SUMMARY& CONCLUSION

Cellular energy metabolism consists of a number of pathways. Mitochondrial respiration and ATP synthesis are two pathways lying at the heart of metabolism. Mitochondrial respiration consists of the oxidation of mitochondrial substrate (NADH) supplied by nutrients which couple the electron transport chain (ETC) to the pumping of proton out across the mitochondrial membrane. The ATP synthase couples the transport of proton across the membrane to the synthesis of ATP inside the mitochondrial matrix. Thus mitochondria convert energy stored in nutrient into ATP/ADP that drives work within the body. However, nothing comes without cost. Molecular oxygen, the final electron acceptor for cytochrome c oxidase, is ultimately reduced to water. However, a small quantity of O_2 may be incompletely reduced as a leakage of single electrons, causes the reduction of O_2 to superoxide ($O_2^{-\bullet}$). Intracellular signaling effectors, including H_2O_2 reflect the balance between the electron leak from the electron transport system, superoxide formation and scavenging of H_2O_2 by endogenous antioxidants in the matrix.

In physiological conditions a homeostatic balance exists between endogenous oxidants, ROS formation and their elimination by endogenous antioxidants. However, under conditions of overnutrition and physical inactivity, typical to those facing metabolic syndrome, the oversupply of substrates generating surplus reducing equivalents could in turn be expected to elevate the redox state and increasing oxidative stress.

We focus in the present work on oxidative stress events leading to individual disease factor appearance in metabolic syndrome patients. Metabolic syndrome patients show altered mitochondrial metabolism as evidenced by more than 100% increase in 8-OHdG (a critical biomarker of oxidative stress) compared to control. Metabolic abnormalities detected in our results defined the presence of abdominal obesity (increased waist circumference), IR along with its associated hyperinsulinemia, elevated TG and fasting glucose, low HDL and high blood pressure.

We obtain also a linear combination of variables, including: waist circumference, hypertension, hyperglycemia, IR, dyslipidemia and oxidative stress markers (8-OHdG, MDA and SOD). Abdominal obesity (increase WC) is consistent with increased oxidative stress markers, representing the trigger for systemic oxidative alterations. Positive correlation between fasting glucose and 8-OHdG suggests a role of hyperglycemia in increase of intracellular glucose metabolism and consequent increase in ROS production.

High plasma oxidative stress markers (8-OHdG& MDA) correlate also positively with elevated TG, LDL and with low HDL. Also, Malondialdehyde (lipid peroxidation index) correlates with low HDL. It is likely that stressors in various forms hit the organism at many different levels (molecular integration between lipid and glucose) and that this adds up to a pathogenic process moving toward insulin resistance obtained in our results. Thus, oxidative stress plays a number of potential mechanisms in the pathophysiology of metabolic syndrome.

Positive correlation between oxidative stress markers and insulin resistance in our metabolic syndrome patients may suggest that several factors that cause insulin resistance have a common pathway in the excessive formation of ROS. Thus, the present work links intracellular metabolic balance to the control of insulin sensitivity. We also place the

etiology of obesity-induced insulin resistance, which is a component of metabolic syndrome in the context of mitochondrial bioenergetics.

Several studies have suggested that increased oxidants by chronic overnutrition coupled with decreased endogenous antioxidant capacity results in oxidative stress. In the present work a significant decrease in total SOD enzyme suggests decreased endogenous mitochondrial capacity in our metabolic syndrome patients.

Accordingly, for many years, interest has focused on strategies that enhance removal of ROS using either antioxidants or drugs that enhance endogenous antioxidant defense. Another object in the present study was to evaluate the prognostic value of oxidative stress markers in a group of metabolic syndrome subjects receiving atorvastatin treatment (a synthetic lipid-lowering agent), and a second group receiving atorvastatin plus vitamin E (as an antioxidant), both groups were followed up for 3 months.

Our results reveal improvements in the quantitative estimation of oxidative stress markers (8-OHdG, MDA and SOD) in both groups. However, this level of supplementation with combined treatment (atorvastatin+ vitamin E) did not alter significantly 8-OHdG, while it decreased significantly MDA and LDL levels when compared with treatment with atorvastatin alone, reflecting the importance of antioxidant vitamins in reducing lipid peroxidation and oxidative stress level.

Therefore, combination therapy that simultaneously addresses multiple mechanisms for the pathogenesis of metabolic disorders is an attractive emerging concept for slowing progression of oxidative stress complications. Combined therapy with statins and antioxidant vitamins demonstrates additive beneficial effects on dyslipidemia and lipid peroxidation when compared with monotherapies in patients with metabolic syndrome and cardiovascular risk factors due to both distinct and interrelated mechanisms. These additive beneficial effects of combined therapies are consistent with laboratory and recent clinical studies. Thus, combination therapy may be an important paradigm for treating and slowing progression of atherosclerosis, coronary heart disease, and co-morbid metabolic disorders characterized by endothelial dysfunction and hyperlipidemia.

We suggest that oxidative stress markers correlate with outcomes in metabolic syndrome patients treated with atorvastatin revealing antioxidant properties of atorvastatin by reducing lipid peroxidation (MDA) and ROS production (8-OHdG). Significant reduction in cholesterol, LDL, TG and obvious increase in HDL in both groups was also obtained.

We can suggest also that the beneficial effect of atorvastatin appear to be greater than that might be expected from changes in lipid levels alone, and as a result of a direct decrease in oxidative stress.

We can suggest that mitochondrial dysfunction leading to increased oxidative stress markers (8-OHdG& MDA) may occur during overnutrition coupled with limited physical activity, and thereby contribute to the genesis and maintenance of the metabolic syndrome.

We suggest a great benefit of dietary intervention for the metabolic syndrome population, i.e. simply reestablishing cellular metabolic balance by limiting caloric intake and/or increasing metabolic demand through increased physical activity. Insulin resistance,

elevated triglycerides and defect of mitochondrial oxidative capacity are all related abnormalities switched-on in response to metabolic stress. We can say also that oxidative stress may be a major determinant factor in the loss of both insulin sensitivity and mitochondrial function associated with overnutrition. Thus oxidative stress plays a number of potential mechanisms in the pathophysiology of metabolic syndrome.

Thus present study suggests that mitochondrial dysfunction leading to oxidative stress as evidenced by increased (8-OHdG& MDA) is a consequence of altered cellular metabolism that develops with nutritional overload. We recommend further testing and exploration of this proposal for the role of mitochondria in the metabolic syndrome which may contribute to our understanding of its origin, maintenance and the development of further therapy.

REFERENCES

1. Grundy SM. Metabolic syndrome pandemic. Arterioscler Thromb Vasc Biology 2008; 28(4): 629-36.
2. Cameron AJ, Shaw JE, Zimmet PZ. The metabolic syndrome: Prevalence in worldwide populations. Endocrinol Metab Clin North America 2004; 33(2): 351-75.
3. Otani H. Oxidative Stress as Pathogenesis of Cardiovascular Risk Associated with Metabolic Syndrome. Antioxidants& Redox Signaling 2011; 15(7): 1911–26.
4. Sliem HA, Ahmed S, Nemr N, El-Sherif I. Metabolic syndrome in the Middle East. Indian J Endocrinol Metab 2012; 16(1): 67-71.
5. Ford ES, Giles WH, Mokdad AH. Increasing prevalence of the metabolic syndrome among U.S. adults. Diabetes Care 2004; 27(10): 2444-9.
6. Alberti KG, Zimmet PZ. Should we dump the metabolic syndrome? No. BMJ 2008; 336(7645): 641.
7. Yubero-Serrano EM, Delgado-Lista J, Peña-Orihuela P, Perez-Martinez P, Fuentes F, Marin C, et al. Oxidative stress is associated with the number of components of metabolic syndrome: LIPGENE study. Exp Mol Med 2013; 45: e28.
8. Gupta A, Gupta V. Metabolic syndrome: what are the risks for humans. BioScience Trends 2010; 4(5): 204-12.
9. Yehia S, Tawfek E, Ibrahim M, Mohamed I. Metabolic syndrome (MetS) in children suffered from Otitis media with effusion. Nature and Science 2013; 11(1): 52-7.
10. Després JP. Body Fat Distribution and Risk of Cardiovascular Disease: An Update. Circulation 2012; 126(10): 1301-13.
11. Das M, Pal S, Ghosh A. Family history of type 2 diabetes and prevalence of metabolic syndrome in adult Asian Indians. Journal of Cardiovascular Disease Research 2012; 3(2): 104-8.
12. Reaven GM. The metabolic syndrome: is this diagnosis necessary. Am J Clin Nutr 2006; 83(6): 1237-47.
13. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabetic Medicine 1998; 15(7): 539–53.
14. Cleeman JI. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults: Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA 2001; 285(19): 2486–97.
15. Balkau B, Charles MA. Comment on the provisional report from the WHO consultation: European Group for the Study of Insulin Resistance (EGIR). Diabetic Medicine 1999; 16(5): 442–3.

16. Alberti KG, Zimmet P, Shaw J. Metabolic syndrome – a new world-wide definition. A Consensus Statement from the International Diabetes Federation. Diabetic Medicine 2006; 23(5): 469–80.

17. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: An American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation 2005; 112(17): 2735-52.

18. Moy FM, Bulgiba A. The modified NCEP ATP III criteria maybe better than the IDF criteria in diagnosing Metabolic Syndrome among Malays in Kuala Lumpur. BMC Public Health 2010; 10: 678.

19. Gill H, Mugo M, Whaley-Connell A, Stump C, Sowers JR. The key role of insulin resistance in the cardiometabolic syndrome. Am J Med Sci 2005; 330(6): 290-4.

20. Isomaa B, Almgren P, Tuomi T, Forsén B, Lahti K, Nissén M, et al. Cardiovascular morbidity and mortality associated with the metabolic syndrome. Diabetes Care 2001; 24(4): 683-9.

21. Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J, et al. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. JAMA 2002; 288(21): 2709-16.

22. Ridker PM, Buring JE, Cook NR, Rifai N. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: An 8-years follow-up of 14,719 initially healthy American women. Circulation 2003; 107(3): 391-7.

23. Lamarche B, Tchernof A, Mauriège P, Cantin B, Dagenais GR, Lupien PJ, et al. Fasting insulin and apolipoprotein B levels and low-density lipoprotein particle size as risk factors for ischemic heart disease. JAMA 1998; 279(24): 1955-61.

24. Lamarche B, Desroches S. Metabolic syndrome and effects of conjugated linoleic acid in obesity and lipoprotein disorders: the Québec experience. Am J Clin Nutr 2004; 79(6): 1149–52.

25. Chuengsamarn S, Rattanamongkoulgul S, Villarroel A. Association between metabolic syndrome and risk of cardiovascular disease, using different criteria and stratified by sex. Int J Diabetes Mellit 2010; 2(2): 78–82.

26. Muñoz MA, Bedoya G, Velásquez CM. An approach to the etiology of metabolic syndrome. Colombia Medica 2013; 44(1); 57-63.

27. Reaven GM. Pathophysiology of insulin resistance in human disease. Physiological Reviews 1995; 75(3): 473–86.

28. Mohamed-Ali V, Pinkney JH, Coppack SW. Adipose tissue as an endocrine and paracrine organ. Int J Obes Relat Metab Disorders 1998; 22(12): 1145-58.

29. Pittas AG, Joseph NA, Greenberg AS. Adipocytokines and Insulin Resistance. J Clin Endocrinol Metabolism 2004; 89(2): 447-52.

30. Kwon H, Pessin JE. Adipokines mediate inflammation and insulin resistance. Frontiers in Endocrinology (Lausanne) 2013; 4: 71-83.

31. Duvnjak M, Lerotić I, Barsić N, Tomasić V, Virović Jukić L, Velagić V. Pathogenesis and management issues for non-alcoholic fatty liver disease. World J Gastroenterol 2007; 13(34): 4539-50.

32. Arita Y, Kihara S, Ouchi N, Maeda K, Kuriyama H, Okamoto Y, et al. Adipocyte-derived plasma protein adiponectin acts as a platelet-derived growth factor-BB-binding protein and regulates growth factor-induced common postreceptor signal in vascular smooth muscle cell. Circulation 2002; 105(24): 2893-8.

33. Chandran M, Phillips SA, Ciaraldi T, Henry RR. Adiponectin: More than just another fat cell hormone. Diabetes Care 2003; 26(8): 2442-50.

34. Feskens EJ, Tuomilehto J, Stengård JH, Pekkanen J, Nissinen A, Kromhout D. Hypertension and overweight associated with hyperinsulinaemia and glucose tolerance: A longitudinal study of the Finnish and Dutch cohorts of the Seven Countries Study. Diabetologia 1995; 38(7): 839-47.

35. Lind L, Berne C, Lithell H. Prevalence of insulin resistance in essential hypertension. Journal of Hypertension 1995; 13(12): 1457-62.

36. Yiannikouris F, Gupte M, Putnam K, Cassis L. Adipokines and blood pressure control. Curr Opin Nephrol Hypertension 2010; 19(2): 195-200.

37. Giugliano D, Ceriello A, Esposito K. Are there specific treatments for the metabolic syndrome. Am J Clin Nutr 2008; 87(1): 8-11.

38. Golbidi S, Mesdaghinia A, Laher I. Exercise in the Metabolic Syndrome. Oxidative Medicine and Cellular Longevity 2012; 2012: 349710-22.

39. Chilibeck PD, Pérez-López FR, Bodary PF, Kang ES, Jeon JY. Adipocytokines, Metabolic Syndrome, and Exercise. International Journal of Endocrinology 2014; 2014: 597162-4.

40. Schenk S, Harber MP, Shrivastava CR, Burant CF, Horowitz JF. Improved insulin sensitivity after weight loss and exercise training is mediated by a reduction in plasma fatty acid mobilization, not enhanced oxidative capacity. J Physiol 2009; 587(20): 4949–61.

