The Relationship between Obesity and Oxidative Stress and Cardiac Markers

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Abstract

Obesity is one of the most even leading issue for the last era but however the reasons for this epidemic could not been explained clearly yet. We aimed to investigate the relationship between obesity, and myoglobin and homocysteine in means of cardiac markers and the levels of nitric oxide in means of oxidative stress and leptin. Study populations consisted of 30 patients with obesity and 30 healthy volunteers as control group. Serum nitric oxide, homocysteine, leptin and myoglobin were higher in obese individuals compared to controls. Nitric oxide level was related to myoglobin levels and seems to alter the myoglobin concentration. A negative and strong correlation was defined for myoglobin with both gender and age. There was a positive correlation between Body Mass Index and homocysteine. This is one of the studies investigating the relationship between nitric oxide as an oxidation marker homocysteine and myoglobin as cardiac markers, and leptin with obesity to lighten the complex relationship for the issue.

Key Words: Obesity, nitric oxide, leptin, myoglobin, homocysteine

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Introduction

Obesity is an important risk factor for cardiovascular disease. By perturbing the vascular function it increases the risk of atherosclerosis. The whole mechanism of obesity could not been explained clearly yet [1,2]. On the other hand free radicals are defined as the molecules having uncoupled electrons, which assumed to play important role in obesity [3]. Oxidative stress is an imbalance between antioxidant defense mechanism and free radical formation, and as a result of disturbance of this balance, serious tissue damage occurs [4].

Leptin has not only a crucial role in regulation of nutrition but also in thermogenesis, immune system, fertility, brain development, respiration and bone density. Both leptin deficiency and resistance were defined to cause human obesity [5]. Nitric oxide (NO) mediated vasodilatation is increased by leptin. Since it inhibits hypothalamic neuropeptide Y (NPY), NO synthesis is decreased by this effect in the brain. Leptin levels are found to be higher in both obese and non-obese individuals with hypertension [6,7]. Nitric oxide activates cytoplasmic granulate cyclase and facilitates dilatation of vascular smooth muscle. The rate of blood stream increases NO synthesis in endothelial cells. The mechanical force based on this increment named shear stress, increases both NO synthesis and secretion. Additionally NO is directly or indirectly related to food intake and supposed to be the central regulator in food consumption which linked to neuropeptides. The increment in NO production in hypothalamic saturation center which is diminished by diet was defined as noticing the effects of NO on central leptin concentrations [8].

NO is necessary for lipolytic activity. Nitric oxide synthase (NOS) inhibits lipolysis. However the function of NO in adipocytes is not fully understood [9]. There is a positive relationship between body mass and NO increment. However this fact was found to reverse in severely obese individuals who could be recovered by weight loss [10]. Additionally intensive exercise was found to increase NO production [11].

Homocysteine decreases defense ability of endothelial cells against free radicals. There is a positive relationship between body mass index (BMI) and homocysteine. Compared to normal obese individuals are found to have higher homocysteine levels. Every increase of 5 kg/m2 in BMI leads 10% increase in homocysteine concentrations. Interesting but also overlappingly more vegetable and fruit consumption and aerobic exercise decrease homocysteine concentrations [12].

Myoglobin is a low weight protein and released by damaged myocardial cells to the circulation which can be detectable in increased concentrations just after the beginning of myocardial infarct [13]. As a result of NO's oxidative reaction with oxymyoglobin, high amounts of NO is consumed in endothelial cells of arterioles and surrounding tissues. NO binds to un-oxygenated myoglobin [14]. Here in this study we aimed to determine nitric oxide, homocysteine, leptin and myoglobin parameters as markers in diagnosis of many diseases and obese individuals; any relationship between obese and non-obese human. To our knowledge this is the first time planned study that all this parameters will be used for obesity interrelationship with incidence, prognosis, and early diagnosis.

