IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 14, Issue 11 Ver. IV (Nov. 2015), PP 38-42
www.iosrjournals.org

Association of cardio metabolic risk factors, serum nitric oxide metabolite and oxidative stress in young obese adults

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Abstract:
Background and objectives: *Obesity is a causative factor for the development of coronary artery disease. The integrity of vascular endothelial function affects vascular homeostasis and development of atherosclerosis and coronary artery disease. Recent studies have indicated that endothelial dysfunction is associated with obesity, serum nitric oxide metabolite and oxidative stress. The aim of the present study was to evaluate serum inflammatory markers, nitric oxide metabolite, oxidative stress and their association with cardio metabolic risk factors in obese male volunteers.*
Material and Methods: *This case-control study included 96 obese male subjects (aged 28 ±5.44; without diabetes mellitus, thyroid disease or coronary artery disease) and 100 age and sex matched healthy controls. Cardio metabolic risk factors (lipid profile, insulin resistance, blood pressure), uric acid, high sensitive C-reactive protein was measured. Anthropometric measurements, height, weight, waist-hip ratio were measured. Serum oxidative stress was estimated by ferrous oxidation products in xylenol orange version 2 (FOX2) and total antioxidant status by ferric reducing capacity FRAP assay. Serum nitrosative stress was estimated by griess method.*
Results: *Compared to controls, obese subjects had significantly high nitrosative and oxidative stress. Nitrosative stress marker correlated positively with oxidative stress markers, BMI, waist-hip ratio, cardiometabolic risk factors, uric acid and high sensitive C-reactive protein.*
Conclusion: *Nitrosative and oxidative stress are higher in young obese males and correlates with cardio metabolic risk factors, inflammatory markers, BMI and waist-hip ratio.*
Key words: *Cardio metabolic risk factors, nitrosative stress, oxidative stress, obesity*

I. Introduction

There is an increase in the number of obese individuals worldwide. Obesity is a major risk factor for cardiovascular diseases (1-3). A body mass index (BMI) more than equal to 30kg/m^2 characterises obesity. Almost 20% of the adult population is obese. Obesity is associated with hyperlipidaemia, and atherosclerosis (4).In obese individuals the agonist stimulated calcium dependent nitric oxide metabolite (NO) production is severely reduced. It has been observed by Steinberg et al that vascular dilation is inversely proportional to obesity. The pathophysiology of atherosclerosis involves complex interaction between vascular endothelium, serum lipids, and inflammatory proteins (5). The most important feature in the pathophysiology of atherosclerosis is endothelium dysfunction. Endothelium dysfunction in obesity is regulated by the bioavailability of nitric oxide, inflammation and reactive oxygen species (6).

In obesity the adipose tissue acts as a paracrine gland producing various proinflammatory molecules such as C reactive protein (CRP), interleukins, and tumour necrosis factor (7). These molecules affect phosphorylation of receptors and directly activate the NAD(P)H oxidase pathwayleading to increased production of reactive oxygen species (ROS), NO and insulin resistance (8). Recent studies imply that metabolic changes in obesity increase the level of very low density lipoprotein (VLDL) and change the quality of low density lipoprotein (LDL). This proatherogenic lipoprotein profile causes endothelial dysfunction in obesity (8).

There has been limited research on cardio metabolic risk factors such as serum lipid profile, insulin resistance, C reactive protein (CRP), uric acid, NO and oxidative stress take in combination and assessing their independent correlation with body mass index (BMI), waist hip ratio WHP. Hence, this study was designed to evaluate and find a correlation between anthropometric measurements of obesity, cardio metabolic risk factors, inflammatory markers, serum nitric oxide metabolites and oxidative stress.

II. Material and methods

Subjects and Sample size

This case-control study included 96 obese male subjects (aged 28 ±5.44; without diabetes mellitus, thyroid disease or coronary artery disease) and 100 age and sex matched healthy controls. The study was conducted in MKCG Medical College, Berhampur during May 2013 to December 2014. The study protocol was approved by the institutional Ethical committee. Informed consent was obtained from all the study participants.

