form of medication were recruited.

Exclusion Criteria All Subjects who are obese (BMI $\geqslant$ 30kg/m2) and non obese already diagnosed as been hypertensive and/or diabetic who are on medications were excluded from this study.

Blood Pressure Measurement Resting blood pressure was taken in a sitting position after a 5-10 minutes rest using a mercury sphygmomanometer according to standard procedures at least four different times and the mean recorded.

BMI Measurement Heights were measured in standing position, with shoulder and buttocks against the wall, the subject looking straight ahead with joined feet, and arms hanging on both sides with a graduated tape. In addition, body weights were measured with a calibrated beam scale. These were used to calculate the BMI which is weight (kg)/height (m2).

Fasting Blood Glucose (FBG) Measurement Preliminary measurement of FBG in all the subjects was done using Glucometer at least two different times with the mean value recorded. Subjects were asked to fast overnight (no food, drink, alcohol or smoking).

Sample Collection and analysis About 12.5mls of fasting blood was collected from each subject with 0.5ml immediately used for preliminary FBG estimation and 2.0mls immediately placed in fluoride oxalate container for FBG estimation spectrophotometrically as these served as basis for comparing FBG levels. Four (4.0) mls was placed in EDTA container for vitamin A and E and NO, TPP, and MDA estimation using standard laboratory procedures.Vitamin A and E were analyzed using the vitamin A and E high pressure liquid chromatography kits from America laboratory company (Alpco) diagnostics, USA . MDA was analysed using the Spectrophotometry method.

Duration of Study The study was conducted within a thirty months period (from July, 2009 to June, 2012). The first 0-6mths was selection of subjects and baseline measurements of BP and FBG, 7-12mths was repeat measurements of BP and FBG, 13-18mths was repeat measurements of BP and FBG, 19-24mths was final measurements of BP and FBG and collection of sample for analysis and 25-30mths was analysis, collation and processing of data

Statistical analysis Data were presented as mean±S.D (standard deviation) and then analyzed using Statistical Package for Social Sciences (SPSS) at a P value of 0.05 and 95% level of confidence and results presented in suitable tables.

RESULTS

Table 1 is a representation of some bio-parameters of the subjects used for this study. Of the 264 subjects examined, those in the NONDH (control) were youngest. However, the age differences between the groups were statistically not significant. In relation to BMI, the obese groups (OD, OH, ODH and ONDH) were having a higher BMI and were statistically significant compared to the control (NONDH; 23.62±0.26 Kg/m2). Although the BMI differences among the obese was not statistically significant (P > 0.05), it was highest among the OD (36.09±0.69 Kg/m2) follows by the ODH (34.73±0.79 Kg/m2), ONDH (33.69±0.57 Kg/m2) and then the OH (33.28±0.62 Kg/m2). In terms of blood pressure, the obese hypertensive presented the highest (163.1±13.90/94.39±4.53 mmHg) and this was follows by the ODH (157.7±7.07/92.39±6.89mmHg), OD (138.9±7.01/79.81±8.15mmHg), ONDH (127.8±11.47/70.09±9.20 mmHg) and then the control (125.5±10.14/70.77±11.29 mmHg). Statistically, the mean blood pressure for the NONDH was significant lower ($p<0.05$) than those of the OH, ODH and OD groups but not significantly different ($p>0.05$) with that of ONDH group. To check for diabetes, the fasting blood glucose was investigated and this was observed to be significantly higher amongst the ODH (12.50±6.41) and OD (10.91±4.65) compared to the OH (4.97±1.37), ONDH (4.09±0.93) and NONDH (3.93±0.85) groups.

