IS OBESITY ASSOCIATED WITH INCREASED PLASMA LIPID PEROXIDATION AND OXIDATIVE STRESS IN WOMEN?

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Abstract

INTRODUCTION: The role of obesity in diabetes mellitus, hyperlipidemia, colon cancer, sudden death and other cardiovascular diseases has been confirmed by many studies. In this study, it was hypothesized that obesity is an independent risk factor for lipid peroxidation and decreased activity of cytoprotective enzymes in humans.

METHODS: To test the study hypothesis, we assessed lipid peroxidation by measuring the concentrations of plasma malondialdehyde (MDA) and the activity of erythrocyte copper-zinc superoxide dismutase (CuZn-SOD), glutathione peroxidase (GPX) and catalase (CAT) in 25 obese women (BMI=30-40 Kg/m²) and 25 women with healthy BMI (19-25Kg/m²).

RESULTS: The concentration of plasma MDA was significantly higher (P<0.001) in obese women (3.4± 0.7 μmol/L) compared to women with healthy BMI (1.4± 0.3 μmol/L). Furthermore, there was a significantly positive correlation (r =0.75, P<0.0001) between BMI and plasma MDA. On the other hand, women with healthy BMI had significantly higher (P<0.001) erythrocyte CuZn-SOD (873± 52 U/g Hb) and GPX (64.7± 14.2 U/g Hb) activity than obese women (660± 39 U/g Hb) and (48.5± 13.1 U/g Hb), respectively. Furthermore, erythrocyte CuZn-SOD and GPX activity were negatively correlated with BMI (r =-0.52, P<0.0001 and r =-0.42, P<0.001), respectively. No significant difference was observed between two groups in erythrocyte CAT activity.

CONCLUSIONS: From these observations, it is concluded that obesity even in the absence of smoking, diabetes, renal or liver disease can decrease the activities of body's protective antioxidants, and can enhance the systemic oxidative stress.

Keywords: Obesity, Lipid peroxidation, Cytoprotective enzymes, Oxidative stress, Women.

Introduction

Obesity is a growing epidemic in affluent nations, with the estimated prevalence ranging from 10 to 50 percent or more in the adult population.[1] Oxidative damage has been implicated in the pathogenesis of many chronic progressive diseases, such as cancer, inflammation, and neurodegenerative disorders.[2] Over the last decade, there has also been considerable interest in the role of oxidative stress in vascular disease. This interest has been driven by a wealth of data indicating that LDL oxidation is a prominent feature of atherosclerosis.[3] Obesity is associated with increased mortality, increased prevalence of cardiovascular disease, diabetes and colon cancer.[4] Although the exact biochemical mechanisms responsible for the association between obesity and the above diseases have not been completely elucidated, it is known that increased production of reactive oxygen species (ROS) at high levels is associated with cellular damage, including oxidation of cell membranes and proteins in conjunction with disturbances of cellular redox homeostasis.[5] Previous studies have reported that myocardial levels of both enzymatic and non-enzymatic antioxidants are elevated following chronic or repeated exposure to ROS.[6]

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Although animal studies in rats have shown that obesity is associated with increased myocardial oxidative stress[7] and lipid peroxidation,[8] to the best of our knowledge there is no report in the literature of the effect of obesity per se on lipid peroxidation and erythrocyte cytoprotection in women. The purpose of this study was to test the hypothesis that obesity increases plasma lipid peroxidation and decreases erythrocyte cytoprotection. Therefore, lipid peroxidation and erythrocyte cytoprotection were assessed by measuring the concentrations of plasma malondialdehyde (MDA) and the activities of erythrocyte copper zinc-superoxide dismutase (CuZn-SOD), glutathione peroxidase (GPX) and catalase (CAT) in 25 women with various grades of obesity but with no confounding factors, and comparing the values with those obtained for 50 age-matched women with healthy body mass index (BMI).