41. Richard C, Couture P, Desroches S, Lichtenstein AH, Lamarche B. Effect of weight loss, independent of change in diet composition, on apolipoprotein AI kinetic in men with metabolic syndrome. J Lipid Res 2013; 54(1): 232-7.

42. Miller M, Stone NJ, Ballantyne C, Bittner V, Criqui MH, Ginsberg HN, et al. Triglycerides and cardiovascular disease: a scientific statement from the American Heart Association. Circulation 2011; 123(20): 2292-333.

43. Previtali E, Bucciarelli P, Passamonti SM, Martinelli I. Risk factors for venous and arterial thrombosis. Blood Transfus 2011; 9(2): 120-38.

44. Schulze MB, Hu FB. Dietary approaches to prevent the metabolic syndrome: quality versus quantity of carbohydrates. Diabetes Care 2004; 27(2): 613-4.

45. Esposito K, Ceriello A, Giugliano D. Diet and the metabolic syndrome. Metab Syndr Relat Disord 2007; 5(4): 291-6.

46. Ivester P, Sergeant S, Danhauer SC, Case LD, Lamb A, Chilton BG, Delar B, Hollingshead ML, Weaver KL, Chilton FH. Effect of a multifaceted, church-based wellness program on metabolic syndrome in 41 overweight or obese congregants. Preventing Chronic Disease 2010; 7(4): 81-8.

47. Oh SW, Yoon YS, Lee ES, Kim WK, Park C, Lee S, et al. Association between cigarette smoking and metabolic syndrome: the Korea National Health and Nutrition Examination Survey. Diabetes Care 2005; 28(8): 2064-6.

48. Slagter SN, van Vliet-Ostaptchouk JV, Vonk JM, Boezen HM, Dullaart RP, Kobold AC, Feskens EJ, van Beek AP, van der Klauw MM, Wolffenbuttel BH. Associations between smoking, components of metabolic syndrome and lipoprotein particle size. BMC Med 2013; 11: 195.

49. Berlin I, Lin S, Lima JA, Bertoni AG. Smoking Status and Metabolic Syndrome in the Multi-Ethnic Study of Atherosclerosis. A cross-sectional study. Tob Induc Dis 2012; 10(1): 9.

50. Nakanishi K, Nishida M, Ohama T, Moriyama T, Yamauchi-Takihara K. Smoking associates with visceral fat accumulation especially in women. Circ J. 2014; 78(5): 1259-63.

51. Tota-Maharaj R, Defilippis AP, Blumenthal RS, Blaha MJ. A practical approach to the metabolic syndrome: review of current concepts and management. Curr Opin Cardiol 2010; 25(5): 502-12.

52. Pichandi S, Rao YY, Farook J, Pasupathi P, Bakthavathsalam G, Subramaniyamd S, et al. Comparative evaluation of the effect of statin drugs in Hyperlipidaemic patients. Int J Cur Biomed Phar Res 2011; 1(2): 6–10.

53. Pichandi S, Pasupathi P, Rao YY, Farook J, Ambika A, Ponnusha BS, et al. The role of statin drugs in combating cardiovascular diseases. Int J Cur Sci Res 2011; 1(2): 47–56.

54. Shepherd J, Cobbe SM, Ford I. West of Scotland Coronary Prevention Study Group. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. N Engl J Med 1995; 333: 1301-1307.

55. Eliasson B, Svensson AM, Miftaraj M, Jonasson JM, Eeg-Olofsson K, Sundell KA, Gudbjörnsdóttir S. Clinical use and effectiveness of lipid lowering therapies in diabetes mellitus--an observational study from the Swedish National Diabetes Register. PLoS One 2011; 6(4): 18744-52.

56. Vaughn CJ, Delanty N. Neuroprotective actions of statins in cerebral ischemia and stroke. Stroke 1999; 30: 1969-73.

57. Black DM. A general assessment of the safety of HMG CoA reductase inhibitors (statins). Curr Atheroscler Rep 2002; 4(1): 34-41.

58. Scirica BM, Cannon CP. Treatment of elevated cholesterol. Circulation 2005; 111(21): 360-3.

59. Castaño G, Mas R, Fernández L, Illnait J, Mesa M, Alvarez E, Lezcay M. Comparison of the efficacy and tolerability of policosanol with atorvastatin in elderly patients with type II hypercholesterolaemia. Drugs& Aging 2003; 20(2): 153-63.

60. Vijayaraghavan K. Treatment of dyslipidemia in patients with type 2 diabetes. Lipids in Health and Disease 2010; 9: 144-55.

61. McCrindle BW, Ose L, Marais AD. Efficacy and safety of atorvastatin in children and adolescents with familial hypercholesterolemia or severe hyperlipidemia: a multicenter, randomized, placebo-controlled trial. J Pediatr 2003; 143(1): 74-80.

62. Brugts JJ, Yetgin T, Hoeks SE, Gotto AM, Shepherd J, Westendorp RG, de Craen AJ, Knopp RH, Nakamura H, Ridker P, van Domburg R, Deckers JW. The benefits of statins in people without established cardiovascular disease but with cardiovascular risk factors: meta-analysis of randomised controlled trials. BMJ 2009; 338: 2376-83.

63. Plosker GL, Lyseng-Williamson KA. Atorvastatin: a pharmacoeconomic review of its use in the primary and secondary prevention of cardiovascular events. Pharmacoeconomics 2007; 25(12): 1031-53.

64. Durazzo AE, Machado FS, Ikeoka DT, De Bernoche C, Monachini MC, Puech-Leão P, Caramelli B. Reduction in cardiovascular events after vascular surgery with atorvastatin: a randomized trial. J Vasc Surg 2004; 39(5): 967-75; discussion 975-6.

65. Nitha B, De S, Adhikari SK, Devasagayam TP, Janardhanan KK. Evaluation of free radical scavenging activity of morel mushroom, Morchella esculenta mycelia: a potential source of therapeutically useful antioxidants. Pharm Biol 2010; 48(4): 453-60.

66. Kehrer JP. Free-radicals as mediators of tissue-injury and disease. Crit Rev Toxicol 1993; 23: 21–48.

67. Shen HM, Shi CY, Shen Y, Ong CN. Detection of elevated reactive oxygen species level in cultured rat hepatocytes treated with aflatoxin B-1. Free Rad Biol Med 1996; 2: 139–46.

68. Ames NB, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative disease of aging. Proc Natl Acad Sci USA 1993; 90(17): 7915–22.

69. Halliwell B, Gutteridge JMC, Cross CE. Free-radicals, antioxidants, and human-disease – Where are we now. J Lab Clin Med 1992; 119: 598–620.

70. Ward JF, Limoli CL, Calabro-Jones P, Evans JW. Radiation versus chemical damage to DNA. In: Anticarcinogenesis and Radiation Protection. Ceruti P.A, Nygaard O.F, Simic M.G (eds). Plenum Press, New York, 1988, pp 321–27.

71. Esterbauer H, Eckl P, Ortner A. Possible mutagens derived from lipids and lipid precursors. Mutat Res 1990; 238(3): 223–33.

72. Bartsch H. DNA adducts in human carcinogenesis: Etiological relevance and structure–activity relationship. Mut Res Rev Genet Toxicol 1996; 340(2-3): 67–79.

73. Winterbourn CC. Superoxide as an intracellular radical sink. Free Rad Biol Med 1993; 14(1): 85–90.

74. Liochev SI, Fridovich I. The role of $O_2^{\bullet -}$ in the production of $HO^{\bullet}$: in vitro and in vivo. Free Rad Biol Med 1994; 16(1): 29–33.

75. Miller DM, Buettner GR, Aust SD. Transition-metals as catalysts of autoxidation reactions. Free Rad Biol Med 1990; 8(1): 95–108.

76. Fridovich I. Biological effects of the superoxide radical. Arch Biochem Biophys 1986; 247: 1–11.

77. Valko M, Izakovic M, Mazur M, Rhodes CJ, Telser J. Role of oxygen radicals in DNA damage and cancer incidence. Mol Cell Biochem 2004; 266(1-2): 37-56.

78. Salvador A, Sousa J, Pinto RE. Hydroperoxyl, superoxide and pH gradients in the mitochondrial matrix: A theoretical assessment. Free Rad Biol Med 2001; 31: 1208–15.

79. Hanukoglu I, Rapoport R, Weiner L, Sklan D. Electron leakage from the mitochondrial NADPH-adrenodoxin reductase-adrenodoxin-P450scc (cholesterol side-chain cleavage) system. Arch Biochem Biophys 1993; 305(2): 489–98.

80. Benzi G, Pastoris O, Marzatico F, Villa RF, Dagani F, Curti D. The mitochondrial electron-transfer alteration as a factor involved in the brain aging. Neurobiol Aging 1992; 13: 361–68.

81. Freeman BA, Crapo JD. Free-radicals and tissue-injury. Lab Invest 1982; 47: 412–26.

82. Kinnula VL, Crapo JD, Raivio KO. Biology of disease-generation and disposal of reactive oxygen metabolites in the lung. Lab Invest 1995; 73: 3–19.

83. Brookes PS, Levonen AL, Shiva S, Sarti P, Darley-Usmar VM. Mitochondria: Regulators of signal transduction by reactive oxygen and nitrogen species. Free Rad Biol Med 2002; 33(6): 755–64.

84. Butler J, Hoey BM. The one-electron reduction potential of several substrates can be related to their reduction rates by cytochrome-P-450 reductase. Biochim Biophys Acta 1993; 1161(1): 73–8.

85. Thannickal VJ, Fanburg BL. Reactive oxygen species in cell signaling. Am J Physiol Lung cell Mol Physiol 2000; 279(6): L1005–28.

86. Moriwaki Y, Yamamoto T, Higashino K. Enzymes involved in purine metabolism – A review of histochemical localization and functional implications. Histol Histopathol 1999; 14(4): 1321–40.

87. Harrison R. Structure and function of xanthine oxidoreductase: Where are we now. Free Rad Biol Med 2002; 33(6): 774–97.

88. Borges F, Fernandes E, Roleira F. Progress towards the discovery of xanthine oxidase inhibitors. Curr Med Chem 2002; 9(2): 195–217.

89. Stohs SJ, Bagchi D. Oxidative mechanisms in the toxicity of metalions. Free Rad Biol Med 1995; 18(2): 321–36.

90. Fahl WE, Lalwani ND,Watanabe T, Goel SK, Reddy JK. DNA damage related to increased hydrogen-peroxide generation by hypolipidemic drug-induced liver peroxisomes. Proc Natl Acad Sci USA; Biol. Sci 1984; 81(24): 7827–30.

91. Thannickal VJ, Fanburg BL. Reactive oxygen species in cell signaling. Am J Physiol Lung Cell Mol Physiol 2000; 279(6): L1005–28.

92. Vignais PV. The superoxide-generating NADPH oxidase: Structural aspects and activation mechanism. Cell Mol Life Sci 2002; 59(9): 1428–59.

93. Van Heerebeek L, Meischl C, Stooker W, Meijer CJ, Niessen HW, Roos D. NADPH oxidase(s): New source(s) of reactive oxygen species in the vascular system. J Clin Pathol 2002; 55(8): 561–8.

94. Jirapongsananuruk O, Malech HL, Kuhns DB, Niemela JE, BrownMR, Anderson-Cohen M, et al. Diagnostic paradigm for evaluation of male patients with chronic granulomatous disease, based on the dihydrorhodamine 123 assay. J Allergy Clin Immun 2003; 111(2): 374–9.

95. Proctor PH. Free radicals and human disease. In: Miquel J, Quintanilha A, Weber H (eds) Handbook of Free Radicals and Antioxidants, CRC Press, Boca Raton, FL, USA, 1989, pp 209-21.

96. Rodriguez AM, Carrico PM, Mazurkiewicz JE, Melendez JA. Mitochondrial or cytosolic catalase reverses the MnSOD-dependent inhibition of proliferation by enhancing respiratory chain activity, net ATP production, and decreasing the steady state levels of H_2O_2. Free Rad Biol Med 2000; 29(9): 801–13.

97. Prutz WA, Butler J, Land EJ. The glutathione free radical equilibrium, GS. + GS– ↔ GSS.–G, mediating electron transfer to FE(III) -cytochrome c. Biophysical Chemistry 1994; 49(2): 101–11.

98. Prousek J. Fenton reaction after a century. Chem Listy 1995; 89(1): 11–21.

99. Kalyanaraman B, Morehouse KM, Mason RP. An electron-paramagnetic resonance study of the interactions between the adriamycin semiquinone, hydrogen-peroxide, iron-chelators, and radical scavengers. Arch Biochem Biophys 1991; 286(1): 164–70.

100. Buettner GR, Jurkiewicz BA. Catalytic metals, ascorbate and free radicals: Combinations to avoid. Rad Res 1996; 145(5): 532–41.

101. Shi XL, Chiu A, Chen CT, Halliwell B, Castranova V, Vallyathan V. Reduction of chromium(VI) and its relationship to carcinogenesis. J Toxicol Environ Health B Crit Rev 1999; 2(1): 87–104.

102. Klug-Roth D, Rabani J. Pulse radiolytic studies on reactions of aqueous superoxide radicals with copper(II) complexes. J Phys Chem 1976; 80: 588–91.

103. Stohs SJ, Bagchi D. Oxidative mechanisms in the toxicity of metalions. Free Rad Biol Med 1995; 18(2): 321–36.

104. Balasubramanian B, Pogozelski WK, Tullius TD. DNA strand breaking by the hydroxylradical is governed by the accessible surface areas of the hydrogen atoms of the DNA backbone. Proc Natl Acad Sci USA 1998; 95(17): 9738–43.

105. Agatsuma S, Nagoshi T, Kobayashi M, Usa M, Watanabe H, Sekino H, et al. Hydroxyl radical-induced characteristic chemiluminescent spectra from plasma of hemodialysis-patients. Clin Chem 1992; 38(1): 48–55.