Table 2 showed the biomarkers of oxidative stress (MDA) and antioxidant status (vit. A. vit. E and NO) of the diabetes and hypertensive subjects compared with control. Although the mean MDA level was lowest in the NONDH group (5.32 1.85) it was not significantly different ($p>0.05$) from those of the obese groups. However, the ODH (5.87±1.06) showed the highest level follows by OD (5.83±1.24), OH (5.63±1.05) and then the ONDH (5.58±1.00). Vitamin A was observed to be highest significantly ($p<0.05$) in the ODH (4.54±11.12) group and higher in the OD (2.78±8.49) and OH (2.14±7.43) groups compared to the ONDH (0.40±0.30) and NONDH (0.45±0.23) groups. On the other hand, vitamin E was observed to be lowest in the ODH (8.95±1.86) but highest in the ONDH (12.96±2.99) group. However, the vitamin E level for the NONDH (11.66±4.27) was not significantly different from those of ONDH (12.96±2.99) and OD (10.39±6.17) but significantly different from those of the OH (9.44±2.42) and ODH (8.95±1.86) groups. Although there were variability in the level of nitric oxide among the groups, the variations were not statistically significant ($p>0.05$). However, the OH (31.27±4.39) group showed the lowest nitric oxide value compared to others while the control group (NONDH; 32.37±2.53) showed the highest value

DISCUSSION

Oxidative stress has been reported to plays critical roles in the pathogenesis of many diseases.[11] It implication in

Table 1: Some bio-parameters in mean ± SD of the obese diabetes and hypertensive subjects compared with the control (NONDH)

Parameter	OD (n=59)	OH (n=59)	ODH (n=41)	ONDH (n=45)	NONDH (n=60)
AGE (years)	42.86±15.78	51.32±15.09	49.29±15.92	43.73±14.78	42.33±14.64
BMI (Kg/m^2)	36.09±0.69[a]	33.28±0.62[a]	34.73±0.79[a]	33.69±0.57[a]	23.62±0.26
BP systolic mmHg)	138.9±7.01[a]	163.1±13.90[a]	157.7±7.07[a]	127.8±11.47	125.5±10.14
BP diastolic (mmHg)	79.81±8.15[a]	94.39±4.53[a]	92.39±6.89[a]	70.09±9.20	70.77±11.29
FBG(mmol/l)	10.91±4.65[abc]	4.97±1.37[b]	12.50±6.41[abc]	4.09±0.93[c]	3.93±0.85

a: significantly different from NONDH (control), b: significantly different from OH c: significantly different from ONDH

Table 1: Biomarkers of Oxidative stress (MDA) and antioxidants status (vit. A, vit. E and NO) in mean ± SD of the obese diabetes and hypertensive subjects compared with the control (NONDH)

Parameter	OD (n=59)	OH (n=59)	ODH (n=41)	ONDH (n=45)	NONDH (n=60)
MDA	5.83±1.24	5.63±1.05	5.87±1.06	5.58±1.00	5.32±1.85
Vit . A	2.78±8.49[ab]	2.14±7.43[ab]	4.54±11.12[ab]	0.40±0.30[b]	0.45±0 .23[a]
Vit . E	10.39±6.17	9.44±2.42[a]	8.95±1.86[a]	12.96±2.99	11.66±4.27[a]
NO	32.10±3.50	31.27±4.39	32.05±3.77	32.07±1.81	32.37±2.53

a: significantly different from NONDH (control), b: significantly different from ONDH

hypertension and diabetic conditions has also been reported. However,[12-15] the underlying mechanisms for the association of obesity with diabetes and/or hypertension are still not fully known.[3,4] In the present study, it was shown that oxidative stress assessed by MDA worsens when obesity is complicated with diabetes and or hypertension but milder in obesity only. This finding is in agreement with previous studies which suggested that systemic oxidative stress correlates with BMI.[16,17,18] Also, the addition of hypertension and diabetes aggravated the MDA level as observed in the present study. Indeed, it is a known fact that the co-existence of the two conditions is a powerful promoter of CVD, accelerating microvascular and macrovascular complications and greatly increasing cardiovascular stroke and end stage renal disease risk. Hence, oxidative stress may be increased in patients with diabetes and or hypertension since persistent hyperglycemia causes increased production of oxygen free radicals through auto-oxidation of glucose and non-enzymatic glycation of proteins.