Materials and methods

The subjects of this study were recruited from the population of women covered by rural health centers of Kerman Province, Iran. In this study, 160 women aged 20-45 years were randomly selected. Body weight was measured to the nearest 0.1 kg with the subjects wearing light clothing and no shoes. Body height was also measured to the nearest 1 cm with the subjects wearing no shoes. The BMI for each individual was calculated as weight divided by height squared and was used to assess obesity. From the data obtained, 25 subjects with healthy BMI (19-25 kg/m²) were chosen randomly and were matched for age and number of pregnancies with 25 subjects with BMI in the range of 30-40 kg/m². In order to find out whether obesity on its own is an independent risk factor for lipid peroxidation and depletion of erythrocyte cytoprotective enzymes, pregnant and lactating women and subjects with history of

smoking, diabetes, hypertension, renal or liver diseases were excluded.

Venus blood samples were obtained from median cubital vein and collected into standard tubes containing ethylenediamine tetra acetic acid (EDTA). Blood samples were centrifuged at 3000 rpm for 10 minutes at 4 °C and plasma was separated for MDA assay. The buffy coat was removed and the remaining erythrocytes were washed three times in cold saline (9.0 g/l NaCl) and hemolyzed by the addition of cold deionized water. The subjects' plasma and hemolysate were stored at -70 °C until analysis.

Plasma MDA concentrations were assayed by measurement of thiobarbituric acid reactive substances (TBARS) according to the Satoh method.[9] The pink chromogen produced by the reaction of thiobarbituric acid with MDA was measured at 530 nm. In order to express the enzyme activity per gram, hemoglobin (Hb) concentration was measured in the hemolysate with a standard kit using the cyanmethemoglobin method (Drabkin's method).

CAT (CAT, E.C.1.11.1.6) activity was determined according to Hygo Aebi.[10] Activity of CAT was determined by following the decomposition of H_2O_2 in phosphate buffer pH 7.2 spectrophotometrically at 230 nm.

GPX (GPX, E.C.1.11.1.9) activity was measured according Paglia and Valentine method[11] and SOD (E.C.1.15.1.1) activity was assayed by kit RAN-SOD (cat.NO.SD 125).

Data were expressed as mean ± standard deviation (mean ± SD). Differences in mean values between the groups were evaluated by Student's t-test. The association between variables was evaluated by Pearson correlation coefficients. Significance level was set at P<0.05. All statistical analyses were done with the SPSS for Windows 12.5.

TABLE 1. Characteristics of the study population*

	Non-obese (n = 25)	Obese (n = 25)
Age	32.4± 5.3	32.8± 6.7
Height (cm)	157.4± 5.9	158.6± 5.3
Weight (Kg)	53.3± 6	79.2± 7.2 †
Body mass index (Kg/m2)	21.5± 2.3	33.2± 2.8 †

* mean ± SD, **†** Significantly different from non- obese: P<0.001

TABLE 2. Concentrations of plasma MDA and activities of erythrocyte cuzn-SOD, GPX and CAT in obese and non-obese subjects

variables	Non-obese (n = 25)	Obese (n = 25)
MDA (µmol/L)	1.4±0.3	3.4± 0.7‡
Cuzn-SOD (U/g Hb)	873± 52	660± 39 ‡
GPX (U/g Hb)	64.7± 14.2	48.5± 13.1 †
CAT (K/g Hb)	194± 59	198± 48

†‡ Significantly different from non- obese: **†**p<0.05, **‡**p<0.001

TABLE 3. Correlation between plasma MDA, activities of erythrocyte cuzn-SOD, GPX and CAT in subjects

	MDA(μmol/L)	cuzn-SOD	GPX	CAT
	n = 50	n = 50	n = 50	n = 50
BMI	r = 0.75	r = -0.52	r = -0.42	r = 0.08
	P<0.0001	P<0.0001	P<0.001	N.S.

Results

As shown in Table 1, the mean age was similar in obese and non-obese groups, hence eliminating the confounding effect of age on lipid peroxidation and enzyme activity.

Table 2 shows that the plasma concentration of MDA in the obese group was significantly (P<0.001) higher (3.4±0.7 μmol/l) than in the non-obese group (1.4±0.3 μmol/l). We also observed an inverse relationship between BMI and erythrocyte CuZn-SOD and GPX activities (Table 2).