106. Schafer FQ, Qian SY, Buettner GR. Iron and free radical oxidations in cell membranes. Cell Mol Biol 2000; 46(3): 657–62.

107. Hempel SL, Buettner GR, Wessels DA, Galvan GM, Omalley YQ. Extracellular iron (II) can protect cells from hydrogen peroxide. Arch Biochem Biophys 1996; 330(2): 401–8.

108. Lloyd RV, Hanna PM, Mason RP. The origin of the hydroxyl radical oxygen in the Fenton reaction. Free Rad Biol Med 1997; 22(5): 885–8.

109. Reddy S, Halliwell B, Jones AD, Longhurst JC. The use of phenylalanine to detect hydroxyl radical production in vivo: A cautionary note. Free Rad Biol Med 1999; 27(11-12): 1465.

110. Halliwell B, Clement MV, Ramalingam J, Long LH. Hydrogen peroxide. Ubiquitous in cell culture and in vivo. IUBMB Life 2000; 50(4-5): 251–7.

111. Kadiiska MB, Mason RP. In vivo copper-mediated free radical production: An ESR spin-trapping study. Spectrochim Acta A, Mol Biomol Spectr 2002; 58(6): 1227–39.

112. Toyokuni S. Iron and carcinogenesis. From Fenton reaction to target genes. Redox Rep 2002; 7(4): 189–97.

113. Imlay JA, Park S, Woodmansee A. What is the intracellular reductant that drives the Fenton reaction. Free Rad Biol Med 2002; 33(suppl 2): 255.

114. Raleigh JA, Shum FY. Hydroxyl radical scavengers and membrane damage – Supplementary role for alpha-tocopherol in scavenging secondary radicals. Rad Res 1983; 94: 664–5.

115. Zastawny TH, Altman SA, Randers-Eichhorn L, Madurawe R, Lumpkin JA, Dizdaroglu M, et al. DNA-base modifications and membrane damage in cultured-mammalian-cells treated with iron ions. Free Rad Biol Med 1995; 18(6): 1013–22.

116. Mao H, Deng ZW, Wang F, Harris TM, Stone MP. An intercalated and thermally stable FAPY adduct of aflatoxin B1 in a DNA duplex: Structural refinement from 1H NMR. Biochemistry 1998; 37(13): 4374–87.

117. Boiteux S, Radicella JP. The human OGG1 gene: Structure, functions, and its implication in the process of carcinogenesis. Arch Biochem Biophys 2000; 377(1): 1–8.

118. Jaruga PW, Rodriguez H, Dizdaroglu M. Measurement of 8-hydroxy-2'-deoxyadenosine in DNA by liquid chromatography/mass spectrometry. Free Rad Biol Med 2001; 31(3): 336–44.

119. Dizdaroglu M, Olinski R, Doroshow JH, Akman SA. Modification of DNA bases in chromatin of intact target human-cells by activated human polymorphonuclear leukocytes. Cancer Res 1993; 53(6): 1269–72.

120. Murata M, Imada M, Inoue S, Kawanishi S. Metal-mediated DNA damage induced by diabetogenic alloxan in the presence of NADH. Free Rad Biol Med 1998; 25(4-5): 586–95.

121. Reiter RJ, Acuna-Castroviejo D, Tan DX, Burkhardt S. Free radical-mediated molecular damage. Mechanisms for the protective actions of melatonin in the central nervous system. Neuroprot Agents Annals NY Acad Sci 2001; 939: 200–15.

122. Gichner T. DNA damage induced by indirect and direct acting mutagens in catalase-deficient transgenic tobacco. Cellular and acellular Comet assays. Mutat Res 2003; 535(2): 187–93.

123. Mistry N, Podmore L, Cooke M, Butler P, Griffiths H, Herbert K, et al. Novel monoclonal antibody recognition of oxidative DNA damage adduct, deoxycytidine-glyoxal. Lab Invest 2003; 83(2): 241–50.

124. Rodriguez H, Valentine MR, Holmquist GP, Akman SA, Termini J. Mapping of peroxyl radical induced damage on genomic DNA. Biochemistry 1999; 38(50): 16578–88.

125. Chen CJ, Bozzelli JW. Thermochemical property, pathway and kinetic analysis on the reactions of allylic isobutenyl radical with O_2: An elementary reaction mechanism for isobutene oxidation. J Phys Chem A 2000; 104(43): 9715–32.

126. Hawkins CL, Davies MJ. Generation and propagation of radical reactions on proteins. Biochim Biophys Acta Bioenerg 2001; 1504(2-3): 196–219.

127. Korytowski W, Zareba M, Girotti AW. Inhibition of free radical-mediated cholesterol peroxidation by diazeniumdiolate-derived nitric oxide: Effect of release rate on mechanism of action in a membrane system. Chem Res Toxicol 2000; 13(12): 1265–74.

128. Kanazawa A, Sawa T, Akaike T, Maeda H. Dietary lipid peroxidation products and DNA damage in colon carcinogenesis. Eur J Lipid Sci Technol 2002; 104(7): 439–47.

129. Razskazovskii Y, Sevilla MD. Reactions of sulphonyl peroxyl radicals with DNA and its components: Hydrogen abstraction from the sugar backbone versus addition to pyrimidine double bonds. Int J Radiat Biol 1996; 69(1): 75–87.

130. Basaga HS. Biochemical aspects of free-radicals. Biochem Cell Biol =Biochimie et Biologie Cellulaire 1990; 68(7-8): 989–98.

131. Alfassi ZB. The Chemistry of Free Radicals: Peroxyl Radicals. Wiley, New York, 1997, pp 546.

132. Willcox JK, Ash SL, Catignani GL. Antioxidants and prevention of chronic disease. Crit Rev Food Sci Nutr 2004; 44(4): 275-95.

133. Powers KM, Oberley LW, Domann FE. The Adventures of Superoxide Dismutase in Health and Disease: Superoxide in the Balance. In: Oxidants in Biology: A Question of Balance. Giuseppe Valacchi, Paul A. Davis (eds), Springer Science + Business Media B.V 2008; pp: 183-201.

134. Marklund SL. Human copper-containing superoxide dismutase of high molecular weight. Proc Natl Acad Sci USA 1982; 79(24): 7634-8.

135. Sayuri Miyamoto, Hirofumi Arai, Junji Terao. Enzymatic antioxidant defenses. In: Biomarkers for antioxidant defense and oxidative damage. Giancarlo Aldini, Kyung-Jin Yeum, Etsuo Niki, Robert M. Russell. Wiley-Blackwell USA. 2010, pp 21-33.

136. Crapo JD, Oury T, Rabouille C, Slot JW, Chang LY. Copper-zinc superoxide dismutase is primarily a cytosolic protein in human cells. Proc Natl Acad Sci USA 1992; 89(21): 10405-9.

137. Elchuri S, Oberley TD, Qi W, Eisenstein RS, Jackson Roberts L, Van Remmen H, Epstein CJ, Huang TT. Cu/Zn SOD deficiency leads to persistent and widespread oxidative damage and hepatocarcinogenesis later in life. Oncogene 2005; 24(3): 367-80.

138. Pick M, Rabani J, Yost F, Fridovich I. The catalytic mechanism of the manganese containing superoxide dismutase of Escherichia coli studied by pulse radiolysis. J Am Chem Soc 1974; 96(23): 7329-33.

139. Ough M, Lewis A, Zhang Y, Hinkhouse MM, Ritchie JM, Oberley LW, et al. Inhibition of cell growth by overexpression of manganese superoxide dismutase (MnSOD) in human pancreatic carcinoma. Free Radic Res 2004; 38(11): 1223-33.

140. Huie RE, Padmaja S. The reaction of NO with superoxide. Free Radic Res Commun 1993; 18(4): 195-9.

141. Brown KA, Chu Y, Lund DD, Heistad DD, Faraci FM. Gene transfer of extracellular superoxide dismutase protects against vascular dysfunction with aging. Am J Physiol Heart Circ Physiol 2006; 290(6): H2600-5.

142. Ralph GS, Radcliffe PA, Day DM, Carthy JM, Leroux MA, Lee DC, et al. Silencing mutant SOD1 using RNAi protects against neurodegeneration and extends survival in an ALS model. Nat Med 2005; 11(4): 429-33.

143. Urushitani M, Sik A, Sakurai T, Nukina N, Takahashi R, Julien JP. Chromogranin-mediated secretion of mutant superoxide dismutase proteins linked to amyotrophic lateral sclerosis. Nat Neurosci 2006; 9(1): 108-18.

144. Huret JL, Delabar JM, Marlhens F, Aurias A, Nicole A, Berthier M, et al. Down syndrome with duplication of a region of chromosome 21 containing the Cu/Zn superoxide dismutase gene without detectable karyotypic abnormality. Human Genet 1987; 75(3): 251-7.

145. Hitchler MJ, Wikainapakul K, Yu L, Powers K, Attatippaholkun W, Domann FE. Epigenetic regulation of manganese superoxide dismutase expression in human breast cancer cells. Epigenet 2006; 1(4): 163-71.

146. Soini Y, Vakkala M, Kahlos K, Paakko P, Kinnula V. MnSOD expression is less frequent in tumour cells of invasive breast carcinomas than in in-situ carcinomas or non-neoplastic breast epithelial cells. J Pathol 2001; 195(2): 156-62.

147. Madamanchi NR, Runge MS. Mitochondrial dysfunction in atherosclerosis. Circ Res 2007; 100(4): 460-73.

148. Muller L, Theile K, Bohm V. In vitro antioxidant activity of tocopherols and tocotrienols and comparison of vitamin E concentration and lipophilic antioxidant capacity in human plasma. Mol Nutr Food Res 2010; 54(5): 731-42.

149. Engin KN. Alpha-tocopherol: Looking beyond an antioxidant. Mol Vision 2009; 15: 855-60.

150. Christopher Min K. Structure and function of alpha-tocopherol transfer protein: implications for vitamin E metabolism and AVED. Vitam Horm 2007; 76: 23-43.

151. Brigelius-Flohe R. Vitamin E: the shrew waiting to be tamed. Free Radic Biol Med 2009; 46(5): 543-54.

152. Maret G Traber. Vitamin E. In: Handbook of vitamins. Janos Zempleni, Robert B Rucker, Donald B McCormick, John W Suttie. 4th ed. CRC Press Taylor & Francis Group, Printed in the United States of America 2007, pp. 153-74.

153. Banks R, Speakman JR, Selman C. Vitamin E supplementation and mammalian lifespan. Mol Nutr Food Res 2010; 54(5): 719-25.

154. Sen CK, Khanna S, Roy S. Tocotrienols in health and disease: The other half of the natural vitamin E family. Mol Aspects Med 2007; 28(5-6): 692-728.

155. Upadhyay J, Misra K. Towards the interaction mechanism of tocopherols and tocotrienols (vitamin E) with selected metabolizing enzymes. Bioinformation 2009; 3(8): 326-31.

156. Huey KA, Fiscus G, Richwine AF, Johnson RW, Meador BM. In vivo vitamin E administration attenuates interleukin-6 and interleukin-1beta responses to an acute inflammatory insult in mouse skeletal and cardiac muscle. Exp Physiol 2008; 93(12): 1263-72.

157. Zou T, Liu N, Li SD, Su YC, Man Y, Lu D. Vitamin E inhibits homocysteine-mediated smooth muscle cell proliferation. Nan Fang Yi Ke DA Xue Xue Bao 2007; 27(6): 783-6.

158. Lee IM, Cook NR, Gaziano JM, Gordon D, Ridker PM, Manson JE, et al. Vitamin E in the primary prevention of cardiovascular disease and cancer: the Women's Health Study: a randomized controlled trial. JAMA 2005; 294(1): 56-65.

159. Dong YH, Guo YH, Gu XB. Anticancer mechanisms of vitamin E succinate. Chinese J of Cancer = Ai Zheng 2009; 28(10): 1114-18.

160. Muller L, Theile K, Bohm V. In vitro antioxidant activity of tocopherols and tocotrienols and comparison of vitamin E concentration and lipophilic antioxidant capacity in human plasma. Mol Nutr Food Res 2010; 54(5): 731-42.

161. Clarke MW, Burnett JR, Croft KD. Vitamin E in human health and disease. Crit Rev Clin Lab Sci 2008; 45(5): 417-50.

162. Brigelius-Flohe R, Galli F. Vitamin E: A vitamin still awaiting the detection of its biological function. Mol Nut Food Res 2010; 54(5): 583-7.

163. Traber MG, Sies H. Vitamin E in humans: demand and delivery. Annu Rev Nutr 1996; 16, 321–47.

164. Drevon CA. Absorption, transport and metabolism of vitamin E. Free Rad Res Commun 1991; 14(4): 229–46.

165. Hosomi A, Arita M, Sato Y, Kiyose C, Ueda T, Igarashi O, et al. Affinity for alpha-tocopherol transfer protein as a determinant of the biological activities of vitamin E analogs. FEBS Lett 1997; 409(1): 105–8.

166. Poljsak B, Šuput D, Milisav I. Achieving the balance between ROS and antioxidants: when to use the synthetic antioxidants. Oxid Med Cell Longev 2013; 2013: 956792-802.

167. Halliwell B, Whiteman M. Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean. Br J Pharmacol 2004; 142(2): 231-55.

168. Uttara B, Singh AV, Zamboni P, Mahajan RT. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. Curr Neuropharmacol 2009; 7(1): 65-74.

169. Hwang ES, Kim GH. Biomarkers for oxidative stress status of DNA, lipids, and proteins in vitro and in vivo cancer research. Toxicology 2007; 229(1-2): 1-10.

170. Samoylenko A, Hossain JA, Mennerich D, Kellokumpu S, Hiltunen JK, Kietzmann T. Nutritional countermeasures targeting reactive oxygen species in cancer: from mechanisms to biomarkers and clinical evidence. Antioxid Redox Signal 2013; 19(17): 2157-96.

