REPORT

OBESITY: AN INDEPENDENT RISK FACTOR FOR SYSTEMIC OXIDATIVE STRESS

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ABSTRACT
The role of obesity in diabetes mellitus, hyperlipidemia, colon cancer, sudden death and other cardiovascular diseases has confirmed in many recent research studies. In present study, it is hypothesized that obesity can serve as an independent risk factor for the decreased activities of cytoprotective antioxidants in humans and for the associated systemic oxidative stress. 150 age matched, female subjects with no history of smoking or biochemical evidence of diabetes mellitus, hypertension, hyperlipidemia, renal or liver disease or cancer were included in the study and were divided into different grades of obesity according to their body mass index (BMI). Hemoglobin and erythrocyte glutathione (GSH) concentrations were measured for each subject. The study suggests that increase BMI was found to be associated with a significant decrease in erythrocyte glutathione concentration. From these observations it is concluded that obesity even in the absence of smoking, diabetes mellitus, hyperlipidemia, renal or liver diseases can decrease the activities of body's protective antioxidants, and can enhance the systemic oxidative stress and should therefore receive the same attention as obesity with complications.

Keywords: Obesity, body mass index, oxidative stress, glutathione.

INTRODUCTION

Obesity is associated with many metabolic and cardiovascular diseases, thereby contributing to increased morbidity and mortality. A direct or indirect relation has been established between obesity and insulin resistance, type II diabetes, dyslipidemia, inflammation, thrombosis, hypertension, atherosclerosis, and stroke (Fava *et al*., 1996; Krauss *et al*., 1998; Visscher and Seidell, 2001; Eckel *et al*., 2002).Obesity is an important risk factor for coronary artery disease (CAD); however, its effect on acute coronary syndrome (ACS) patients' long-term clinical and economic outcomes has not been quantified (Eisenstein *et al*., 2002). Recent reports from the National Center for Health Statistics show that the prevalence of overweight and obesity are extremely high, reaching epidemic proportions (Flegal *et al*., 2002; Freedman *et al*., 2002). In Pakistan the overall prevalence of obesity and over weight in educated population is 8 and 29.6 % respectively and together with over weights this prevalence is found to be sufficiently high (Khan *et al*., 2003).

The association between obesity and coronary atherosclerosis has not been fully elucidated. It is known that obesity is a strong risk factor for the development of CAD (Fava *et al*., 1996; Krauss *et al*., 1998; Visscher and Seidell, 2001; Eckel *et al*., 2002). Most experts believed that obesity operated indirectly through other CAD risk factors, such as hypertension, dyslipidemia, and impaired glucose tolerance or non-insulin-dependent diabetes mellitus (Reavin, 1988; Lew and Garfinkel, 1979). Obesity also found to be associated with a state of excess oxidative stress a contributing mechanism for excess cardiovascular diseases (CVD) with obesity (Keaney *et al*., 2003). Obesity itself is identified as a state of chronic oxidative stress and inflammation even in the absence of other CVD risk factors (Higdon and Frei, 2003). Oxidation is a prominent feature of atherosclerosis (Witztum and Steinberg, 1991). Studies have also suggested that oxidative stress is a feature of many risk factors for premature atherosclerosis, such as diabetes (Gopaul *et al*., 1995), hypertension (Griendling *et al*., 2000) and smoking (Marrow *et al*., 1995). Animal studies have shown that obesity is associated with increased myocardial oxidative stress (Vincent *et al*., 1999) and increased lipid peroxidation (Dobrian *et al*., 2000). Blood plasma, the transport medium of low density lipoprotein (LDL), contains a vast array of antioxidant defenses (Stocker and Frei, 1991) including a number of antioxidant proteins, mainly metal-binding proteins and enzymes, such as extracellular superoxide dismutase, catalase, and glutathione peroxidase. Oxidative stress can be monitored with several biomarkers (antioxidants and pro-oxidants) which can be assessed in plasma and/or erythrocytes (Passi *et al*., 2001). Following study is design to elaborate the possible relation ship between increasing body mass index (BMI) and the body's anti oxidative processes.

MATERIAL AND METHODS

Collection of samples

Subjects

The subjects selected for study were normal healthy female individuals of age 20-25 years. All subjects were explained the criteria of study and written consent was obtained from each subject before the study. In the survey proper, a total of 150 age matched subjects were interviewed. Weight was recorded on a measuring scale calibrated daily at the

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beginning of each working day. The individual was requested to wear light dress and the weight was recorded with the individual barefooted by taking two successive readings to the nearest 100 g, the mean of which was recorded. The BMI for each individual was calculated from the formula (weight in kg)(height in metres)^2. From the data obtained, subjects were divided into three groups according to their BMI, group I (n=50) with normal BMI (18.5-24.9 kgm^2), group II (n=50) are overweight with BMI of 25.0-29.9 kg/m^2, group III (n=50) include obese with BMI of 30.0-34.9 kgm^2 (Kenchaiah *et al*., 2002). From the detailed history and routine biochemical investigations done on each subject during the survey, only subjects with no history of smoking or biochemical evidence of diabetes, hypertension, dyslipidemia, renal or liver diseases were included in this study in order to find out whether obesity on its own is an independent risk factor for oxidative stress or not.

