Research Paper

Effect of Diet on Superoxide Dismutase Enzyme and Its Relation with Anthropometric Parameters

Dr. Nita Sahi
M.Sc. Medicine Biochemistry, PhD
Associate Professor, Department of Biochemistry
Pacific Medical College and Hospital
Bhillo Ka Bedla, Udaipur

Disha Sahi
Student- MBBS First Year
Pacific Medical College and Hospital
Bhillo Ka Bedla, Udaipur

Meenakshi Sharma
Student- MSc Medicine Biochemistry
Pacific Medical College and Hospital
Bhillo Ka Bedla, Udaipur

Address for Correspondence
Dr Nita Sahi
sahi_mukesh1973@yahoo.co.in

INTRODUCTION

Obesity is defined as a complicated, heritable, heterogeneous group of disorders. It develops as a result of complex interactions between polygenic multifactorial trait, environmental factors and behavior characterized by long term energy imbalance which can be due to excess consumption of calories, energy output can be insufficient, sedentary style of routine, low resting metabolic rate. Obesity is a disorder of body weight regulatory system characterized by an accumulation of excess energy in the form of body fat which impairs health. The hallmark of obesity is excess adipose tissue with a relatively absolute excess fat store.

Superoxide dismutase (SOD):

Superoxide dismutase (SOD) is an antioxidant metalloproteinase enzyme catalyzing the conversion of superoxide radical to hydrogen peroxide and molecular oxygen. This enzyme acts as a first line of defense to protect cells from the injurious effects of superoxide (Fridovich, 1975). [12]

SOD is a tetramer of approximately 80,000 molecular weight protein with each subunit being about 23 KD in size. There are three different types of SOD: The function of SOD seems to be that of protecting aerobic organisms against the potential deleterious effects of superoxide [2].

SOD has a major role in superoxide (O_2^-) anion radical metabolism. O_2^- is converted into hydrogen peroxide (H_2O_2) by SOD. [2].

SOD is a primary antioxidant.

$$O_2^- + O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2$$

Angelika *et al.* (2005) [2] showed that the mean erythrocyte CuZn-SOD activity in obese women (660±39 U/g Hb) were significantly lower than in the group of non-obese female (873±52 U/g Hb).

According to Duangkamol *et al.* (2000) [10], significantly lower SOD activity was seen in overweight and obese subjects as compared to control subjects (1613 U/g Hb & 2750 U/g Hb respectively in male and 1571 U/g Hb & 2528 U/g Hb in female.

Olusi *et al.* (2002)[18] reported an inverse relationship between body mass index, the enzyme. Statistically significant negative association ($r = -0.566$; $P < 0.005$) between erythrocyte CuZn-SOD and body mass index was observed, which suggest that in obesity there is a low activity of the enzyme.

REVIEW OF LITERATURE:

Obesity, Oxidative Stress and Antioxidant Enzymes:

Olusi et al (2002) [18] studied the relationship between obesity and the stress He finally concluded that obesity is the main cause behind systemic oxidative stress along with improper regulation of adipocytokines which is the main reason of metabolic syndrome.

Vincet et al (2006) [23] determined the red blood cells antioxidant enzymes in context to overweight. Reactive oxygen species (ROS) which can cause the oxidative stress can react with unsaturated lipids and also leads to chain reactions called as lipid peroxidation in the membrane, which results in oxidation of lipids which are more atherogenic, which can decrease half life of bio-molecules, protein functions can be deranged; loss of functions of membrane phospholipids can be there, some toxic products can be accumulated; oxidized LDL, MDA, malondialdehyde proteins can be made MDA- DNA adducts can be formed. Our body's defence system can protect the cells against oxidative damage. There are some enzymes that directly metabolize ROS like superoxide dismutase, glutathione peroxidase and catalase. Superoxide dismutase plays a very important part in the ROS metabolism by directly dismuting the superoxide anion radical to H2O2 that can be further scavenged by catalase in the cells. In obese people who have (BMI>30.0kg/m^2), there were significantly positive relationship between systolic blood pressure and WHR, and superoxide dismutase could be related to weight, body mass index as well as catalase while opposite observations were seen for age and superoxide dismutase .

Free radical and antioxidant status in urban and rural population and relation with obesity and body fat distribution was done by Reddy et al (1997) [22] and significant increases in plasma lipid peroxides and free radicals (superoxide anion and hydrogen peroxide), and DNA damage was indicated.