171. Cooke MS, Evans MD, Dizdaroglu M, Lunec J. Oxidative DNA damage: mechanisms, mutation, and disease. FASEB J 2003; 17(10): 1195-214.

172. Rim KT. Oxidative DNA damages by chemical exposures at work. Advances in Bioscience and Biotechnology 2012; 3(7): 957-71.

173. Sampath H, Vartanian V, Rollins MR, Sakumi K, Nakabeppu Y, Lloyd RS. 8-Oxoguanine DNA glycosylase (OGG1) deficiency increases susceptibility to obesity and metabolic dysfunction. PLoS One 2012; 7(12): e51697.

174. Valavanidis A, Vlachogianni T, Fiotakis C. 8-hydroxy-2' -deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 2009; 27(2): 120-39.

175. Ock CY, Kim EH, Choi DJ, Lee HJ, Hahm KB, Chung MH. 8-Hydroxydeoxyguanosine: not mere biomarker for oxidative stress, but remedy for oxidative stress-implicated gastrointestinal diseases. World J Gastroenterol 2012; 18(4): 302-8.

176. Herczenik E, Gebbink MF. Molecular and cellular aspects of protein misfolding and disease. FASEB J 2008; 22(7): 2115-33.

177. Dalle-Donne I, Rossi R, Giustarini D, Milzani A, Colombo R. Protein carbonyl groups as biomarkers of oxidative stress. Clin Chim Acta 2003; 329(1-2): 23-38.

178. Arteel GE, Briviba K, Sies H. Protection against peroxynitrite. FEBS Letters 1999; 445(2-3): 226-30.

179. Piconi L, Quagliaro L, Ceriello A. Oxidative stress in diabetes. Clin Chem Lab Med 2003; 41(9): 1144-9.

180. Roberts LJ, Milne GL. Isoprostanes. J Lipid Res 2009; 50 Suppl: S219-23.

181. Grimsrud PA, Xie H, Griffin TJ, Bernlohr DA. Oxidative stress and covalent modification of protein with bioactive aldehydes. J Biol Chem 2008; 283(32): 21837-41.

182. Wong-Ekkabut J, Xu Z, Triampo W, Tang IM, Tieleman DP, Monticelli L. Effect of lipid peroxidation on the properties of lipid bilayers: a molecular dynamics study. Biophys J 2007; 93(12): 4225-36.

183. Montuschi P, Barnes PJ, Roberts LJ 2nd. Isoprostanes: markers and mediators of oxidative stress. FASEB J 2004; 18(15): 1791-800.

184. Fortuño A, San José G, Moreno MU, Beloqui O, Díez J, Zalba G. Phagocytic NADPH oxidase overactivity underlies oxidative stress in metabolic syndrome. Diabetes 2006; 55(1): 209-15.

185. Hansel B, Giral P, Nobecourt E, Chantepie S, Bruckert E, Chapman MJ, et al. Metabolic syndrome is associated with elevated oxidative stress and dysfunctional dense high-density lipoprotein particles displaying impaired antioxidative activity. J Clin Endocrinol Metab 2004; 89(10): 4963-71.

186. Roberts CK, Barnard RJ, Sindhu RK, Jurczak M, Ehdaie A, Vaziri ND. Oxidative stress and dysregulation of NAD(P)H oxidase and antioxidant enzymes in diet-induced metabolic syndrome. Metabolism 2006; 55(7): 928-34.

187. Ford ES, Mokdad AH, Giles WH, Brown DW. The metabolic syndrome and antioxidant concentrations: findings from the Third National Health and Nutrition Examination Survey. Diabetes 2003; 52(9): 2346-52.

188. Perticone F, Ceravolo R, Candigliota M, Ventura G, Iacopino S, Sinopoli F, et al. Obesity and body fat distribution induce endothelial dysfunction by oxidative stress: protective effect of vitamin C. Diabetes 2001; 50(1): 159-65.

189. Montero D, Walther G, Perez-Martin A, Roche E, Vinet A. Endothelial dysfunction, inflammation, and oxidative stress in obese children and adolescents: markers and effect of lifestyle intervention. Obes Rev 2012; 13(5): 441-55.

190. Rector RS, Warner SO, Liu Y, Hinton PS, Sun GY, Cox RH, et al. Exercise and diet induced weight loss improves measures of oxidative stress and insulin sensitivity in adults with characteristics of the metabolic syndrome. Am J Physiol Endocrinol Metab 2007; 293(2): E500-6.

191. Roberts CK, Won D, Pruthi S, Kurtovic S, Sindhu RK, Vaziri ND, et al. Effect of a short-term diet and exercise intervention on oxidative stress, inflammation, MMP-9, and monocyte chemotactic activity in men with metabolic syndrome factors. J Appl Physiol 2006; 100(5): 1657-65.

192. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest 2004; 114(12): 1752-61.

193. Houstis N, Rosen ED, Lander ES. Reactive oxygen species have a causal role in multiple forms of insulin resistance. Nature 2006; 440(7086): 944-8.

194. De Mattia G, Bravi MC, Laurenti O, Moretti A, Cipriani R, Gatti A, Mandosi E, Morano S. Endothelial dysfunction and oxidative stress in type 1 and type 2 diabetic patients without clinical macrovascular complications. Diabetes Res Clin Pract 2008; 79(2): 337-42.

195. de Oliveira J, Hort MA, Moreira EL, Glaser V, Ribeiro-do-Valle RM, Prediger RD, et al. Positive correlation between elevated plasma cholesterol levels and cognitive impairments in LDL receptor knockout mice: relevance of cortico-cerebral mitochondrial dysfunction and oxidative stress. Neuroscience 2011; 197: 99-106.

196. Marques de Mattos A, Marino LV, Ovidio PP, Jordão AA, Almeida CC, Chiarello PG. Protein oxidative stress and dyslipidemia in dialysis patients. Ther Apher Dial 2012; 16(1): 68-74.

197. Zelzer S, Fuchs N, Almer G, Raggam RB, Prüller F, Truschnig-Wilders M, et al. High density lipoprotein cholesterol level is a robust predictor of lipid peroxidation irrespective of gender, age, obesity, and inflammatory or metabolic biomarkers. Clin Chim Acta 2011; 412(15-16): 1345-9.

198. Andreadou I, Farmakis D, Prokovas E, Sigala F, Zoga A, Spyridaki K, et al. Short-term statin administration in hypercholesterolaemic rabbits resistant to postconditioning: effects on infarct size, endothelial nitric oxide synthase, and nitro-oxidative stress. Cardiovasc Res 2012; 94(3): 501-9.

199. Ansari JA, Bhandari U, Pillai KK, Haque SE. Effect of rosuvastatin on obesity-induced cardiac oxidative stress in Wistar rats—a preliminary study. Indian J Exp Biol 2012; 50(3): 216-22.

200. Ward NC, Hodgson JM, Puddey IB, Mori TA, Beilin LJ, Croft KD. Oxidative stress in human hypertension: association with antihypertensive treatment, gender, nutrition, and lifestyle. Free Radic Biol Med 2004; 36(2): 226-32.

201. Redón J, Oliva MR, Tormos C, Giner V, Chaves J, Iradi A, et al. Antioxidant activities and oxidative stress byproducts in human hypertension. Hypertension 2003; 41(5): 1096-101.

202. Wang D, Strandgaard S, Iversen J, Wilcox CS. Asymmetric dimethylarginine, oxidative stress, and vascular nitric oxide synthase in essential hypertension. Am J Physiol Regul Integr Comp Physiol 2009; 296(2): R195-200.

203. Abdilla N, Tormo MC, Fabia MJ, Chaves FJ, Saez G, Redon J. Impact of the components of metabolic syndrome on oxidative stress and enzymatic antioxidant activity in essential hypertension. J Hum Hypertens 2007; 21(1): 68-75.

204. Chen HJ, Pan WH. Probable blind spot in the International Diabetes Federation definition of metabolic syndrome. Obesity (Silver Spring) 2007; 15(5): 1096-100.

205. Monami M, Marchionni N, Masotti G, Mannucci E. IDF and ATP-III definitions of metabolic syndrome in the prediction of all-cause mortality in type 2 diabetic patients. Diabetes Obes Metab 2007; 9(3): 350-3.

206. Sanchez R, Fischer P, Cuniberti L, Masnatta LD, Ramírez AJ. Vascular oxidative stress is associated with insulin resistance in hyper-reninemic nonmodulating essential hypertension. J Hypertens 2007; 25(12): 2434-40.

207. Cottone S, Mulè G, Nardi E, Vadalà A, Lorito MC, Guarneri M, et al. C-reactive protein and intercellular adhesion molecule-1 are stronger predictors of oxidant stress than blood pressure in established hypertension. J Hypertens 2007; 25(2): 423-8.

208. Somers MJ, Mavromatis K, Galis ZS, Harrison DG. Vascular superoxide production and vasomotor function in hypertension induced by deoxycorticosterone acetate-salt. Circulation 2000; 101(14): 1722-8.

209. Landmesser U, Cai H, Dikalov S, McCann L, Hwang J, Jo H, et al. Role of p47(phox) in vascular oxidative stress and hypertension caused by angiotensin II. Hypertension 2002; 40(4): 511-5.

210. Heitzer T, Wenzel U, Hink U, Krollner D, Skatchkov M, Stahl RA, et al. Increased NAD(P)H oxidase-mediated superoxide production in renovascular hypertension: evidence for an involvement of protein kinase C. Kidney Int 1999; 55(1): 252-60.

211. Rajagopalan S, Kurz S, Münzel T, Tarpey M, Freeman BA, Griendling KK, et al. Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone. J Clin Invest 1996; 97(8): 1916-23.

212. World Medical Association General Assembly. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. J Int Bioethique 2004; 15(1): 124-9.

213. Marketou ME, Zacharis EA, Nikitovic D, Ganotakis ES, Parthenakis FI, Maliaraki N, et al. Early effects of simvastatin versus atorvastatin on oxidative stress and proinflammatory cytokines in hyperlipidemic subjects. Angiology 2006; 57(2): 211-8.

214. Shishehbor MH, Brennan ML, Aviles RJ, Fu X, Penn MS, Sprecher DL, et al. Statins promote potent systemic antioxidant effects through specific inflammatory pathways. Circulation 2003; 108(4): 426-31.

215. Kebapci N, Efe B, Akyuz F, Sunal E, Demirustu C. Oxidative Stress & Antioxidant Therapy in Type 2 Diabetes Mellitus. Turkish Journal of Endocrinology & Metabolism 1999; 4: 153-62.

216. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen accepter. Ann Clin Biochem 1969; 6: 24-7.

217. Starr JI, Mako ME, Juhn D, Rubenstein AH. Measurement of serum proinsulin-like material: cross-reactivity of porcine and human proinsulin in the insulin radioimmunoassay. J Lab Clin Med 1978; 91(4): 683-92.

218. Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem 1974; 20(4): 470-5.

219. Grove TH. Effect of reagent pH on determination of high-density lipoprotein cholesterol by precipitation with sodium phosphotungstate-magnesium. Clin Chem 1979; 25(4): 560-4.

220. Fukuyama N, Homma K, Wakana N, Kudo K, Suyama A, Ohazama H, et al. Validation of the Friedewald Equation for Evaluation of Plasma LDL-Cholesterol. J Clin Biochem Nutr 2008; 43(1): 1-5.

221. Fossati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem 1982; 28(10): 2077-80.

222. Kasai H. Analysis of a form of oxidative DNA damage, 8-hydroxy-2'-deoxyguanosine, as a marker of cellular oxidative stress during carcinogenesis. Mutat Res 1997; 387(3): 147-63.

223. Yagi K. Simple assay for the level of total lipid peroxides in serum or plasma. Methods Mol Biol 1998;108:101-6.

224. Ukeda H, Maeda S, Ishii T, Sawamura M. Spectrophotometric assay for superoxide dismutase based on tetrazolium salt 3'--1--(phenylamino)-carbonyl--3, 4-tetrazolium]-bis(4-methoxy-6-nitro)benzenesulfonic acid hydrate reduction by xanthine-xanthine oxidase. Anal Biochem 1997; 251(2): 206-9.

225. Galassetti P. Inflammation and oxidative stress in obesity, metabolic syndrome, and diabetes. Exp Diabetes Res 2012; 2012:943706.

226. Hutcheson R, Rocic P. The metabolic syndrome, oxidative stress, environment, and cardiovascular disease: the great exploration. Exp Diabetes Res 2012; 2012: 271028-40.

227. Chung SW, Kang SG, Rho JS, Kim HN, Song IS, Lee YA, et al. The Association between Oxidative Stress and Metabolic Syndrome in Adults. Korean J Fam Med 2013; 34(6): 420-8.

228. Youn JY, Siu KL, Lob H, Itani H, Harrison DG, Cai H. Role of Vascular Oxidative Stress in Obesity and Metabolic Syndrome. Diabetes 2014; "In press".

229. Bartlett HE, Eperjesi F. Nutritional supplementation for type 2 diabetes: a systematic review. Ophthalmic Physiol Opt 2008; 28(6): 503-23.

230. Rocha M, García-Bou R, Enguix N, Hernández-Mijares A, Esplugues JV, Vícto VM. Mitochondria-Targeted Vitamin E Antioxidant: An Agent for Cardiovascular Protection. Vascular Disease Prevention 2009; 6: 36-46.

231. Dröge W. Free radicals in the physiological control of cell function. Physiol Rev 2002; 82(1): 47-95.

232. Kowaltowski AJ, Vercesi AE. Mitochondrial damage induced by conditions of oxidative stress. Free Radic Biol Med 1999; 26(3-4): 463-71.

233. Brown GC. Control of respiration and ATP synthesis in mammalian mitochondria and cells. Biochem J 1992; 284(Pt1): 1-13.