The pathological role of oxidative stress in vascular diseases is well recognized.[19] The present findings showed that diabetes have a more worsen potential on MDA compared to hypertension. However, hypertensive condition has been found to produce increased blood lipid peroxidation values and lower total antioxidant capacity (TAC) levels.[20, 21] a fact that was supported by the present study. Series of clinical and experimental studies have also shown that oxidative stress, through free radical generation, plays a major role in the onset of diabetes and hypertension.[22,23] The deleterious effect of which can be prevented by antioxidants.[24,25] But the effectiveness of antioxidants enzymes in scavenging free radicals depends on their antioxidant cofactors.

Recall that in healthy individuals, oxidative damage to tissues is prevented by a system of defenses which includes antioxidant enzymes and vitamins.[26] Also, decreased antioxidant activity and reduced levels of reactive oxygen species (ROS) scavengers (vitamins E and C and glutathione) contribute to oxidative stress. These reports are justified in the present study when one takes a clue from the result on nitric oxide and vitamin C. However, the observation in terms of Vitamin A may be that the worsen MDA in the ODH may have stimulated the production of Vitamin A from other mechanism to combat the increased oxidative stress.

Considering the fact that increased oxidative stress in vascular walls is involved in the pathogenesis of hypertension,[27] atherosclerosis and hepatic steatosis,[28] the reduce vitamins E and the increase in MDA in obese diabetes and hypertensive subject may make them prone to CVD. It become more scary considering that oxidative stress impair insulin secretion by pancreatic â cells,[15] glucose transport in muscle and in obesity, adipose tissue.[13,14] By implication, as the diseases progress, there is tendency for the oxidative damages to deepen via way of positive feedback mechanism.

Interesting, accompanying changes in diet and lifestyle are leading to growing epidemic of overweight/obesity, type 2 diabetes mellitus and other related cardiovascular disease (CVD).[29]
Hence, dietary supplements with antioxidant and related anti-inflammatory effects may present a novel strategy of controlling and reducing complication of obesity at the population level.[30,31]

REFERENCES

1. Ashley FW, Kannel WB. Relation of weight change to changes in atherogenic traits: the Framingham study. J Chronic Dis. 1974; 27:103–114.
2. Cerulli J, Malone M. Outcomes of pharmacological and surgical treatment for obesity. Pharmaco-economics. 1998;14:269–283.
3. Porte, D. and Kahn, S.E. Beta-cell dysfunction and failure in type 2 diabetes: potential mechanisms. Diabetes,2001; 50(Suppl 1):S160-S163.
4. Goodman E, Dolan L.M, Morrison J.A. The Factor

Analysis of clustered cardiovascular risks in adolescence: obesity is the predominant correlate of risk among youth. Circulation; 2005; 111:1970–1977.