Dexter et al (2005) [9] found that there is a relation between lipid per oxidation, vitamin C and superoxide dismutase in obesity. He found a reduced level of antioxidant vitamin and superoxide dismutase was significantly low in overweight patients. This clearly proves that higher oxidant stress is associated with a reduced antioxidant status accompanying hypercholesterolemia and hypertriglyceridemia which can lead to atherosclerosis.

Beltowski et al (2000) [5] studied the effect of obesity which is mainly due to diet on peroxidation of lipids and antioxidant enzymes and stated that obesity is clearly a risk factor for atherosclerosis.

OBESITY AND DIETARY FIBER

Anderson et al (2003) [1] studied plant fiber in context with metabolism of carbohydrates and lipids. Plant fibers are ingested as parts of plant foods and are not digested by the human body. They are neglected by the people because they have no nutritional value. In the last decade, attention has been there on the plant fibers as they influence our gastrointestinal physiology. They reported that whole grains are protective against atherosclerotic CVD. Diets rich in whole grains decrease serum LDL-C and triglyceride levels and also blood pressure and increases serum HDL-C levels along with positively altering the antioxidant enzyme status. They also reported that dietary fiber prevents carbohydrate induced hypertriglyceridemia.

Fernandez et al (2001) [11] reported that by ingesting soluble fiber, a mean lowering of 9% in LDL-C can be achieved.

Slavin (2009) [19] studied the dietary fiber and body weight and found that dietary fiber intake prevents obesity, intake of fiber diet is inversely proportional to body weight and body fat, fiber intake is inversely related with BMI.

Jenkins et al (2001) [13] tested the effects of a high fiber diet on the disorder, and concluded that high fiber diet resulted in the largest reduction in LDL-C (33%+/-4%, $p<0.001$). They concluded that very high fiber intakes reduce risk factors for cardiovascular diseases. They also reported that viscous fibers are hypocholesterolemic and have been associated with higher HDL-C levels and reduced incidences of cardiovascular disease.

Liu et al (2002) [15] reported that the more is the ingestion of fiber diet, lower is the risk of cardiovascular diseases and myocardial infarction.

Newby et al (2003) [17] studied dietary patterns and changes in body mass index and waist circumference in adults. Consuming a diet high in fiber was associated with smaller gains in body mass index and waist circumference. Diet rich in whole and unrefined foods contain high concentration of fibers that may be protective against chronic diseases.

Antioxidants can counteract the reactive oxygen species before free radicals arise in the body from different sources. (Singh, 2009) [20]

AIMS AND OBJECTIVES

- To study classification of the subjects according to various anthropometric parameters.
- To study prevalence of Total, boys and girls according to age.
- To study antioxidant enzyme status in total subjects, boys and girls before and after fiber diet.
- To study anthropometric parameters in total subjects, boys and girls with statistical evaluation before and after fiber diet.
- To study average anthropometric characteristics in total subjects, boys and girls according to age.
- Study of antioxidant profile in relation to fiber diet.
- Relation between anthropometric parameters and fiber diet.

MATERIAL AND METHOD

The present study includes anthropometry and a clinical study with fiber diet in 100 students aged between 18 to 30 years to evaluate and establish the correlation between anthropometric parameters, antioxidant enzyme before and after fiber diet. The practical work was done in Department of Biochemistry, PMCH, Udaipur

SELECTION OF GIRLS FOR THE STUDY

This study was conducted on 100 students living in different institutional hostels from different states; they were randomly selected irrespective of their caste and creed. The subjects with any clinical evidence of liver, kidney or endocrine disease and those on treatment that would affect the metabolism of lipid were not included in the study.

Normal subjects of same age group with that of respective obese group acted as control.

(1) Skin fold thickness:

The instrument used was Harpenden skin fold caliper.

(2) Total Body Fat Percent:

Total body fat percent was calculated using the following formula: (Young men Christian association)[19,20,]

$$\text{Body Fat \%} = \frac{-76.76+4.15 \times \text{Waist} - 0.082 \times \text{Weight} \times 100}{\text{Weight}}$$

(3) Body Fat:

Body Fat: Multiply body weight (kg) with body fat percentage.

(4) Lean Body Mass (LBM):

Lean Body Mass (LBM): Subtract the body fat (kg) from total body weight [21].

Fiber Diet

Every individual in each group was told to replace the wheat chapatti by fiber diet for one month.

COLLECTION OF BLOOD SAMPLES

The venous blood samples were analyzed for different parameters before and after fiber diet. Haemolysate was prepared.