234. Stone JR, Yang S. Hydrogen peroxide: a signaling messenger. Antioxid Redox Signal 2006; 8(3-4): 243-70.

235. Brea D, Roquer J, Serena J, Segura T, Castillo J. Oxidative stress markers are associated to vascular recurrence in non-cardioembolic stroke patients non-treated with statins. BMC Neurol 2012; 12: 65.

236. Anderson EJ, Lustig ME, Boyle KE, Woodlief TL, Kane DA, Lin CT, et al. Mitochondrial H_2O_2 emission and cellular redox state link excess fat intake to insulin resistance in both rodents and humans. J Clin Invest 2009; 119(3): 573-81.

237. James AM, Collins Y, Logan A, Murphy MP. Mitochondrial oxidative stress and the metabolic syndrome. Trends Endocrinol Metab 2012; 23(9): 429-34.

238. Whaley-Connell A, McCullough PA, Sowers JR. The role of oxidative stress in the metabolic syndrome. Rev Cardiovasc Med 2011; 12(1): 21-9.

239. Henriksen EJ, Diamond-Stanic MK, Marchionne EM. Oxidative stress and the etiology of insulin resistance and type 2 diabetes. Free Radic Biol Med 2011; 51(5): 993-9.

240. Bonnard C, Durand A, Peyrol S, Chanseaume E, Chauvin MA, Morio B, et al. Mitochondrial dysfunction results from oxidative stress in the skeletal muscle of diet-induced insulin-resistant mice. J Clin Invest 2008; 118(2): 789-800.

241. Murphy MP. How mitochondria produce reactive oxygen species. Biochem J 2009; 417(1): 1-13.

242. Korshunov SS, Skulachev VP, Starkov AA. High protonic potential actuates a mechanism of production of reactive oxygen species in mitochondria. FEBS Lett 1997; 416(1): 15-8.

243. Liu SS. Cooperation of a "reactive oxygen cycle" with the Q cycle and the proton cycle in the respiratory chain--superoxide generating and cycling mechanisms in mitochondria. J Bioenerg Biomembr 1999; 31(4): 367-76.

244. Liu SS. Generating, partitioning, targeting and functioning of superoxide in mitochondria. Biosci Rep 1997; 17(3): 259-72.

245. Lee I, Bender E, Kadenbach B. Control of mitochondrial membrane potential and ROS formation by reversible phosphorylation of cytochrome c oxidase. Mol Cell Biochem 2002; 234-235(1-2): 63-70.

246. Schafer FQ, Buettner GR. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radic Biol Med 2001; 30(11): 1191-212.

247. Domenicali M, Vendemiale G, Serviddio G, Grattagliano I, Pertosa AM, Nardo B, et al. Oxidative injury in rat fatty liver exposed to ischemia-reperfusion is modulated by nutritional status. Dig Liver Dis 2005; 37(9): 689-97.

248. Grattagliano I, Palmieri VO, Portincasa P, Moschetta A, Palasciano G. Oxidative stress-induced risk factors associated with the metabolic syndrome: a unifying hypothesis. J Nutr Biochem 2008; 19(8): 491-504.

249. Davì G, Ciabattoni G, Consoli A, Mezzetti A, Falco A, Santarone S, et al. In vivo formation of 8-iso-prostaglandin f2alpha and platelet activation in diabetes mellitus: effects of improved metabolic control and vitamin E supplementation. Circulation 1999; 99(2): 224-9.

250. Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, et al. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. Nature 2000; 404(6779): 787-90.

251. Bonnefont-Rousselot D. Glucose and reactive oxygen species. Curr Opin Clin Nutr Metab Care 2002; 5(5): 561-8.

252. Donohoe CL, Doyle SL, Reynolds JV. Visceral adiposity, insulin resistance and cancer risk. Diabetol Metab Syndr 2011; 3: 12.

253. Jensen MD. Role of body fat distribution and the metabolic complications of obesity. J Clin Endocrinol Metab 2008; 93(11 Suppl 1): S57-63.

254. Després JP. Body fat distribution and risk of cardiovascular disease: an update. Circulation 2012; 126(10): 1301-13.

255. Van Kruijsdijk RC, van der Wall E, Visseren FL. Obesity and cancer: the role of dysfunctional adipose tissue. Cancer Epidemiol Biomarkers Prev 2009; 18(10): 2569-78.

256. Hardie DG. AMPK: a key regulator of energy balance in the single cell and the whole organism. Int J Obes (Lond) 2008; 32 Suppl 4: S7-12.

257. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, et al. Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest 2001; 108(8): 1167-74.

258. Fryer LG, Parbu-Patel A, Carling D. The Anti-diabetic drugs rosiglitazone and metformin stimulate AMP-activated protein kinase through distinct signaling pathways. J Biol Chem 2002; 277(28): 25226-32.

259. Nabha L, Garbern JC, Buller CL, Charpie JR. Vascular oxidative stress precedes high blood pressure in spontaneously hypertensive rats. Clin Exp Hypertens 2005; 27(1): 71-82.

260. Reaven GM, Lithell H, Landsberg L. Hypertension and associated metabolic abnormalities--the role of insulin resistance and the sympathoadrenal system. N Engl J Med 1996; 334(6): 374-81.

261. Evans JL, Maddux BA, Goldfine ID. The molecular basis for oxidative stress-induced insulin resistance. Antioxid Redox Signal 2005; 7(7-8): 1040-52.

262. Schmitz-Peiffer C, Whitehead JP. IRS-1 regulation in health and disease. IUBMB Life 2003; 55(7): 367-74.

263. Lundgren M, Svensson M, Lindmark S, Renström F, Ruge T, Eriksson JW. Fat cell enlargement is an independent marker of insulin resistance and 'hyperleptinaemia'. Diabetologia 2007; 50(3): 625-33.

264. Fujita K, Nishizawa H, Funahashi T, Shimomura I, Shimabukuro M. Systemic oxidative stress is associated with visceral fat accumulation and the metabolic syndrome. Circ J 2006; 70(11): 1437-42.

265. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. Endocr Rev 2002; 23(5): 599-622.

266. Lowell BB, Shulman GI. Mitochondrial dysfunction and type 2 diabetes. Science 2005; 307(5708): 384-7.

267. Morino K, Petersen KF, Shulman GI. Molecular mechanisms of insulin resistance in humans and their potential links with mitochondrial dysfunction. Diabetes 2006; 55 Suppl 2: S9-15.

268. Mootha VK, Lindgren CM, Eriksson KF, Subramanian A, Sihag S, Lehar J, et al. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat Genet 2003; 34(3): 267-73.

269. Patti ME, Butte AJ, Crunkhorn S, Cusi K, Berria R, Kashyap S, et al. Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: Potential role of PGC1 and NRF1. Proc Natl Acad Sci USA 2003; 100(14): 8466-71.

270. Ritov VB, Menshikova EV, He J, Ferrell RE, Goodpaster BH, Kelley DE. Deficiency of subsarcolemmal mitochondria in obesity and type 2 diabetes. Diabetes 2005; 54(1): 8-14.

271. Boushel R, Gnaiger E, Schjerling P, Skovbro M, Kraunsøe R, Dela F. Patients with type 2 diabetes have normal mitochondrial function in skeletal muscle. Diabetologia 2007; 50(4): 790-6.

272. Mogensen M, Sahlin K, Fernström M, Glintborg D, Vind BF, Beck-Nielsen H, Højlund K. Mitochondrial respiration is decreased in skeletal muscle of patients with type 2 diabetes. Diabetes 2007; 56(6): 1592-9.

273. Grattagliano I, Wieland P, Schranz C, Lauterburg BH. Disposition of glutathione monoethyl ester in the rat: glutathione ester is a slow release form of extracellular glutathione. J Pharmacol Exp Ther 1995; 272(2): 484-8.

274. Day BJ. Catalytic antioxidants: a radical approach to new therapeutics. Drug Discov Today 2004; 9(13): 557-66.

275. Hadi NR, Abdelhussein MA, Alhamami OM, Rudha AR, Sabah E. Antioxidant Effect of Atorvastatin in Type 2 Diabetic Patients. Pharmacology & Pharmacy 2010; 1: 53-59

276. Olanlokun JO. Protective influence of vitamin E on the antioxidant defence system in the whole blood and liver of normal and alloxan-induced diabetic rats. Indian J Clin Biochem 2008; 23(1): 62-6.

277. Heeba G, Hassan MK, Khalifa M, Malinski T. Adverse balance of nitric oxide/peroxynitrite in the dysfunctional endothelium can be reversed by statins. J Cardiovasc Pharmacol 2007; 50(4): 391-8.

278. Liao JK, Laufs U. Pleiotropic effects of statins. Annu Rev Pharmacol Toxicol 2005; 45: 89-118.

279. John S, Schlaich M, Langenfeld M, Weihprecht H, Schmitz G, Weidinger G, et al. Increased bioavailability of nitric oxide after lipid-lowering therapy in hypercholesterolemic patients: a randomized, placebo-controlled, double-blind study. Circulation 1998; 98(3): 211-6.

280. Feron O, Dessy C, Desager JP, Balligand JL. Hydroxy-methylglutaryl-coenzyme A reductase inhibition promotes endothelial nitric oxide synthase activation through a decrease in caveolin abundance. Circulation 2001; 103(1): 113-8.

281. Landmesser U, Engberding N, Bahlmann FH, Schaefer A, Wiencke A, Heineke A, et al. Statin-induced improvement of endothelial progenitor cell mobilization, myocardial neovascularization, left ventricular function, and survival after experimental myocardial infarction requires endothelial nitric oxide synthase. Circulation 2004; 110(14): 1933-9.

282. Förstermann U, Sessa WC. Nitric oxide synthases: regulation and function. Eur Heart J 2012; 33(7): 829-37.

283. Wassmann S, Laufs U, Müller K, Konkol C, Ahlbory K, Bäumer AT, et al. Cellular antioxidant effects of atorvastatin in vitro and in vivo. Arterioscler Thromb Vasc Biol 2002; 22(2): 300-5.

284. Wagner AH, Köhler T, Rückschloss U, Just I, Hecker M. Improvement of nitric oxide-dependent vasodilatation by HMG-CoA reductase inhibitors through attenuation of endothelial superoxide anion formation. Arterioscler Thromb Vasc Biol 2000; 20(1): 61-9.

285. Nissen SE, Nicholls SJ, Sipahi I, Libby P, Raichlen JS, Ballantyne CM, et al. Effect of very high-intensity statin therapy on regression of coronary atherosclerosis: the ASTEROID trial. JAMA 2006; 295(13): 1556-65.

286. Patel TN, Shishehbor MH, Bhatt DL. A review of high-dose statin therapy: targeting cholesterol and inflammation in atherosclerosis. Eur Heart J 2007; 28(6): 664-72.

287. Wenzel P, Daiber A, Oelze M, Brandt M, Closs E, Xu J, et al. Mechanisms underlying recoupling of eNOS by HMG-CoA reductase inhibition in a rat model of streptozotocin-induced diabetes mellitus. Atherosclerosis 2008; 198(1): 65-76.

288. Wu JH, Ward NC, Indrawan AP, Almeida CA, Hodgson JM, Proudfoot JM, et al. Effects of alpha-tocopherol and mixed tocopherol supplementation on markers of oxidative stress and inflammation in type 2 diabetes. Clin Chem 2007; 53(3): 511-9.

289. Lagadu S, Lechevrel M, Sichel F, Breton J, Pottier D, Couderc R, et al. 8-oxo-7,8-dihydro-2'-deoxyguanosine as a biomarker of oxidative damage in oesophageal cancer patients: lack of association with antioxidant vitamins and polymorphism of hOGG1 and GST. J Exp Clin Cancer Res 2010; 29: 157.

290. Garelnabi M, Veledar E, White-Welkley J, Santanam N, Abramson J, Weintraub W, et al. Vitamin E differentially affects short term exercise induced changes in oxidative stress, lipids, and inflammatory markers. Nutr Metab Cardiovasc Dis 2012; 22(10): 907-13.

291. Thomas SR, Stocker R. Molecular action of vitamin E in lipoprotein oxidation: implications for atherosclerosis. Free Radic Biol Med 2000; 28(12): 1795-805.

292. Han SN, Adolfsson O, Lee CK, Prolla TA, Ordovas J, Meydani SN. Vitamin E and gene expression in immune cells. Ann N Y Acad Sci 2004; 1031: 96-101.

293. Scott JA, King GL. Oxidative stress and antioxidant treatment in diabetes. Ann N Y Acad Sci 2004; 1031: 204-13.

294. Singh U, Jialal I. Anti-inflammatory effects of alpha-tocopherol. Ann N Y Acad Sci 2004; 1031: 195-203.

295. Devaraj S, Leonard S, Traber MG, Jialal I. Gamma-tocopherol supplementation alone and in combination with alpha-tocopherol alters biomarkers of oxidative stress and inflammation in subjects with metabolic syndrome. Free Radic Biol Med 2008; 44(6): 1203-8.

296. Ward NC, Wu JH, Clarke MW, Puddey IB, Burke V, Croft KD, et al. The effect of vitamin E on blood pressure in individuals with type 2 diabetes: a randomized, double-blind, placebo-controlled trial. J Hypertens 2007; 25(1): 227-34.

APPENDIX

Table (I): Age (years), body mass index (kg/m^2), waist circumference (cm), waist-hip ratio (WHR), systolic and diastolic blood pressure (B.P) of normal control subjects.