Inclusion and exclusion criteria

The subjects included in the study were obese with a BMI of more than equal to $30Kg/M^2$. These individuals did not suffer from thyroid diseases, Diabetes mellitus, hypertension or coronary artery disease. Persons on steroids, OC pills, thyroxin, antidepressants or other medication were excluded. The anthropometric measurements such as height, weight, waist circumference (measured midway between lower rib margin and iliac crest in horizontal plane) and hip circumference were recorded. The blood pressure was recorded. Each subject was seated in a quiet and comfortable position for 5 min, with feet on the floor and arm supported at heart level and then two readings of BP were measured on the right arm, 5 min apart with a mercury sphygmomanometer (cuff size 12.5 X 40 cm) with auscultatory method of BP measurement. BP readings were confirmed in the contralateral arm at the same time. The SBP and DBP were read to the nearest 2 mm Hg. First and fifth phases of Korotkoff's sounds were taken as criteria for SBP and DBP respectively. The average of the two consecutive readings was recorded.

Measurement of biochemical parameters: All the biochemical parameters were estimated in the clinical biochemistry laboratory at the regional diagnostic centre of MKCG medical college. Fasting venous sample was collected and the biochemical parameters were measured by using commercial kits adapted to Toshiba 120 FRAutoanalyser. Cardio metabolic risk factors (lipid profile, insulin resistance, blood pressure), uric acid, high sensitive C-reactive protein was measured using kits from Aggape diagnostics. Glucose was estimated using glucose oxidase peroxidase method, lipid profile parameters such as total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol was measured using kits from Agappe diagnostics.

Oxidative stress parameters: The oxidative stress was evaluated by estimating the amount of oxidant load of lipid peroxides was determined by ferrous oxidation products in xylenol orange assay in conjunction with triphenylphosphine version 2 (FOX2 assay) (9). The inter assay and intra assay coefficient of variation for FOX2 were 4.9% and 2.7% respectively.Antioxidant power of serum was measured by ferric reducing ability of serum (FRAP assay) (10). Inter and intra assay coefficient of variation for FRAP were 3 and 1% respectively. Serum nitrosative stress was estimated by griessmethod (11).

Statistical analysis:The data is expressed as mean ± standard deviation (SD). The data was analysed by student's't' test for unpaired data. Correlation was derived by Pearson's correlation analysis. A p value <0.05 was considered significant. Statistical analysis was done using SPSS version 19.

III. Results

The anthropometric data is represented in Table 1. The mean age of obese individuals was 28.2 ± 5.44 years and that of controls 29.2 ± 5.42 years. There was no statistical difference in the age of the two groups. A significant difference ($p < 0.05$) was observed between BMI of cases (36.2 ±1.9 kg/m^2 vs 20.1 ± 0.6 kg/m^2) and WHR (0.96 ± 0.4 vs 0.71 ±0.2). Table 2 depicts the comparison of cadiometabolic risk factors and inflammatory markers between obese young adults and healthy volunteers. A significant difference ($p < 0.001$) was observed between lipid profile, insulin resistance, uric acid, high sensitive C- reactive protein in obese young adults as compared to healthy volunteers. We also observed a significant increase in oxidant load, nitric oxide metabolites and a lower antioxidant capacity in obese adults, table 3. Table 4 shows the correlation of cardiometabolic risk factors and inflammatory markers with oxidative stress and nitrosative stress.

Table 1 shows the comparison of anthropometric data.

Parameters	Obese young adults	Healthy volunteers Controls	P value	Significance
Age(years)	28.2±5.44	29.2±5.42	0.22	Not significant
BMI (Kg/m^2)	36.2±1.9	20.1±0.6	<0.001	Significant
WHR	0.96±0.4	0.71±0.2	<0.05	Significant

All the data is represented as mean ±SD. Statistical comparison by unpaired student's t test shows a significant increase in the BMI and WHR in young obese adults than healthy volunteers.

Table 2 represents the comparison of cardio metabolic risk factors and inflammatory markers in young obese adults and healthy volunteers.

Cardiometabolic risk factors and inflammatory markers	**Obese young adults**	**Healthy volunteers Controls**	**P value**
Serum total cholesterol (mg%)	288.25 ±12.68	156.06 ± 16.5	<0.001
Serum triglyceride (mg%)	378.65± 15.12	151.81±32.19	<0.001
Serum LDL cholesterol	185.86±20.4	86.07±13.85	<0.001
Serum HDL cholesterol	40.7 ±3.7	46.65±6.0	<0.001
Serum Very low density lipoprotein (mg%)	75.73±3.2	31.5±6.4	<0.001
Insulin resistance (IR)	5.4±0.56	2.7 ±0.12	<0.001
systolic blood pressure (mm of Hg)	136 ±6.8	122±2.2	<0.001
diastolic blood pressure (mm of Hg)	100±20.4	86.32±11.2	<0.001
Serum uric acid (mg %)	6.8±0.2	3.2±0.12	<0.001
Serum high sensitive C reactive protein	6.4±3.2	4.26±2.65	<0.001

All the data is represented as mean ±SD. Statistical comparison by unpaired student's t testshows a significant increase in the cardio metabolic risk factors and inflammatory markers in young obese adults than healthy volunteers.