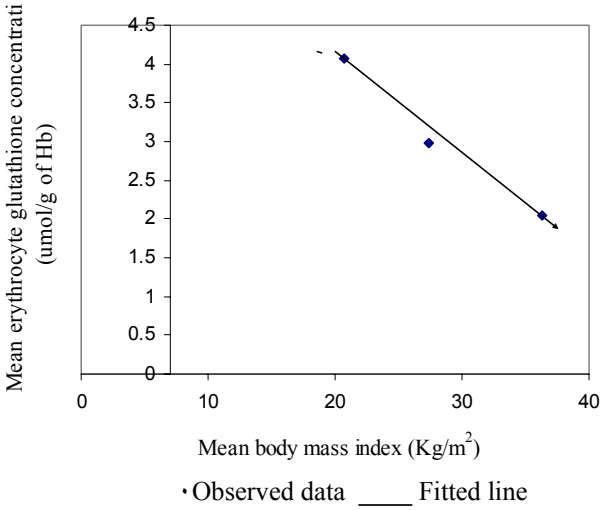

Fig. 1: BMI related changes of Erythrocyte glutathione concentration.

Sample preparation

Heparinized blood was drawn from each subject after an overnight fast in resting conditions.

Measurement of hemoglobin concentration

Hemoglobin concentration was determined using Drabkin's reagent on an automatic hemoglobinometer (Osaka, Sieki, Japan).

Erythrocyte Glutathione concentration

Glutathione concentration of red blood cells was determined by the method of Beutler *et al* (1963).

Data and Statistical Analysis

All values in this paper are presented as mean ± SE. The difference between the study groups was determined with t-test. Significance was accepted at $P < 0.005$. Correlation coefficient (r) was also calculated between the groups where it is required.

RESULTS

In the present study the erythrocyte glutathione concentration was found to have negative correlation with the BMI (fig. 1). Mean erythrocyte glutathione concentration in normal subjects having healthy BMI (18.5-24.9kg/m2) was 4.08 ± 0.005 µmol/g of Hb. In overweight individuals, as the BMI increases upto 25.0-29.9 kg/m2, this value significantly ($P<0.05$) reduces to 2.99± 0.006 µmol /g of Hb. Obese individuals further showed a significant decrease in erythrocyte glutathione concentration to 2.04 ± 0.005 µmol/g of Hb ($r = - 0.64$).

DISCUSSION

Overweight and obesity are associated with a significantly increased mortality from atherosclerotic cardiovascular disease and other causes (National task force on the prevention and treatment of Obesity, 2000; Eckel *et al*., 2002). A report from Keaney *et al* provides evidence that increased systemic oxidative stress may be an important mechanism by which obesity increases the incidence of atherosclerotic cardiovascular disease through lipid peroxidation (Keaney *et al*., 2003). The basic emphasize of the present study is to find out that whether the obesity alone, without the confounding factors like hypertension, diabetes, hyperlipidaemia and smoking, causes significant systemic oxidative stress in humans. The notion that obesity is associated with a state of heightened oxidative stress is not without precedent. There are at least three main ways by which obesity, acting independently, can produce lipid peroxidation. Obesity increases the mechanical and metabolic loads on the myocardium, thus increasing myocardial oxygen consumption (Olusi *et al*; 2002). A negative consequence of the elevated myocardial oxygen consumption is the production of reactive oxygen species such as superoxide, hydroxy radical and hydrogen peroxides from the increased mitochondrial respiration (Turrens, 1997). The second mechanism by which obesity can independently cause increased oxidative stress 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, which generates reactive oxygen species from the tissues (Lechieitner *et al*., 2000). A third possible mechanism 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 consequently may cause lipid peroxidation. If the production of these oxygen species exceeds the antioxidant capacity of the cell, oxidative stress resulting in lipid peroxidation may occur and the process then leads to atherosclerosis.

Erythrocyte glutathione concentration considers as the principle marker of systemic oxidative stress. Dobrian and his colleagues reported an increase in this red cell antioxidant levels during oxidative stress induction in rat model of diet induced obesity (Dobrian *et al*., 2000). How ever in our present study we found low levels of this red cell antioxidant in obese individuals. Our results were in agreement, however, with those of Burgos *et al* (1992), who found decreased antioxidant levels in obese adults, and those of Kuno *et al* (1998), who found decreased levels in girls, Decsi *et al* (1997) who found decreased levels in boys, and Strauss (Strauss, 1999) who found decreased serum concentrations of α-tocopherol and β-carotine in obese compared with non-obese children. The discrepancy between our results and those of Dobrian *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 enzyme activity will be stimulated. However, once the obesity is established, the sources of the antioxidant enzymes become depleted, leading to a low level of activity (Olusi *et al*; 2002). In conclusion, this study has demonstrated that obesity in humans, in the absence of other confounding factors such as smoking, hypertension, diabetes and hyperlipidaemia, is an independent risk factor for the depletion of cytoprotective antioxidants.

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