Case number	Age (years)	BMI (kg/m^2)	WC (cm)	WHR	Systolic BP (mmHg)	Diastolic BP (mmHg)
1	40	24.51	81	0.85	120	80
2	45	22.66	93	0.86	130	80
3	44	24.15	86	0.868	120	75
4	40	25.56	89	0.89	110	70
5	43	24.49	93	0.86	120	80
6	46	25.10	96	0.9142	130	85
7	44	25.73	90	0.86	130	80
8	52	25.62	90	0.9	125	85
9	53	25.90	95	0.87	120	80
10	49	25.85	92	0.89	120	85
11	56	27.46	94	0.903	130	90
12	49	25.06	87	0.805	120	80
13	47	24.86	87	0.887	130	85
14	47	23.74	86	0.886	120	80
15	49	23.84	85	0.885	135	80
16	48	26.37	93	0.89	120	80
17	43	25.85	89	0.88	120	80
18	46	24.30	88	0.846	110	75
19	55	24.15	85	0.876	120	85
20	49	25.28	92	0.8679	110	70
Range	40-56	22.66-27.46	81-96	0.81-0.91	110-135	70-90
Mean ±SD	47.25 ±4.45	25.02 ±1.09	89.55±3.94	0.87 ±0.02	122 ±7.14	80.25 ±4.99

Table (II): Age (years), body mass index (kg/m^2), waist circumference (cm), waist-hip ratio (WHR), systolic and diastolic blood pressure (B.P) of metabolic syndrome patients.

Case number	Age (years)	BMI (kg/m^2)	WC (cm)	WHR	Systolic BP (mmHg)	Diastolic BP (mmHg)
1	57	45.87	127	1.09	170	110
2	56	44.46	124	1.04	160	105
3	49	43.07	122	1.04	150	105
4	49	41.53	122	1.15	150	105
5	49	41.45	121	1.13	140	100
6	54	40.79	117	1.08	140	100
7	47	40.70	121	1.02	140	100
8	43	40.30	119	1.03	140	100
9	45	39.91	115	1.19	140	95
10	44	39.41	119	1.11	135	95
11	43	38.39	121	1.16	135	95
12	43	38.20	117	1.08	135	95
13	47	38.01	119	1.07	130	95
14	41	35.19	114	1.06	130	95
15	40	31.74	107	1.13	130	80
16	56	48.90	130	1.10	180	110
17	55	47.02	125	1.0869	165	110
18	52	45.33	123	1.05	145	105
19	49	43.25	122	1.119	140	100
20	49	42.30	120	1.13	140	100
21	48	41.82	113	1.0089	140	100
22	47	40.96	119	1.09	140	100
23	45	40.48	119	1.0258	140	100
24	45	39.91	118	1.216	140	100
25	44	38.62	116	1.08	140	95
26	43	38.27	115	1.1057	140	95
27	43	37.11	114	1.055	140	95
28	42	35.26	120	1.08	135	95
29	41	33.13	111	1.0277	135	95
30	40	32.69	108	1.1739	130	90
Range	40–57	31.74-48.9	107-130	1.01-1.22	130-180	80-110
Mean ±SD	46.87 ±5	40.14±4.09	118.6±5.13	1.09 ±0.05	142.5±11.87	98.83±6.25

Table (III): Systolic and diastolic blood pressure (B.P) of metabolic syndrome subgroups (IIa)& (IIb) after treatment with either atorvastatin or (atorvastatin + vitamin E).

Case number	MS patients subgroup (IIa)		MS patients subgroup (IIb)	
	Systolic B.P	Diastolic B.P	Systolic B.P	Diastolic B.P
1	170	105	170	110
2	160	105	170	110
3	145	110	140	100
4	155	105	135	100
5	140	100	130	105
6	140	100	135	90
7	150	100	140	100
8	140	100	140	95
9	135	95	140	110
10	135	90	140	100
11	140	95	140	90
12	130	90	145	95
13	130	95	140	100
14	120	90	130	90
15	120	90	120	85
Range	120 – 170	90 – 110	120 – 170	85 – 110
Mean ±SD	140.66 ±13.87	98 ±6.49	141 ±13.25	98.66 ±7.89

Table (IV): Fasting serum glucose (mg/dL), serum insulin (µIU/mL) and homeostasis model assessment-insulin resistance (HOMA-IR) of normal control subjects.

Case number	Fasting glucose (mg/dL)	Serum insulin (µIU/mL)	HOMA-IR
1	95	9	2.11
2	84	8.5	1.76
3	88	9	1.96
4	93	7	1.61
5	85	7.5	1.57
6	82	10	2.02
7	87	8	1.72
8	86	9.2	1.95
9	100	9.8	2.42
10	81	6	1.20
11	79	9.6	1.87
12	84	5	1.04
13	92	8	1.82
14	97	7.4	1.77
15	75	4.4	0.81
16	83	7	1.43
17	86	9	1.91
18	96	7.4	1.75
19	91	8.6	1.93
20	90	9	2.00
Range	75 – 100	4.4 – 10	0.81 – 2.42
Mean ±SD	87.7 ±6.48	7.97 ±1.53	1.73 ±0.378

Table (V): Fasting serum glucose (mg/dL), serum insulin (µIU/mL) and homeostasis model assessment-insulin resistance (HOMA-IR) of metabolic syndrome subgroup (IIa) before and after treatment with atorvastatin.

Case number	Fasting glucose (mg/dL)		Serum insulin (µIU/mL)		HOMA-IR	
	Before treat.	After treat.	Before treat.	After treat.	Before treat.	After treat.
1	240	245	12	11.8	7.11	7.11
2	200	190	14	14.3	6.91	6.7
3	143	152	17	15	6	5.63
4	140	145	17	16.6	5.88	5.94
5	129	138	19	18.6	6.05	6.34
6	128	129	19	22	6	7.01
7	120	114	20	19.6	5.93	5.52
8	118	123	20	23	5.83	6.99
9	114	124	22	23	6.19	7.04
10	113	106	14	21.5	3.91	5.63
11	110	120	24	19	6.52	5.63
12	109	99	24	23.5	6.46	5.74
13	107	101	26	31	6.87	7.73
14	105	110	27	26.5	7	7.2
15	94	97	29	30.5	6.73	7.3
Range	94 – 240	97 – 245	12 – 29	11.8 – 31	3.91 – 7.11	5.52 – 7.73
Mean ±SD	131.33±39.03	132.86±39.44	20.26±5.04	21.06±5.55	6.22±0.78	6.5±0.75

Table (VI): Fasting serum glucose (mg/dL), serum insulin (µIU/mL) and homeostasis model assessment-insulin resistance (HOMA-IR) of metabolic syndrome subgroup (IIb) before and after treatment with atorvastatin + vitamin E.

Case number	Fasting glucose (mg/dL)		Serum insulin (µIU/mL)		HOMA-IR	
	Before treat.	After treat.	Before treat.	After treat.	Before treat.	After treat.
1	160	179	14	16	5.53	7.07
2	150	130	17	17.4	6.3	5.59
3	140	132	18	20	6.22	6.52
4	130	120	19	19.5	6.1	5.76
5	126	133	20	19.6	6.22	6.44
6	121	119	20	20.5	5.98	6.02
7	117	110	21	19.8	6.07	5.38
8	115	109	22	22.5	6.25	6.06
9	113	109	23	23.6	6.42	6.35
10	110	112	17	22.4	4.62	6.21
11	109	102	24	25	6.46	6.3
12	108	103	25	22	6.67	5.6
13	104	119	27	28.6	6.93	8.4
14	95	99	29	28.4	6.8	6.94
15	88	85	31	30	6.74	6.3
Range	88 – 160	85 – 179	14 – 31	16 – 30	4.62 – 6.93	5.38 – 8.4
Mean ±SD	119.06 ±19.59	117.4 ±21.51	21.8 ±4.75	22.35 ±4.13	6.22 ±0.57	6.32 ±0.74

Table (VII): High-density lipoprotein (HDL mg/dL), triglycerides (TG mg/dL), total cholesterol (mg/dL) and low-density lipoprotein (LDL mg/dL) of normal control subjects.

Case number	HDL (mg/dL)	Triglycerides (mg/dL)	T.Cholest (mg/dL)	LDL (mg/dL)
1	40	142	160	91.6
2	49	129	174	99.2
3	65	137	166	73.6
4	61	121	153	67.8
5	52	149	135	53.2
6	36	143	185	120.4
7	60	139	149	61.2
8	44	134	178	107.2
9	39	133	163	97.4
10	44	136	144	72.8
11	48	140	175	99
12	59	135	139	53
13	50	139	145	67.2
14	55	129	159	78.2
15	47	144	164	88.2
16	52	141	155	74.8
17	56	130	190	108
18	62	146	182	90.8
19	54	137	141	59.6
20	47	126	162	89.8
Range	36 – 65	121 – 149	135 – 190	53 – 120.4
Mean ±SD	51 ±8.1	136.5 ±7.05	160.95 ±16.06	82.65 ±19.35

Table (VIII): High-density lipoprotein (HDL mg/dL), triglycerides (TG mg/dL), total cholesterol (mg/dL) and low-density lipoprotein (LDL mg/dL) of metabolic syndrome subgroup (IIa) before treatment with atorvastatin.

Case number	HDL (mg/dL)	Triglycerides (mg/dL)	T.Cholest (mg/dL)	LDL (mg/dL)
1	24	490	333	211
2	30	330	295	199
3	32	420	310	194
4	35	259	290	203.2
5	37	280	300	207
6	36	310	305	207
7	36	250	290	204
8	30	220	260	186
9	30	210	270	198
10	31	230	260	183
11	32	190	250	180
12	33	230	260	181
13	34	210	235	159
14	39	180	240	165
15	46	168	240	160.4
Range	24 – 46	168 – 490	235 – 333	159 – 211
Mean ±SD	33.66 ±5	265.13 ±90.3	275.86 ±29.67	189.17 ±17.41

Table (IX): High-density lipoprotein (HDL mg/dL), triglycerides (TG mg/dL), total cholesterol (mg/dL) and low-density lipoprotein (LDL mg/dL) of metabolic syndrome subgroup (IIa) after treatment with atorvastatin.

Case number	HDL (mg/dL)	Triglycerides (mg/dL)	T.Cholest (mg/dL)	LDL (mg/dL)
1	26	390	233	129.02
2	31	284	207	118.39
3	33	361	217	111.32
4	37	223	203	121.877
5	41	188	198	119.04
6	38	267	214	122.56
7	38	215	203	122.38
8	34	147	172	108.52
9	31	181	189	121.53
10	35	154	172	106.06
11	33	163	175	108.88
12	37	154	172	103.82
13	37	165	163	93.1
14	41	110	161	98.245
15	48	144	168	91.034
Range	26 – 48	110 – 390	161 – 233	91 – 129.02
Mean ±SD	36 ±5.19	209.73 ±82.06	189.8 ±22.51	111.71 ±11.64

Table (X): High-density lipoprotein (HDL mg/dL), triglycerides (TG mg/dL), total cholesterol (mg/dL) and low-density lipoprotein (LDL mg/dL) of metabolic syndrome subgroup (IIb) before treatment with atorvastatin + vitamin E.

Case number	HDL (mg/dL)	Triglycerides (mg/dL)	T.Cholest (mg/dL)	LDL (mg/dL)
1	29	250	280	201
2	30	408	320	208.4
3	33	270	300	213
4	31	230	280	203
5	28	220	285	213
6	28	350	326	228
7	35	300	295	200
8	30	260	280	198
9	30	200	230	160
10	32	210	265	191
11	33	215	240	164
12	34	230	240	160
13	35	200	240	165
14	39	155	260	190
15	44	150	240	166
Range	28 – 44	150 – 408	230 – 326	160 – 228
Mean ±SD	32.73 ±4.33	243.2 ±68.56	272.06 ±30.41	190.69 ±22.3

Table (XI): High-density lipoprotein (HDL mg/dL), triglycerides (TG mg/dL), total cholesterol (mg/dL) and low-density lipoprotein (LDL mg/dL) of metabolic syndrome subgroup (IIb) after treatment with atorvastatin + vitamin E.

Case number	HDL (mg/dL)	Triglycerides (mg/dL)	T.Cholest (mg/dL)	LDL (mg/dL)
1	35.67	175	173.6	102.93
2	36.9	285.6	198.4	104.38
3	40.59	189	186	107.61
4	38.13	161	173.6	103.27
5	34.44	154	176.7	111.46
6	34.44	245	202.12	118.68
7	39.2	201	194.7	115.3
8	36.9	182	173.6	100.3
9	33.6	134	151.8	91.4
10	39.36	147	164.3	95.54
11	40.59	150.5	148.8	78.11
12	41.82	161	148.8	74.78
13	41.6	140	148.8	79.2
14	47.97	108.5	161.2	91.53
15	45.98	129	168	96.22
Range	33.6 – 47.97	108.5 – 285.6	148.8 – 202.12	74.78 – 118.68
Mean ±SD	39.14 ±4.14	170.84 ±45.86	171.36 ±18.03	98.04 ±13.31

Table (XII): Serum 8-hydroxy-2'-deoxyguanosine (8-OHdG ng/mL), malondialdehyde (MDA nmol/mL) and superoxide dismutase (SOD unit/mL) of normal control subjects.

Case number	8-OHdG (ng/mL)	MDA (nmol/mL)	SOD (U/mL)
1	7.7	2	13
2	5.8	2.4	11.1
3	5.4	3	11
4	5.5	2.9	10.9
5	5	3.3	10.6
6	7.5	4.5	7.9
7	5.6	3.5	9.9
8	6.3	4.2	8
9	7.2	4.1	8.3
10	5.3	2.6	9.4
11	6.5	3.9	8.5
12	4.9	3.1	9.2
13	4.8	3.3	9
14	4.7	3.3	8.8
15	4.5	2.9	8.7
16	4.3	2.4	8.6
17	4	3.4	9.3
18	3.1	6	9.6
19	4.1	2.5	9.8
20	1.8	2	10.4
Range	1.8 – 7.7	2 – 6	7.9 – 13
Mean ±SD	5.2 ±1.44	3.26 ±0.94	9.6 ±1.27

Table (XIII): Serum 8-hydroxy-2'-deoxyguanosine (8-OHdG ng/mL), malondialdehyde (MDA nmol/mL) and superoxide dismutase (SOD unit/mL) of metabolic syndrome subgroup (IIa) before and after treatment with atorvastatin.