Table 3 shows the comparison of oxidative stress and nitric oxide metabolites in young obese adults and healthy volunteers.

Parameters	**Obese young adults**	**Healthy volunteers Controls**	**P value**	**Statistical significance**
FOX2 (µmol/L) equivalent of hydrogen peroxide	15.50 ± 5.9*	4.54 ± 0.7	<0.05	significant
FRAP (µmol/L) equivalent of Ferrous Sulphate	99.98 ± 7.74*	418± 12.42	<0.05	significant
Nitric oxide metabolite (µmol/L)	64.76± 6.74*	42.76± 6.74*	<0.05	significant

The young obese adults had significantly higher oxidant load and nitric oxide metabolites and significantly lower total antioxidants.

Table 4 shows the correlation of cardio metabolic risk factors with oxidative stress and nitric oxide metabolite.

Cardiometabolic risk factors	FOX2 (µmol/L) equivalent of hydrogen peroxide	Nitric oxide metabolite (µmol/L)
Serum total cholesterol (mg%)	PC 0.846 P <0.001	PC 0.901 P <0.001
Serum triglyceride (mg%)	PC 0.907 P <0.001	PC 0.908 P <0.001
Serum LDL cholesterol	PC 0.921 P <0.001	PC 0.895 P <0.001
Serum HDL cholesterol	PC – 0.270 P <0.05	PC -0.299 P <0.05
Serum Very low density lipoprotein (mg%)	PC 0.851 P <0.001	PC 0.846 P <0.001
Insulin resistance (IR)	PC 0.903 P <0.001	PC 0.911 P <0.001
systolic blood pressure (mm of Hg)	PC 0.841 P <0.001	PC 0.801 P <0.05
diastolic blood pressure (mm of Hg)	PC 0.866 P <0.001	PC 0.803 P <0.05
Serum uric acid (mg %)	PC 0.921 P <0.001	PC 0.942 P <0.001
Serum high sensitive C reactive protein	PC 0.881 P <0.001	PC 0.905 P <0.001

We observed a positive correlation of cadiometabolic risk factors and inflammatory markers with oxidative stress and serum nitric oxide metabolite level.

Figure 1 shows the positive correlation of oxidant load with nitric oxide metabolite.

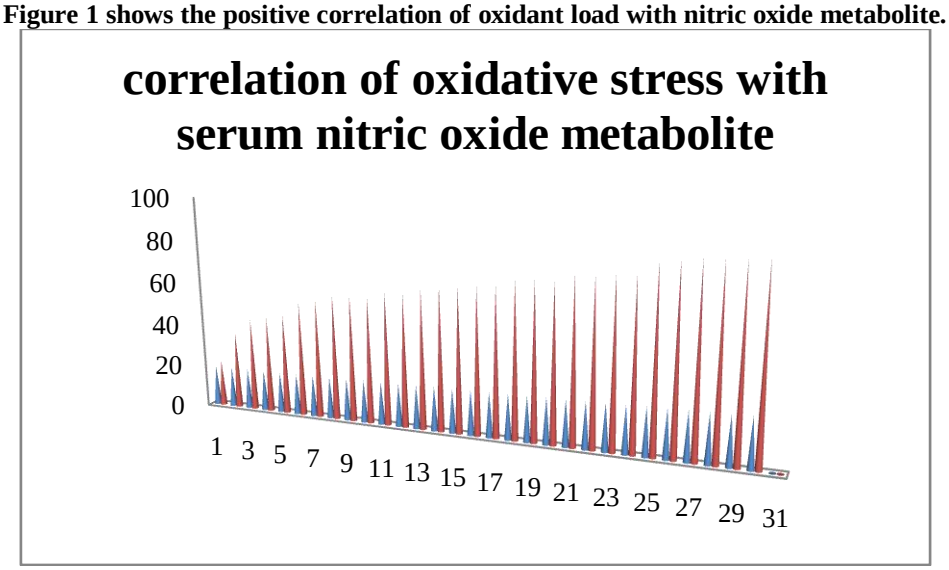
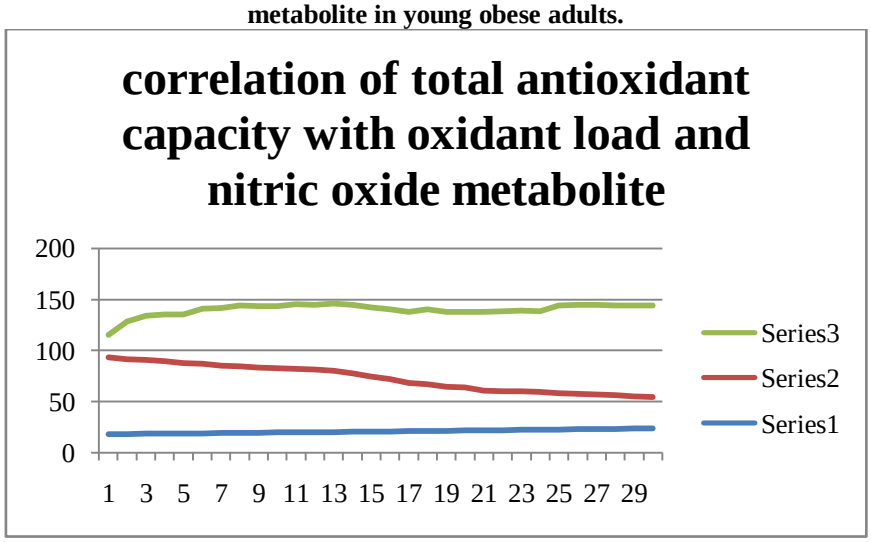