ANALYSIS OF BLOOD FOR SOD

Superoxide Dismutase (SOD) (Randox kit method) (Wollians et al, 1983) [24]

The role of superoxide dismutase is to increase the dismutation of the toxic superoxide radical (O^{2-}), which was formed by oxidative processes, and convert to hydrogen peroxide and molecular oxygen. This method uses xanthine and xanthine oxidase to generate superoxide radical which reacts with 2, 4 iodo phenyl 3.4 nitrophenyl 5 phenyl tetrazolium chlorides (I.N.T) to form a red formazen dye. The superoxide dismutase activity is then measured by the degree of inhibition of this reaction.

$$\text{Xanthine} \xrightarrow{XOD} \text{Uric acid} + O_2^-$$

$$\text{I.N.T.} \xrightarrow{O_2} \text{Formazen dye}$$

$$\text{Or } O_2^- + O_2^- + 2H+ + SOD \longrightarrow O_2 + H_2O_2$$

Calculations:

$$\frac{A2 - A1}{3} = \Delta A/\text{min of standard or sample}$$

All standard rates and diluted sample rates must be converted into percentages of the sample diluent rate and subtracted from 100% to give a % inhibition.

$$100 - \frac{(\Delta A \text{ Std/min} * 100)}{(\Delta S1/\text{min})} = \% \text{ inhibition}$$

$$100 - \frac{(\Delta A \text{ Sample/min} * 100)}{(\Delta S1/\text{min})} = \% \text{ inhibition}$$

Plot percent inhibition for each standard, against log10. Use % inhibition of sample to obtain units of SOD from standard curve.

SOD units/ml of whole blood = SOD units/ml from standard curve * dilution factor

Converting to SOD units/gm Hemoglobin = (SOD Units/ml) / (g Hemoglobin)

The activity of SOD expressed in enzyme unit. (EU)

Normal range: 1000-1600 U/gm Hb.

STATISTICALANALYSIS

1. Mean (X) = $\frac{\text{Sum of observations}}{\text{Total No. of cases}}$

2. Standard Deviation (S.D) = $\sqrt{\frac{\text{Sum +square of term-(mean)}^2}{n-1}}$
   n=number of obs.

3. Students "t" test:
   $T = \frac{\text{Mean 1-Mean 2}}{\text{SED}}$

4. $SED = \sqrt{((SE1 \times SE1) + (SE2 \times SE2)}$

Now P value was determined. P value if more than 0.05; it is not significant, if it was less than 0.05 then it is significant. SPSS software was used for statistical data.

DISCUSSION

The WHO has drawn attention to fact that obesity is our modern non communicable "epidemic" that is, disease that affects population not an unavoidable attribute of aging [11].

Our results were in agreement with those of Kuno et al [14] who found decreased levels in girls, Decri, [8] who found decreased levels in boys.

Vincet [23] concluded a high activity of SOD; the variation or discrepancy between our results and those of Vincet is because of obesity duration. In the early days of obesity, antioxidant enzyme activity could be increased. But if it persists for longer, the sources of antioxidant enzyme become less or exhausted, which can result in low level of enzyme activity which we found in our study.

All these reports are in accordance with the present study where antioxidant enzyme showed low acceptable ranges when matched against high body mass index, waist circumference, waist hip ratio, blood pressure, body fat percent and showed high acceptable values against low and normal weight category along with normal body mass index and waist hip ratio. There was a positive effect of the diet on the status of antioxidant enzyme in all the categories. SOD, showed increased levels in category of WHR after the diet was taken (1354.43 U/gmHb v/s 1357.25 U/gmHb;

All these results indicate that fiber helps control weight and related parameters like body mass index, waist hip ratio, body fat percent, which indirectly improves antioxidants status of

the individuals which is in accordance with our observations and results.

RESULTS

There was not much variation seen for the anthropometric parameters for age, inhabitance and socio economic status. Although waist circumference, total body fat percent and total body fat were higher in urban girls than rural girls.SOD improved with the diet although the change was not significant. The level of enzymatic antioxidants decreased with increasing age. SOD was 1329.64U/gmHb for age <20 and 1304.29U/gmHb for age >20;

SUMMARY AND CONCLUSION

We distributed the total subjects into two groups, boys and girls, to find out the prevalence according to different categories 52 were boys and 48 were girls, in the second part of the study they were examined for antioxidant status; the parameter selected to evaluate antioxidant profile parameters was superoxide dismutase

1. The level of enzymatic antioxidants decreased with increasing age. SOD was 1329.64U/gmHb for age <20 and 1304.29U/gmHb for age >20;
2. SOD showed decreasing trend with increasing TBF% (1365 U/gmHb v/s 1125.76 U/gmHb).
3. SOD improved with the diet although the change was not significant.