Case number	8-OHdG (ng/mL)		MDA (nmol/mL)		SOD (U/mL)	
	Before treat.	After treat.	Before treat.	After treat.	Before treat.	After treat.
1	19.4	13.8	12.0	8.4	5.0	6.1
2	18.6	13.3	11.9	7.6	5.0	6.1
3	18.4	12.0	11.3	7.9	5.1	6.3
4	16.9	12.1	11.2	7.8	5.4	6.6
5	15.4	11.0	11.2	7.6	5.6	6.8
6	13.7	9.8	11.2	6.7	5.6	6.8
7	13.2	9.1	11.0	7.7	5.7	7.0
8	13.0	9.3	11.0	6.7	5.7	7.0
9	12.3	8.8	10.9	7.6	5.8	7.1
10	11.8	7.7	10.6	6.5	6.0	7.3
11	11.0	7.9	10.5	7.4	6.1	7.5
12	9.8	7.0	10.2	7.1	6.1	7.5
13	6.4	4.1	10.0	7.0	6.1	7.5
14	5.6	4.0	8.3	5.8	6.6	8.0
15	4.4	4.6	7.7	5.4	6.9	8.4
Range	4.4 – 19.4	3.2 – 13.8	7.7 – 12	5.4 – 8.4	5 – 6.9	6.1 – 8.4
Mean ±SD	12.66 ±4.7	8.87 ±3.31	10.6 ±1.19	7.14 ±0.81	5.78 ±0.54	7.06 ±0.65

Table (XIV): Serum 8-hydroxy-2'-deoxyguanosine (8-OHdG ng/mL), malondialdehyde (MDA nmol/mL) and superoxide dismutase (SOD unit/mL) of metabolic syndrome subgroup (IIb) before and after treatment with atorvastatin + vitamin E.

Case number	8-OHdG (ng/mL)		MDA (nmol/mL)		SOD (U/mL)	
	Before treat.	After treat.	Before treat.	After treat.	Before treat.	After treat.
1	18.87	12.27	12	7.32	4.898	6.24
2	18.38	11.95	11.9	7.26	5.084	6.27
3	17.65	11.37	11.6	7.08	5.27	6.47
4	15.93	10.36	11.3	6.89	5.332	6.54
5	14.22	10.15	11.2	6.40	5.456	6.92
6	13.48	8.76	11	5.94	5.58	6.98
7	13.24	6.35	11	5.97	5.642	7.14
8	12.26	7.97	10.5	5.94	5.766	7.38
9	12.01	7.81	10.5	6.05	5.952	7.46
10	11.52	5.53	10	5.67	6.076	7.62
11	10.54	6.85	10	5.49	6.448	7.91
12	7.60	3.65	9.9	5.42	6.51	8.32
13	6.13	3.98	9.8	5.40	6.758	8.33
14	4.90	3.19	9	5.40	6.82	8.65
15	4.17	4.5	8.9	5.34	8.06	10.32
Range	4.17 – 18.87	2 – 12.27	8.9 – 12	5.34 – 7.32	4.9 – 8.06	6.24 – 10.32
Mean ±SD	12.06 ±4.71	7.47 ±3.34	10.57 ±0.96	6.1 ±0.71	5.97 ±0.829	7.5 ±1.09

التلف التأكسدي للحمض النووي (دي إن إيه) عند المرضى بمتلازمة الأيض

Oxidative DNA damage in patients with metabolic syndrome

خطة بحث مقدمة إلى
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BACKGROUND

It is estimated that around a quarter of the world's adult population have metabolic syndrome[1] and they are twice as likely to die from and three times as likely to have a heart attack or stroke compared with people without the syndrome.[2]

A person is defined as having the metabolic syndrome if he has central obesity (waist circumference≥94cm for European men and≥80 cm for European women) with ethnicity specific values for other groups plus two of the following four factors:

a) Raised triglycerides level: ≥150mg/dL (1.7mmol/L) or specific treatment for this lipid abnormality.
b) Reduced high density lipoprotein cholesterol<40mg/dL (1.03mmol/L) in males and <50mg/dL (1.29mmol/L) in females or specific treatment for this lipid abnormality.
c) Raised blood pressure: systolic blood pressure≥130 or diastolic blood pressure≥85mmHg or treatment of previously diagnosed hypertension.
d) Raised fasting plasma glucose≥100mg/dL (5.6mmol/L) or previously diagnosed type II diabetes. If above 5.6mmol/L or 100mg/dL, oral glucose tolerance test is strongly recommended but is not necessary to define the presence of this syndrome.[3]

Hypertriglyceridemia, hyperglycemia, and insulin resistance are closely linked to elevated systemic oxidative stress in metabolic syndrome, which is, in turn, reflected in the impairment of HDL antioxidative properties. Dysfunctional HDL subfractions play a central role in the expression of elevated oxidative stress in metabolic syndrome. Because oxidative stress is a key component of endothelial dysfunction, and thus of elevated cardiovascular risk in metabolic syndrome, these results lead us to propose that early treatment of metabolic

syndrome patients, targeted at reduction of oxidative stress and normalization of HDL function, is necessary, even in the absence of diabetes.[4]

Lipid peroxidation is frequently investigated in biomedical research, and malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acids peroxidation in the cells. [5]

The transfer of a single electron to O_2 forms superoxide anion, whereas the transfer of two electrons yields peroxide. Superoxide Dismutase (SOD) catalyzes the conversion of superoxide anion (O_2^-.) to hydrogen peroxide (H_2O_2) and oxygen (O_2), and provides the primary cellular defense against the toxicity of superoxide anion.[6]

The free radicals activity is evaluated by measuring their pathophysiologic products.[7] DNA is probably the most biologically significant target of oxidative attack, and it is widely thought that continuous oxidative damage to DNA is a significant contributor to the age-related development of the major cancers, such as those of the colon, breast, rectum, and prostate.[8]

Recently, it was found that reactive oxygen species, irrespective of their origin, may interact with cellular biomolecules, such as DNA, leading to modification and potentially serious consequences for the cell.[9]

Deoxyguanosine (dG) is one of the constituents of DNA and when it is oxidized, it is altered into 8-hydroxy-2'-deoxyguanosine (8-OH-dG).[10] (8-OH-dG) is a quantitative measurement of the oxidative DNA adducts in various biological samples such as DNA serum, urine etc. Numerous evidences have indicated that urinary (8-OH-dG) not only is a biomarker of DNA damage but might also be a risk factor for cancer, atherosclerosis and diabetes.[11]

During the repair of damaged DNA in vivo by exonucleases, the resulting 8-OH-dG is excreted without further metabolism, and can be measured as a biomarker of oxidative DNA damage.[12]

Statins (hydroxyl-B-methylglutaryl coenzyme-A reductase inhibitors) were developed as cholesterol-lowering agents and found to have antioxidative effects. Various studies have also demonstrated that statins decrease the frequency of coronary heart disease. The antioxidative effect exhibited by statins is considered to be one of the mechanisms behind the anti-atherogenic effect.[13]

Several large-scale clinical trials have assessed the efficacy of atorvastatin in the primary and secondary prevention of cardiovascular events in patients with diabetes mellitus and/or metabolic syndrome.[14]

AIM OF THE WORK

The objective of the present study is the measurement of the serum level of 8-hydroxydeoxyguanosine (8-OHdG), Malondialdehyde (MDA), which reflects the tissue lipid peroxidation level and Superoxide Dismutase (SOD), representing the activity of hepatic antioxidant enzymes, in patients with metabolic syndrome. The effect of Atorvastatin and Vitamin E on these parameters in different groups will be also studied.

SUBJECTS AND METHODS

This study will be performed on a total of 30 obese male patients who fulfilled the definition criteria of metabolic syndrome according to the International Diabetes Federation (IDF) definition, their ages range from 40-60 years.

Patients with metabolic syndrome will be assigned to two groups randomly. One group will be administered 10mg/day of atorvastatin, and the other group 10mg/day of atorvastatin plus 1000mg/day of vitamin E for 3 months.

A group of 20 age-matched healthy non-obese males will be used as a control group.

All patients and controls will be subjected to the following:

A) Complete medical history taking with special emphasis on: age, special habits (smoking, diet, exercise, medications,…), hypertension, diabetes mellitus, dyslipidemia, ischemic heart disease.

B) Thorough clinical examination with special emphasis on: blood pressure, height, weight, waist circumference, hip circumference.

Body mass index will be calculated:

Body mass index (BMI)= weight in Kilograms divided by the square of height in Meters; (obesity BMI>30).

Blood pressure will be measured in a duplicate by mean of a standard sphygmomanometer.

C) Laboratory analysis: blood samples will be collected after an overnight fasting (12-h), serum will be used for the following:

- Fasting and 2 hours post prandial blood glucose by colorimetric method.[15]

- Lipid profile by colorimetric method.[16,17,18,19]
- Fasting insulin concentration by ELISA.
- Estimation of serum (8-OHdG) as a marker of oxidative stress.
- Estimation of serum (MDA) as a marker of oxidative stress.
- Estimation of serum (SOD) as a marker of antioxidant liver enzymes.

The patients with metabolic syndrome will be subjected to all investigations twice, pre and post oral treatment with atorvastatin (group I), and with atorvastatin plus vitamin E (group II) for three months.

In this study, we will use atorvastatin tablets, once/day (Ator 10mg) (EPICO- 10th of Ramadan city, Egypt). Also we will use vitamin E capsules, once/day (E1000) (SEDICO- 6th of October city, Egypt).

Patients with metabolic syndrome will be selected from the outpatients' clinics. Healthy individuals will be recruited consecutively among physicians and co-workers, represent normal-weight, control subjects.

All subjects will give written informed consent to participate in this study, which will be approved by the local ethical committee; in accordance with the Declaration of Helsinki.

Estimation of serum (8-OHdG):[20]

This will be done by Cell Biolabs' Oxidative DNA Damage ELISA Kit. (USA). It is a competitive enzyme immunoassay developed for rapid detection and quantitation of 8-OHdG in urine, serum, or other cell or tissue DNA samples.

Estimation of fasting insulin concentration:[21]

By ELISA kit provided by (Biosource Europe S.A, Nivelles, Belgium).

Estimation of serum (MDA):[22,23]

This will be done by Cayman's TBARS Assay Kit.

Estimation of serum (SOD):[24]

By Cayman Chemical Superoxide Dismutase Assay Kit (USA), using activity method.

ANALYSIS OF RESULTS

The results obtained will be statistically evaluated by comparing them with the data obtained from the corresponding control groups. A t-test will be used for two group comparisons. ANOVA will be used for comparisons of multiple means. *P* values less than 0.05 will be considered significant.

REFERENCES

1- Dunstan DW, Zimmet PZ, Welborn TA. The rising prevalence of diabetes and impaired glucose tolerance. The Australian Diabetes, Obesity and Lifestyle Study. Diabetes Care 2002; 25: 829-34.

2- Isomaa B, Almgren P, Tuomi T. Cardiovascular morbidity and mortality associated with the metabolic syndrome. Diabetes Care 2001; 24(4): 683-9.

3- Susanne Moebus, Jens Ulrich Hanisch, Pamela Aidelsburger, Peter Bramlage, Jürgen Wasem, Karl-Heinz Jöckel. Impact of 4 different definitions used for the assessment of the prevalence of the Metabolic Syndrome in primary healthcare: The German Metabolic and Cardiovascular Risk Project (GEMCAS). Cardiovasc Diabetol. 2007; 6: 22.

4- Boris Hansel, Philippe Giral, Estelle Nobecourt, Sandrine Chantepie, Eric Bruckert, M. John Chapman, Anatol Kontush. Metabolic Syndrome Is Associated with Elevated Oxidative Stress and Dysfunctional Dense High-Density Lipoprotein Particles Displaying Impaired Antioxidative Activity. The Journal of Clinical Endocrinology & Metabolism. 2004; 89(10): 4963-71.

5- Balahan Makay, Ozer Makay, Cigdem Yenisey, Gokhan Icoz, Gokhan Ozgen, Erbil Unsal, Mahir Akyildiz, Enis Yetkin. The Interaction of Oxidative Stress Response with Cytokines in the Thyrotoxic Rat: Is There a Link? Mediators Inflammation. 2009; 2009: 391682-8.

6- Francesco Addabbo, Monica Montagnani, Michael Goligorsky S. Mitochondria and Reactive Oxygen Species. Hypertension. 2009; 53: 885-92.

7- Potepsk J, Tuszkiewicz Misztal E. Selected parameters of the oxidative-antioxidative balance in children with chronic severe bronchial asthma. Pneumonal-Alergo-Pol. 2001; 69(9-10): 553-63.

8- Barry Halliwell. Why and how should we measure oxidative DNA damage in nutritional studies? How far have we come? Am J Clin Nutr 2000; 72: 1082–7.

9- Charles L. Valon. New Developments in Mutation Research. Nova Biomedical 2007; 241-55.

10- Ann E. Aust, Jamie F. Eveleigh. Mechanisms of DNA oxidation. Proceedings of the Society for Experimental Biology and Medicine 1999; 222: 246-52.

11- Isabella Dalle-Donne, Ranieri Rossi, Roberto Colombo, Daniela Giustarini, Aldo Milzani. Biomarkers of Oxidative Damage in Human Disease. Clinical Chemistry 2006; 52(4): 601-23.

12- Patel PR, Bevan RJ, Mistry N, Lunec J. Evidence of oligonucleotides containing 8-hydroxy-2′-deoxyguanosine in human urine J. Free Radic Biol Med. 2007; 42: 552-8.

13- Miida T, Hirayama S, Nakamura Y. Cholesterol-independent effects of statins and new therapeutic targets: ischemic stroke and dementia. J Atheroscler Thromb. 2004; 11: 253-64.

14- Arca, Marcello. Atorvastatin Efficacy in the Prevention of Cardiovascular Events in Patients with Diabetes Mellitus and/or Metabolic Syndrome. Drugs 2007; 67(12): 43-54.