Figure 2 shows the negative correlation of total antioxidant capacity with oxidant load and nitric oxide metabolite in young obese adults.



IV. Discussion

We observed a significantly higher BMI and WHR in the obese young adults. These obese young adults also exhibited higher fasting total cholesterol, triglyceride, LDL- cholesterol and insulin resistance. This observation can be explained by the fact that insulin plays a major role in decreasing the serum triglyceride level by activating hormone sensitive lipoprotein lipase. Insulin also mediates the synthesis and secretion of VLDL by the liver. Thus, in insulin resistance these events are impaired and ahyperlipidemic state is seen. This is in concurrence with previous studies (12, 13).

The pathophysiology of atherosclerosis involves a complexinteraction between hyperlipedemia, inflammation and endothelial dysfuction (6, 7, 8). An increased level of inflammation is associated with hyperlipidemia in obesity (14). In obesity hyperlipidemia induces macrophages and adipocytes to secrete proinflammatory cytokines and produce a chronic low grade inflammation (14). We observed an increase in hsCRP and uric acid in positive correlation to hyperlipidemia.

C-reactive protein belongs to the pentraxin family of oligomeric proteins and along with uric acid are nonspecific markers of inflammation. These are associated with increased leukocyte reactivity, complement fixation, platelet activation and inflammation (14).

We observed an increased oxidative stress in obese individuals which is similar to previous studies (15,16).We observed a decreased antioxidant levels (FRAP) and increased level of oxidant along with hyperlipidemiawhich is similar to the studies by Aviram et al (17). Oxidative stress and hyperlipedemiaimpair the bioavailability of nitric oxide (NO) and lead to endothelial dysfuntion (18).

Nitric oxide is a second messenger, synthesised by the enzyme nitric oxide synthase from L-arginine and molecular oxygen. NO is associated with various physiological and pathological processes. It maintains blood pressure, relaxes vascular smooth muscles, and induces secretion of growth hormone, insulin and pancreatic polypeptide. Increased NO is also associated with various pathologic conditions such as ischemic stroke type 2 diabetes mellitus, septic shock, inflammatory arthritis and colitis etc (17-20). Studies have suggested increased NO and oxidative stress lead to endothelial dysfunction (19, 20). Our observations are similar to the above studies. We found increased oxidative stress and NO which exhibited a positive correlation with obesity.

Hyperlipidemia, oxidative stress and increased NO decrease insulin sensitivity (14, 21, 22). We observed insulin resistance in obese young adults which correlates positively with hyperlipidemia, oxidative stress and increased NO levels. Insulin resistance along with hyperlipidemia, oxidative stress, endothelial dysfunction, inflammation is considered the core component for thedevelopment of metabolic syndrome and cardiovascular diseases in obesity (23).

In conclusion, our study implicates interplay of the cardiometabolic risk factors, oxidative stress, increased nitric oxide levels,hyperlipidemia and insulin resistance could contribute to the development of metabolic syndrome and cardiovascular diseases in the young obese adults.

Acknowledgement

Authors acknowledge the cooperation received from patients, volunteers and technicians and the help received from the scholars whose articles are cited and included in the references of this manuscript.

Conflict of interest

None

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