Material and Method

Local ethical committee consent was obtained and all the human subjects were fully informed about the study protocol. Written and informed consent were obtained. None of the participants had a history of diabetes mellitus, alcohol and cigarette consumption, had received any medicine and diet treatment. Thirty obese (15 male and 15 female; BMI>30 kg/m2) patients applied to Inonu University Turgut Ozal Medical Center outpatient endocrinology clinic were enrolled into the study. Control group was composed of healthy and volunteer 30 individuals (15 male and 15 female; BMI<25 kg/m2). Five milliliters of blood was obtained. High Density Lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL) , very low density cholesterol (VLDL) and *triglyceride levels were measured in both patient and control groups.*

Chemical Material

Disodium tetra borate (Na2B4O7), glycine (H2NCH2COOH), sodium hydroxide (NaOH), Cu sulphate (CuSO4.5H2O), zinc sulphate (ZnSO4.7H2O), sulphanamyde (C6H8N2O2S), sulphyric asit (36-38%) (H2SO4), cadmium granules (99,9%) were used. Hettich Universal 320 R marked centrifuge, Medisis marked pipette, Thermo Orion 420 marked pH meter, Denver APX-153 marked sensitive balance Shimadzu UV-1201V marked spectrometer were used.

Five milliliters of blood was obtained from both patient and control groups. Then samples were centrifuged at 3000 rpm for 10 min in 4 0C, upper plasma was obtained by pastor pipette and put in separate Eppendorf tubes and were stored in -80 0C. Triglyceride, glycose, cholesterol and

HDL-cholesterol measurements were accomplished by Architect c Systems-Aeroset Systems enzymatically.

Nitrate is reduced to nitrite and NOS activity is measured by considering nitrite amount by cadmium. The colored compound which is formed when NO formed by NOS activity in the medium reacts with Griess reactive via nitrite was measured in 545 nm spectrophotometrically [14].

Leptin measurement in serum; standard measurements were performed with Human Leptin Enzyme Linked ImmunoSorbent Assay (ELISA) reactive in Brio Seac Radim analyzer in biochemistry laboratory. The intensity of color formed is proportional to the leptin concentration in patient samples [15]. Serum homocysteine measurement was performed by Shimadzu (Japan) high-performance liquid chromatography (HPLC) analyzer by precipitation reactive, sample is deproteinized. Then precipitant is removed by centrifuged. At the end analyte is bound with flurosant marker and chromatographic differentiation is performed. For differentiation reverse phase column is used. Analyte is measured by ultraviolet (UV) detector [16].

Myoglobin measurements were performed in PATHFAST analyzer (Mitsubishi Kagaku Latron, Inc. Tokyo, Japan) which based on Chemi-luminescence Enzyme Immuno Assay (CLEIA) method. [17].

Statistical Analysis

SPSS (Statistical Package for Social Sciences) for Windows 13.0 program is used for statistical analysis. Unpaired T-test and Pearson correlation analysis were used for comparison of data. Results were given as mean ± standart deviation and $p<0.05$ was defined as significant.

Results

The demographic [age in years, height in centimeters, and weight in kilogram and body mass index (BMI kg/m2)] and biochemical parameters of the subjects (glycose, triglyceride, total cholesterol, LDL-cholesterol HDL-cholesterol and VLDL-cholesterol, all in mg/dl) are documented on Table 1. All biochemical parameters (glycose, triglyceride, total cholesterol, LDL-cholesterol HDL-cholesterol and VLDL-cholesterol, all in mg/dl) and body mass index were higher in obese group (Group1) compared to control group (Group 2). There were

significant differences between study and control groups and also a very strong correlation between BMI, weight, cholesterol, LDL, HDL and VLDL according to correlation analysis results of obese and control group ($p<0.05$).(Table 1)

Table 1. Statistics for age, height, weight, BMI, glycose, tryglyseride, cholesterol, LDL, HDL and VLDL variations according to Obese and Control groups.

	Age (y)	Height (cm)	Weight (kg)	BMI (kg/m^2)	Glucose (mg/dl)	Trigliserid (mg/dl)	Total cholesterol (mg/dl)	LDL-C (mg/dl)	HDL- C (mg/dl)	VLDL-C (mg/dl)
Group 1	35.8± 7.4	161.6± 9.3	104.7±26.8	40.8± 10.3	100.8± 13.1	210.6± 93.2	220.4± 37.1	142.53± 30.4	114.1 ± 19.2	152.82± 20.1
Group 2	29.6±7.8	163.1± 8.7	57.2± 9.6*	22.1± 2.4*	87.1± 8.5	88.63±29.1	156.4± 19.1*	69.78±24.32*	64.9 ± 10.9*	21.1 ± 9.9*

*=Statistically significant differencies between the groups ($p<0.05$)

All leptin, NO, homocysteine and myoglobin were significantly higher in obese group (Group1) compared to control group (Group 2). The results are documented on Table 2. There was a positive and significant correlation between myoglobin, total nitrite and leptin with age. However such a correlation could not be defined for homocysteine. The results are documented on Table 3. There is a positive relationship between BMI and homocysteine. Compared to normal obese individuals are found to have higher homocysteine levels. There is a not significant correlation with age and Myoglobin, Homocysteine, Total Nitrite and Leptin levels ($p>0.05$).