5. Sjöström CD, Lissner L, Sjöström L. Relationships between changes in body composition and changes in cardiovascular risk factors: the SOS Intervention Study: Swedish Obese Subjects. Obes Res. 1997; 5:519–530.
6. MacDonald KG Jr, Long SD, Swanson MS, Brown BM, Morris P, Dohm GL, Pories WJ. The gastric bypass operation reduces the progression and mortality of non-insulin-dependent diabetes mellitus. J Gastrointest Surg. 1997; 1:213–220.
7. Gariballa Salah, Bachar Afandi, Mamoun AbuHaltem, Javed Yassin, Hosam Habib and Wissam Ibrahim. Oxidative damage and inflammation in obese diabetic Emirati subjects supplemented with antioxidants and B-vitamins: a randomized placebo-controlled trail. Nutrition & Metabolism, 2013;10:21
8. Russo C, Olivieri O, Girelli D, Faccini G, Zenari ML, Lombardi S, Corrocher R. Anti-oxidant status and lipid peroxidation in patients with essential hypertension. J Hypertens. 1998;16:1267–1271.
9. Sagar S, Kallo IJ, Kaul N, Ganguly NK, Sharma BK. Oxygen free radicals in essential hypertension. Mol Cell Biochem. 1992;11:103–108.
10. Van Gaal LF, Zhang A, Steijaert MM, De Leeuw IH. Human obesity: from lipid abnormalities to lipid oxidation. Int J Obes Relat Metab Disord. 1995; 19(suppl 3):S21–S26.
11. Brownlee, M. Biochemistry and molecular cell biology of diabetic complications. Nature; 2001; 414:813-820.
12. Lip, G.Y., Laudary M.J., Edmunds E., and Hughes B.A. Abnormal LDL Subtractions profile in patients with untreated hypertension, Qs Med, 2003; 95:165-171.
13. Maddux, BA. Protection against oxidative stress-induced insulin resistance in rat L6 muscle cells by micromolar concentrations of á-lipoic acid. Diabetes, 2001; 50:404-410.
14. Rudich, A. Prolonged oxidative stress impairs insulin-induced GLUT4 translocation in 3T3-L1 adipocytes. Diabetes.1998; 47:1562-1569.
15. Matsuoka, T. Glycation-dependent, reactive oxygen species-mediated suppression of the insulin gene promoter activity in HIT cells. J. Clin. Invest., 1997; 99:144-150.
16. Shigetada, F., Takuya, F., Michio, S., Masanori, I., Yukio, Y., Yoshimitsu, N., Osamu, N., Makoto, M., Morihiro, M. and Iichiro, S. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest., 2004; 114(12):1752–1761.
17. Keaney, J.F., Larson, M.G. and Vasan, R.S. Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham Study. Arteriosclerosis, Thrombosis and Vascular Biolog, 2003; 23:434–439.
18. Olusi, S.O. Obesity is an independent risk factor for plasma lipid peroxidation and depletion of erythrocyte cytoprotective enzymes in humans. Int. J. Obes. Relat. Metab. Disord, 2002; 26:1159-1164.
19. Madamanchi, N.R., Vendrov, A. and Runge, M.S. Oxidative stress and vascular diseases. Arterioscler Thromb Vasc Biol, 2005; 25:29-38.
20. Kashyap, M.K., Yadav, V., Sherawat, B.S., Jain, S., Kumari, S., Khullar, M.et al. Different antioxidant status, total antioxidant power and free radicals in essential hypertension. Mol Cell Biochem., 2005; 27(7): 89-99.
21. Sun, L., Gao, Y.H., Tian, D.K., Zheng, J.P., Zhu, C.Y., Ke, Y. and Bian, K. Inflammation of different tissues in spontaneously hypertensive rats. Acta Physiol Sinica. 2006; 58:318-323.
22. Maritim AC, Sanders RA, Watkins III JB. Diabetes, oxidative stress and antioxidants: a review, Journal of Biochemical and Molecular Toxicology. 2003, 17: 24–38
23. Zhou XJ, Vaziri ND, Wang XQ, Silva FG, Laszik Z. Nitric oxide synthase expression in hypertension induced by inhibition of glutathione synthase. Journal of Pharmacology and Experimental Therapy,2002;300: 762-767.
24. Vaziri ND. Pathogenesis of lead-induced hypertension: Role of oxidative stress. American Journal of Hypertension 2002, 20 (S): 15-20.
25. Lim CS, Vaziri ND. The effects of iron dextran on the oxidative stress in cardiovascular tissues of rats with chronic renal failure. Kidney International 2004, 65: 1802-1809.
26. Prakash S, Sudha S. Lipid peroxidation and antioxidant status in South Indian patients with Type 2 Diabetes Mellitus. Int Res J Pharmacy. 2012; 3: 132-134.
27. Nakazono, K. Does superoxide underlie the pathogenesis of hypertension? Proc. Natl. Acad. Sci, 1991; 88:145-148.
28. Ohara Y., Peterson E., Harrison D. Hypercholesterolemia increases endothelial superoxide anion production. J. Clin. Invest. 1993; 91:2546-2551.
29. Malik A, Babir A, Abi Saab B, Roglic G, King H: Glucose intolerance and associated factors in the UAE. Diabetes Res Clin Pract 2005, 69:188-195.
30. Evans JL: Antoxidants: do they have a role in the treatment of insulin resistance? Indian J Med Res 2007, 125:355-372.
31. Evans J, Goldfine ID, Maddux BA, Grodsky GM. Oxidative stress and stress activated signalling pathways: a unifying hypothesis of type 2 diabetes. Endocr Rev., 2002;23:599-622.