REFERENCES

1. Anderson W (2003): Atherosclerosis and dietary fiber. Metabolic Research Group, Pubmed.
2. Angelika Mohn, Mariangela Catino, Rita Capanna et al (2005): Increased oxidative stress in severely obese children. Journal of Clinical Endocrinology and Metabolism; 90: 2653-2658.
3. Arcaro G, Zamboni M, Rossi L, Turcato E, Covi G, Armellini F, Bosello O, Lechi A (1999): Body fat distribution. *Int J Obes Relat Metab Disord*; 23: 936–942.
4. Ardern CI, Janssen I, Ross R, Katzmarzyk PT (2004): Development of waist circumferances thresholds within BMI categories. Obes Res; 12: 1094-.
5. Beltowski J et al (2004): Oxidative stress, nitric oproduction and renal sodium handling in leptin induced hypertension. Life Sci; 74: 2987-3000.
6. Bray GA (1992): Pathophysiology of obesity. Am J Clin Nutr; 55: 4885-4945. Fridovich I (1975): Superoxide dismutase. Ann Rev Biochem, 44:147.
7. Daniels SR, Kimball TR, Morrison JA, Khoury P, Witt S, Meyer RA (1995): Effect of lean body mass, fat mass, blood pressure. Statistical, biological, and clinical significance. *Circulation*; 92: 3249–3254.
8. Decri Divitiis O, Fazio S, Petitto M, Maddalena G, Contaldo F, Mancini M (1981) : Obesity and cardiac function. *Circulation*; 64: 477–482.
9. Dexter Canoy, Nicholas Wareham, Ailsa Welch, Sheila Bingham, Robert Luben, Nicholas Day, Kay Tee Khaw (2005) : Plasma ascorbic acid concentrations and fat distribution. American Journal of Clinical Nutrition; 82: 1203-1209.
10. Duangkamol V, Praneet P, Rungsunn T, Benjaluck P, Venus S, Niyomsri V, Frank P (2000) : Erythrocyte antioxidant enzymes and blood pressure in relation to overweight and obese Thai. Nutrition; 31: 2.
11. Fernandez, Maria Luz (2001): Soluble fiber and non digestible carbohydrate effects on plasma lipids and cardiovascular risks. Curr Opin Lipidology; 12: 35-40.
12. a. Fridovich I (1975): Superoxide dismutase. Ann Rev Biochem, 44:147.
12. b. Friedewald WT, Levi RI, and Fredrikson DS (1972): Clin Chem 18:499-502.
13. Jenkins DJ, Kendall CW, Axelsen M, Augustin LS, Vuksan V (2000): Viscous and non viscous fibers. Curr Opin Lipidol. 11: 49-56.
14. Kuno T, Hozumi M, Morinobu T, Murata T, Mingci Z, Tamai H (1998): Antioxidant vitamin levels in plasma of obese girls. Free Radic Res; 28: 81-6.
15. Liu S (2002): Intake of refined carbohydrates and whole grain foods in relation to Coronary heart disease. J Am Coll Nutr; 21: 298-306.
16. Morrison JA, James FW, Sprecher DL (1999): Am J Public Health; 89: 1708-1714.
17. Newby PK, Denis Muller, Judith Hallfrisch, Ning Qiao, Reubin Andres Katherine L (2003): Dietery patterns and changes in BMI. American Journal of Clinical Nutrition; 77: 1417-1425.
18. Olusi SO (2002): Obesity is an independent risk factor for plasma lipid peroxidation and depletion of erythrocyte cytoprotective enzymes in humans. Obesity; 26: 1159-1164.
19. Slavin J (2009): Dietary fiber and body weight. Nutrition; 21: 411-4
20. Singh (2009): *JAMA*; 240: 115–119.
21. Starr JW, Wagner GS, Behar VS, Walston A II, Greenfield JC (1974): *Circulation*; 49: 829–836.
22. Reddy KK, Ramamurthy R, Somasekaraiah Kumara Reddy TP, Papa Rao (1997): Free radical and antioxidant status in urban and rural Tirupati men. Asia Pacific J Clin nutr 6: 296-311.
23. Vincet HK, Bourquignon C, Vincet KR (2006): Resistance training lowers oxidative stress in obese. Obesity; 14:1921-30.
24. Wollians JA, Wrener G, Anderson PH, McMurrey CH (1983): Research in vertinary science. 34:253-256.