Table 2. Statistics for Miyoglobine, Homocystein, Totale Nitrite and Leptin variations according to Obese and Control groups.

	Group 1 (n=30)	**Group 2 (n=30)**	**P value**
Miyoglobin (ng/ml)	47,03* ± 16,34	13,07 ± 3,82	0.001
Homocystein (µmol/L)	21,51* ± 12,59	10,58 ± 2,00	0.001
Total Nitrite (µmol/L)	50,92* ± 4,92	14,08 ± 1,19	0.001
Leptin (ng/ml)	43,82* ± 20,07	3,01 ± 1,65	0.001

* = Statististically significant differencies with group 1 and 2 (p= 0.001)

Table 3. Statistics for Myoglobine, Total Nitrite and Leptin variables

		Miyoglobin	Homocystein	Total Nitrite	Leptin
	r	0,468**	0,230	0,479**	0,514**
Age	**p**	0,000	0,077	0 ,000	0,000
	n	30	30	30	30

** Statistically not significant correlation with age and Myoglobin, Homocystein, Total Nitrite and Leptin levels (p>0.05).

Discussion

Obesity is among the important risk factors of cardiovascular morbidity and mortality. Biochemical mechanism of obesity has not been clearly understood yet. In obese individuals, because of increased free radicals and reactive oxygen species (ROS), advanced oxidative stress is observed [1,3]. Bakker et al [18] hypothesized that deficiency of pancreatic β cells cause hyperglycemia in obese individuals, which induces oxidative stress. As supporting the issue we found very strong correlations between BMI and weight with total cholesterol, LDL_C, HDL-C and VLDL-C.

In our study we searched nitric oxide, leptin, homocysteine and myoglobin together. The data we obtained by studying these four parameters separately or together can be useful in treatment and prediction the prognosis of obesity in addition with other studies. We aimed to investigate how oxidative stress influence these parameters, reason for significant correlation between leptin and nitric oxide, correlation of myoglobin with nitrite and other parameters.

LDL accumulation in blood vessels increases arterial damage. Here in we obtained parallel results to this process and we observed significantly high LDL, low HDL plasma levels in obese individuals compared to healthy lean subjects as supporting the foresight of increased cardiovascular morbidity in obese individuals.

In obesity some elements secreted by adipose tissue are considered to make the individual resistant to insulin. Increase of triglyceride in adipose tissue also decreases the responses of adipose and muscular tissue to physiological stimuli. According to some studies, a decrease in number of insulin receptors or an enlargement of cellular surface cause this failure [2].

Altunkaynak et al [19] reported that circulating leptin levels have a positive relationship with BMI and weight. In the study conducted through obese mice by Jang et al [20], serum leptin levels were observed higher in obese mice compared to non-obese. Additionally women produce more leptin than men. The variation of fat accumulation between genders and low leptin levels are attributed to suppressive effect of testosterone on leptin secretion. Herein we also observed a positive and significant correlation between plasma leptin concentrations and BMI. We didn't examine leptin concentrations between genders. We also demonstrated strong and positive relationships between leptin values and age, weight, BMI, triglyceride, cholesterol, LDL, HDL, VLDL, homocysteine and NO (p=0.001). Since leptin has a cytokine like structure this strong positive relationship between leptin and NO suggests the activation of nuclear factor kappa-B (NFkB) and tumor necrosis factor (TNF-α). Adipose tissue produces TNF and this production is excessive in obese individuals [19]. TNF-α induces lipolysis and increases the release of free fatty acids. Moreover by stimulating NFkB it induces preadipocyte genes [20]. In metabolism, long term presence of increased levels of oxidized LDL causes attachment of monocytes to endothelium which is stimulated by increased TNF-α. As a result of this stimulation reactive oxygen species (ROS) production is increased by monocyte/mononuclear cells. Long term increase in ROS causes increased oxidative stress [21].