15- Giampietro O, Pilo A, Buzzigoli G, Boni C, Navalesi R. Four methods for glucose assay compared for various glucose concentrations and under different clinical conditions. Clin Chem. 1982; 28: 2405-7.

16- Kayamori Y. Endpoint Colorimetric Method for Assaying Total Cholesterol in Serum with Cholesterol Dehydrogenase. Clin. Chem. 1999; 45: 2158-63.

17- Grove T.H. Effect of reagent pH on Determination of HDL Cholesterol by precipitation with Sodium Phosphotungstate-magnesium. Clin Chem 1979; 25: 560.

18- McGowan M.W. A peroxidase-coupled method for the colorimetric determination of serum triglycerides. Clin. Chem. 1983; 29: 538.

19- Anirban Maitra, Shaina V. Hirany, Ishwarlal Jialal. Comparison of two assays for measuring LDL cholesterol. Clinical Chemistry 1997; 43(6): 1040–7.

20- Lily L.Wu, Chiuan-Chian Chiou, Pi-Yueh Chang, James T. Wu**.** Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics**.** Clinica Chimica Acta 2004; 339: 1-9.

21- Temple R.C., Clark P.M., Hales C.N. Measurement of insulin secretion in type2 diabetes: problems and pitfalls. Diabetic medicine. 1992; 9: 503-12.

22- Mark Hodges, John M. DeLong, Charles F. Forney, Robert K. Prange. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta 1999; 207(4): 604-11.

23- Yagi K. Simple assay for the level of total lipid peroxides in serum or plasma. Methods in Molecular Biology 1998; 108: 101-6.

24- Shruti S. Khanzode, Suchet D. Khanzode, Ganesh N. Dakhale. Serum and plasma concentration of oxidant and antioxidants in patients of Helicobacter pylori gastritis and its correlation with gastric cancer. Cancer Letters 2003; 195(1): 27-31.

الملخص العربي

قدرة الإنسان على استعمال الأكسجين في عملية التنفس أمدته بفائدة استعمال أيض المواد الغذائية (دهون- بروتينات- كربوهيدرات) في إنتاج الطاقة.

تتكون عملية التمثيل الغذائي للطاقة داخل الخلايا من العديد من مسارات التفاعلات الكيميائية، حيث تقع عمليتي التنفس الخلوي وتكوين جزيئات الطاقة واللتان تتمان في الميتوكندريا في صلب عملية الأيض.

عملية التنفس الخلوي في الميتوكندريا تتم بأكسدة مادة (NADH) الممولة من المواد الغذائية عن طريق النقل الإلكتروني، وبالتالي ضخ البروتون خارج غشاء الميتوكندريا، حيث يعمل إنزيم (ATP synthase) الموجود في غشاء الميتوكندريا على الربط بين عبور البروتون وتكوين مركب الطاقة (ATP) داخل جسم الميتوكندريا. وبذلك تكون الميتوكندريا قد حولت الطاقة الكيميائية المختزنة في المواد الغذائية إلى مركب الطاقة (ATP) ليستعملها الجسم.

وعلى الرغم من أهمية هذه العمليات الحيوية فإنه لاشئ يأتي من دون ثمن، فمركب الأكسجين (وهو المستقبل الأخير للإلكترون) يتم في النهاية إختزاله إلى ماء، ولكن جزء يسير حوالي ٥ % من الأكسجين قد لا يتم أكسدته بشكل كامل نتيجة هروب بعض الإلكترونات، مما قد ينتج عنه إختزال غير تام للأكسجين مكونا فوق الأكسيد ($O_2^{\cdot -}$) وبعض المؤكسدات التي تعمل كمؤثرات داخلية في نقل الإشارات بين الخلايا، بما فيها فوق أكسيد الهيدروجين (H_2O_2). حيث يعكس معدل فوق أكسيد الهيدروجين (H_2O_2) التوازن بين كل من التسرب الإلكتروني وتكوُّن فوق الأكسيد وإزالته بواسطة مضادات التأكسد الداخلية الموجودة داخل الميتوكندريا.

في الأحوال الوظيفية الطبيعية للأعضاء يوجد إتزان ما بين عملية تكوين المؤكسدات الداخلية وتكون الشقوق الحرة من جانب، وعملية التخلص منها عن طريق مضادات الأكسدة الداخلية من جانب آخر. ولكن في بعض حالات التغذية المفرطة وعدم استهلاك الطاقة (نتيجة عدم بذل نشاط) وهي الصورة النمطية للمرضى بمتلازمة الأيض، في هذه الحالة تنشأ عن زيادة المواد الغذائية زيادة في المكافئات المختزلة، مما يرفع حالة التأكسد ويزيد من وطأتها.

وقد اختص هذا البحث بدراسة وطأة التأكسد المصاحب للإفراط في تناول الغذاء مع عدم استهلاك الطاقة، مما يؤدي إلى الإصابة ببعض الظواهر المرضية عند المرضى بمتلازمة الأيض. وقد أظهرت النتائج زيادة واضحة في معدلات الأيض داخل الميتوكندريا، حيث ارتفع (8-OHdG) بنسبة تفوق المائة في المائة، وهو أحد الدلالات الهامة لوطأة التأكسد الناتج عن أكسدة وتلف الحمض النووي (DNA)، وكذلك ارتفع مؤشر أكسدة الدهون (المالون داي ألدهيد) (MDA) مقارنة بالمجموعة الضابطة.

كما تم رصد وجود سمنة في منطقة البطن (Waist Circumference)، ومقاومة الإنسولين (Insulin Resistance) مع ما يصاحبها من زيادة في تركيز الإنسولين. كذلك زيادة مستوى الدهون الثلاثية (TG)، والبروتين الدهني منخفض الكثافة (LDL)، وانخفاض البروتين الدهني مرتفع الكثافة (HDL)، وارتفاع ضغط الدم.

وقد حصلنا على ارتباط خطي يسير في نفس الاتجاه بين المتغيرات التالية: محيط البطن، ارتفاع ضغط الدم، ارتفاع السكر في الدم، مقاومة الإنسولين، وكذلك دلالات وطأة التأكسد. حيث تتناسب السمنة البطنية طرديا مع الزيادة في دلالات وطأة التأكسد. كما لوحظ وجود علاقة إيجابية بين مستوى السكر الصائم ووطأة التأكسد مما قد يشير إلى الدور الذي يلعبه ارتفاع السكر في زيادة أيض السكر داخل الخلايا، وبالتالي زيادة إنتاج أنواع المؤكسدات التفاعلية.

كما أشارت نتائج هذا البحث أيضا إلى أن زيادة دلالات وطأة التأكسد في الدم (MDA & 8-OHdG) يتناسب طرديا أيضا مع ارتفاع الدهون الثلاثية (TG)، والبروتين الدهني منخفض الكثافة (LDL)، وعكسيا مع البروتين الدهني مرتفع الكثافة (HDL). كذلك فإن الـ(MDA) (مؤشر أكسدة الدهون) يتناسب مع النقص في البروتين الدهني مرتفع الكثافة (HDL). وبذلك تكون المؤثرات المختلفة التي تصيب الإنسان في مستويات مختلفة نتيجة الخلل الناتج عن التكامل الجزيئي في الأيض بين الدهون والسكريات دافعا إلى حالة مقاومة الإنسولين. لذلك تلعب وطأة التأكسد العديد من الآليات القوية في الخلل الوظيفي للمرضى بمتلازمة الأيض.

وقد أظهرت النتائج وجود علاقة طردية بين دلالات وطأة التأكسد ومقاومة الإنسولين في المرضى المصابين بمتلازمة الأيض مما يشير إلى أن العوامل المتعددة التي تسبب مقاومة الإنسولين تشترك جميعها في زيادة تكوين أنواع المؤكسدات التفاعلية. ولذلك فإن هذا البحث يربط بين توازن التمثيل الغذائي داخل الخلايا والتحكم في حساسية الإنسولين.

كما قمنا برصد أسباب مقاومة الإنسولين الناجمة عن السمنة، والتي تعد مكوناً من متلازمة الأيض في سياق تفاعلات الطاقة الحيوية داخل الميتوكوندريا.

كما أوضحت العديد من الدراسات أن زيادة التأكسد بفعل زيادة التغذية مرتبط بالإنخفاض في القدرة الذاتية المضادة للأكسدة (Endogenous Antioxidant)، مما ينجم عنه وطأة التأكسد. لذلك اختص هذا البحث بدراسة السعة الداخلية لمضادات التأكسد داخل الميتوكندريا عند المرضى بمتلازمة الأيض عن طريق قياس أحد الإنزيمات المضادة للتأكسد وهو إنزيم السوبر أكسيد ديسميوتاز (SOD) حيث لوحظ حدوث انخفاض في هذا الإنزيم.

كما تم التركيز في الأعوام الماضية على بعض الوسائل التي تحفز التخلص من أنواع المؤكسدات التفاعلية إما باستعمال مضادات الأكسدة أو العقاقير التي تحفز دفاعات مضادات الأكسدة الذاتية. لذلك شمل هذا البحث أيضا على محاولة تحفيز التخلص من المؤكسدات التفاعلية في مجموعتين من المرضى بمتلازمة الأيض عن طريق استعمال عقار الأتورفاستاتين (Atorvastatin) في المجموعة الأولى وهو عقار خافض لدهون الدم. وكذلك الجمع بين عقار الأتورفاستاتين وفيتامين هـ المضاد للأكسدة في المجموعة الثانية، حيث تمت متابعة كلتا المجموعتين لمدة ثلاثة أشهر متتالية.

وقد أظهرت نتائجنا حدوث تحسن كمي في دلالات وطأة التأكسد (SOD, MDA, 8-OHdG) في كلتا المجموعتين. ومع ذلك فإن العلاج الثنائي في المجموعة الثانية (Atorvastatin + فيتامين هـ) لم يحدث تغيرا ملحوظا في مستوى (8-OHdG)، بينما نجح في تخفيض مستوى كل من ال(MDA) والبروتين الدهني منخفض الكثافة (LDL) عند مقارنته بالعلاج الأحادي (Atorvastatin) مما يعكس دور الفيتامينات المضادة للأكسدة في خفض وطأة تأكسد الدهون.

ولذلك تظهر بصورة واضحة أهمية العلاج المركب (من أكثر من نوع من مضادات الأكسدة) والذي يستهدف العديد من الآليات المتعددة في علاج مسببات الأمراض الخاصة بالخلل التمثيلي وإبطاء تطور مضاعفات وطأة التأكسد. حيث تفوق العلاج الثنائي باستعمال الستاتينز (Statins) مع الفيتامينات مضادات التأكسد في علاج ارتفاع وتأكسد الدهون عند مقارنته بالعلاج الأحادي في المرضى بمتلازمة الأيض نتيجة التأثيرات المحددة والمرتبطة لكل منهم. مما يفتح الباب في استعمال العلاجات المركبة في علاج وتأخير مضاعفات تصلب الشرايين، الناتجة عن ارتفاع الدهون وتلف الأوعية الدموية.

وقد أوضحت النتائج وجود تأثير فعال لعقار الـ (Atorvastatin) كمضاد للأكسدة وليس فقط لمعالجة زيادة دهون الدم، حيث نجح في تقليل وطأة التأكسد لدى المرضى بمتلازمة الأيض، عن طريق تقليل إنتاج أنواع المؤكسدات التفاعلية، مما أفاد في تقليل مؤشر أكسدة الحمض النووي (8-OHdG) وكذلك مؤشر أكسدة الدهون (MDA) إلى جانب خفض الكولسترول والدهون الثلاثية (TG) والبروتين الدهني منخفض الكثافة (LDL)، وزيادة البروتين الدهني مرتفع الكثافة (HDL).

ويكون من أهم نتائج هذا البحث ظهور التأثير الواضح لعقار الـ (Atorvastatin) في إبطاء تطور مضاعفات وطأة التأكسد، حيث أظهرت النتائج أن له دورا مضادا للأكسدة عن طريق تحسين معدلات الأيض داخل الميتوكندريا إلى جانب دوره في خفض الدهون في الدم. كما خلص هذا البحث إلى أن الخلل الوظيفي للميتوكندريا عند المرضى بمتلازمة الأيض والمؤدي إلى زيادة وطأة التأكسد (المثبتة بزيادة دلالات وطأة التأكسد (MDA& 8-OHdG)) سببه الإفراط في الطعام. لذلك نوصي بمزيد من الدراسة عن الخلل الوظيفي في معدلات الأيض داخل الميتوكندريا عند مرضى متلازمة الأيض مما قد يزيد من القدرة على فهمه بصورة أوضح والوصول إلى علاجات جديدة، للتمكن من إحداث التوازن بين تكوين المؤكسدات الداخلية وتكون الشقوق الحرة من جانب والتخلص منها من جانب آخر.

التلف التأكسدي للحمض النووي (دي إن إيه)
عند المرضى بمتلازمة الأيض

رسالة علمية

مقدمة لقسم الكيمياء الطبية التطبيقية

معهد البحوث الطبية - جامعة الإسكندرية
ايفاءا جزئيا لشروط الحصول على درجة

الدكتوراه

في

الكيمياء الطبية التطبيقية

من

عمر إبراهيم محمود الغمراوي
بكالوريوس علوم (شعبة علم حيوان)
كلية العلوم
جامعة القاهرة– ١٩٩٦م

ماجستير الكيمياء الطبية التطبيقية
معهد البحوث الطبية
جامعة الإسكندرية– ٢٠٠٧م

معهد البحوث الطبية
جامعة الإسكندرية

٢٠١٤م

التلف التأكسدي للحمض النووي (دي إن إيه)
عند المرضى بمتلازمة الأيض

رسالة علمية مقدمة من

عمر إبراهيم محمود الغمراوي

للحصول على درجة الدكتوراه

في

الكيمياء الطبية التطبيقية

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