The mean erythrocyte CuZn-SOD and GPX activities in the group of obese women (660±39 U/g Hb, 48.5± 13.1 U/g Hb, respectively) were significantly lower than in the group of non-obese women (873±52 U/g Hb, 64.7±14.2 U/g Hb, respectively). No significant difference was observed between the two groups in erythrocyte CAT activity (Table 2).

Table 3 shows a statistically negative correlation between erythrocyte CuZn-SOD (r = -0.52; P<0.0001) and GPX (r = -0.42; P<0.001) activities, and BMI. We also observed a highly positive relationship between plasma MDA concentration and BMI (r = 0.75; P<0.0001).

Discussion

Lipid peroxidation is a free radical-generating process which occurs on every membranous structure of the cell. Free radicals are known to be involved in a number of human pathologies including atherosclerosis,[12] cancer,[13] and hypertension.[14] Our data support the hypothesis that obesity promotes increased plasma lipid peroxidation. In this study, we confirmed the finding in experimental rat models[7,8] that obesity is associated with lipid peroxidation. There are several potential explanations for this observation.

Obesity increases the mechanical and metabolic load on the myocardium, thus increasing myocardial oxygen consumption.

A negative consequence of the elevated myocardial oxygen consumption is the production of ROS such as superoxide, hydroxyl radical and hydrogen peroxides from the increased mitochondrial respiration.[15] Indeed, leakage of electrons out of the mitochondrial electron transport chain promotes a one-electron reduction of molecular oxygen resulting in the formation of superoxide radicals.[16] The animal model of Vincent et al[7] demonstrated this quite clearly and our results confirmed that the same process can occur in humans.

The second mechanism by which obesity can independently cause increased lipid peroxidation is by progressive and cumulative cell injury resulting from pressure from the large body mass. Cell injury causes the release of cytokines, especially tumor necrosis factor alpha (TNF-α) which generates ROS from the tissues which in turn cause lipid peroxidation.[17]

A third possible mechanism to explain this observation is through the diet. Nutritional obesity which is the predominant form in our study population implies the consumption of hyperlipidemic diets which may be involved in oxygen metabolism.

Double bonds in the fatty acid molecules are vulnerable to oxidation reactions and may consequently cause lipid peroxidation. In addition, human studies have reported that reduced tissue vitamin E levels are associated with obesity.[18]

However, since vitamin E levels were not measured in our subjects, it is unknown whether plasma vitamin E concentrations were compromised in the obese group. It is known that a reduction in membrane vitamin E content compromises the cell's ability to defend against hydroxyl radicals.[19]

Cells contain both enzymatic and non-enzymatic antioxidants that work as a collective unit to remove ROS and other oxidants.[18]

In a rat model of diet-induced obesity, Dobrian and his colleagues[8] reported increases in the activities of erythrocyte CuZn-SOD and GPX after 10 weeks on the diet. They attributed the increase in these erythrocyte cytoprotective enzymes, which are antioxidants, to their stimulation by oxidative stress. Similarly, Vincent et al.[7] in their study of obese Fatty Zucker rats reported increased activities of erythrocyte CuZn-SOD and GPX.

In this study, however, we found a decrease in the activities of these enzymes in obese women. In the present study, mean CAT activity did not differ significantly in obese and non-obese women. A change of erythrocyte CAT activity is dependent on

oxygen consumption[20] and obesity increases oxygen consumption.[15]

However, our results were in agreement with those of Khan[21] who found decreased antioxidant levels in obese women. The discrepancy between our results and those of Dobrian et al. and Vincent et al. could be due to the duration of the obesity. It is likely that, in the early days of the development of obesity, antioxidant enzymes activity will be stimulated. However, when obesity persists for a long time, as in humans, the sources of the antioxidant enzymes become depleted, leading to a low level of activity, as we found in our study. The consequence of the low activity of the cytoprotective enzymes in human obesity is progressive tissue damage, which may eventually lead to atherosclerosis, cancer and other diseases.

From these observations, we conclude that obesity, even in the absence of smoking, diabetes, renal or liver disease can decrease the activities of the body's protective antioxidants, and can enhance the systemic oxidative stress.

Acknowledgements

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