NO which is synthesized from L- arginine activates guanilate cyclase in endothelium, elevates cGMP concentration and increases relaxation in smooth muscles. It inhibits adhesion and aggregation. Supporting the previous numerous studies we observed higher NO levels in obese group. Golan MD et al (22) reported that, endothelial NO mediated dilatation increased with leptin [22]. In our study we observed a strong and positive significant correlation between leptin and NO (p=0.001). When hemoglobin is in oxy form it oxidizes NO to nitrate (NO3). NO becomes ineffective that is to say oxymyoglobine in circulation is inhibitor of NO [23]. In our study we observed a strong positive correlation between myoglobin and NO (p=0.001).

Maniscalco et al [21]. Reported a positive correlation between BMI and NO, and suggested the possibility of endothelial dysfunction in case of a NO synthesis disorder. NO is related to food intake and with weight loss its production increases. L-Arginine also regulates NO synthesis and thus cardiovascular functions [23]. In our study we observed a strong and positive correlation between BMI and weight, and NO (p=0.001). The study evaluating adipose tissue development in obesity performed by Kim et al [7]. Revealed that lipolysis was inhibited by NOS. In our

study we observed significant increase in plasma total nitrite levels in obese individuals (p=0.001). We observed not only a strong positive correlation between NO and weight and BMI, but also with glycose, triglyceride, cholesterol, LDL-C, HDL-C, VLDL-C, homocysteine and leptin with NO (p=0.001).

Every increase of 5 kg/m2 in BMI reported to result a 10% increase in homocysteine concentration [24]. Supporting the fact we observed a positive correlation between homocysteine and BMI (p=0.001). Tyagi et al. [25] reported a decreased NO bioavailability and disturbed vasodilation with hyperhomocysteinemia. Consequently an increase in blood pressure was also observed. They reported 10% increase in risk of cardiovascular diseases and 20% in risk of stroke for every increase of 3% of plasma homocysteine concentration. Herein this study we defined a positive correlation between homocysteine and NO (p=0.001). As homocysteine levels increase in endothelial cells, ROS increase as well which can be considered to have a vanishing act on antioxidant enzymes. According to many studies, hyperhomocysteinemia causes endothelial functional disorders and triggers cardiovascular diseases [26]. In the mentioned study about homocysteine metabolism performed by Tyagi N. et al [27] it was reported that vascular reactivity decreases, endothelial functional disorders come to existence and cardiovascular diseases are triggered in hyperhomocysteinemia.

In our study we observed significant differences between groups in terms of homocysteine concentration. In obese individuals, homocysteine levels were significantly higher (p=0.001). We also observed a positive correlation between homocysteine and weight, BMI, triglyceride, total cholesterol, LDL-C, HDL-C, VLDL-C, myoglobin, NO (p=0.001) and leptin (p=0.004).

Myoglobin (Mb) has a NO collecting role in striated muscles. When NO concentration increases; total oxygenated myoglobin (MbO2) concentration decreases and also, ferric Mb (MetMb) formation increases. NO can be degraded in low concentrations. When MetMb decreases it turns to MbO2 and so its accumulation can be inhibited effectively and rapidly [28].

Lack of Mb in cardiac muscle affects NO concentration and consequently important changes occur. According to data we obtained in this study, significant results are observed between myoglobin and NO. In obese individuals myoglobin together with NO are detected in high levels (p=0.001). A strong and positive correlation between myoglobin with age, weight, BMI, glycose,

triglyceride, total cholesterol, LDL-C, HDL-C, VLDL-C, homocysteine, NO and leptin (p=0.001) were defined.

Conclusion

In conclusion; to our knowledge there is none or few studies evaluating the multifaceted interactions of different parameters in obesity, as we performed here in this study. Most of the researches are based on one or two molecules and their relationships. Especially we could not find enough evaluation on the correlation with myoglobin and obesity and other well-known obesity markers which we recommend to be evaluated in further studies regards of assessing even the prognosis of cardiovascular diseases related to obesity. Beyond the role of oxidative stress in obesity; these markers can be used either as markers of following the success of different treatment modalities or considering the role of antioxidant treatment in obesity. Additionally serial leptin measurements during the follow up of treatment process offers to be helpful in obesity. Since obesity is considered to be influenced by many factors, implementation of extensive forthcoming studies with more and new parameters will contribute to our study with wide perspective.

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