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Original article

THE BALANCE BETWEEN SUPEROXIDE DISMUTASE AND CATALASE ACTIVITIES IN SERA OF OBESE IRAQI MEN.

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Summary

Obesity is a multifaceted disorder stemming from an imbalance in the homeostasis of energy, which leads to an accumulation of excess energy as fat. It has become of increasing concern in the Iraqi population since 2003, when obesity was identified as a significant factor in relation to several diseases. The objective of this study is to investigate the association between oxidative stress and obesity in blood serum of obese Iraqi men.

This study was conducted on two different groups with matching age ranges (25-35 years). The obese group consisted of thirty-six obese subjects with varying grades of obesity (BMI 40 ± 5 kg/m^2). The control group included thirty-six non-obese subjects (BMI 25 ± 3 kg/m^2). There are two parallel components to this study: The first involves Superoxide dismutase activity, catalase activity and total thiol group levels as a marker of antioxidants. The second involves the end product of lipid peroxidation (MDA) as a marker of oxidative stress.

When compared with the control group, SOD, CAT activity, and MDA were significantly increased, whereas TTG was significantly decreased. There is a balance between CAT activity compared with SOD activity and polyunsaturated fatty acids hydroxyl peroxide that play a vital role in the regulation of ROS and the body's defense system in obese men and in the human body in general.

Introduction

The marked improvement in the Iraqi living standard after 2003, due to the elimination of the sanctions that were imposed under the previous regime, resulted in most Iraqis possessing cars, as well as an increase in marriages and childbirth. Considering also the warm weather and Iraqis' habits of drinking tea and soft drinks, all of which are contributing factors to the current prevalence of obesity which has become a real problem in Iraqi society - in particular, obesity linked to metabolic syndrome and

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heart disease (1).

The Health and Social Care Information Centre (HSCIC) released its annual statistical report of 2013 entitled "Statistics on Obesity, Physical Activity and Diet: England, 2013" (2). This report claimed that, according to a variety of sources, obesity in England had markedly increased in recent years, citing an increase in the percentage of adults' body mass index (BMI) from 1993 to 2011 (among men an increase from 34% to 41% and among women from 39% to 50%, respectively). The report also mentioned that the percentage of overweight people, including obese, increased from 58% to 65% in men and from 49% to 58% in women, respectively, between 1993 and 2011 (2).

Previous studies have indicated that the prevalence of obesity in the Iraqi population is increasing, especially since 2003. A study carried out on non-pregnant women in Baghdad city from 2002-2003, to evaluate the prevalence and factors associated with being overweight and obesity, found that the percentage of obese women was 25% and overweight women was 39%, respectively (3).

Al-Hilaly *et al.* (4), conducted a study in Karbala city in 2008 with 1545 individuals. They found that 29.3% of the study population was obese and 31.4% was overweight. Another study was conducted in Baghdad city in 2009 on pre- and post- menopausal women to evaluate the prevalence of obesity. The study determined that the prevalence of obesity in pre-menopausal women was 29.7% and in post-menopausal women was 36.5%, respectively (5).

One of the major contributing risk factors to cardiovascular diseases is obesity. The mechanisms relating cardiovascular diseases to obesity are ambiguous. Two mechanisms are utilized to explain the morbidity of obesity, inflammation and oxidative stress (6). As biochemists, we are interested in the oxidative stress hypothesis. Reactive oxygen species (ROS) are produced in the normal metabolism of oxygen as a byproduct and they play a key role in cell signaling and homeostasis. Oxidative stress results from the imbalance between production of ROS and the cellular antioxidant defense mechanisms (7). Genetic interactions and environmental factors have a role in oxidative stress mediated pathologies (8). De Marchi *et al.* (7) propose a hypothesis about the role of the $p66^{Shc}$ protein and protein kinase C (PKC) in the intracellular regulation of redox balance and levels of oxidative stress in this pathogenesis.

This study involves two parallel topics. The first consists of Superoxide dismutase (SOD EC 1.15.1.1) activity, catalase (CAT EC 1.11.1.6) activity and total thiol groups (TTG) levels as a marker of antioxidants. The second topic includes the end product of lipid peroxidation (MDA) as a marker of oxidative stress. The objective of the present study was to investigate the association between oxidative stress and obesity, as well as their intercorrelation in blood serum of obese Iraqi men.

Materials and methods

Thirty-six obese men with a body mass index of 40 ± 5 and between 25-35 years old were compared with thirty men with a normal body mass index 25 ± 3 and between 25-35 years old, which was the a control group. All men are employees and post-graduate students of the University of Babylon, Hillah, IRAQ. The study lasted from September 2013 to November 2013. Fasting blood samples collected from both the obese group and the control group were collected at 8:00-8:30 am. After blood clotting, the serum was separated by centrifuging for 10 minutes at a relative centrifugal force (RCF) of 2000 X g (9). The analytical methods that are described below were performed immediately after serum separation.

Methods

Determination of SOD activity

1000 µL of 75 mM of tris (hydroxymethyl) aminomethane - EDTA: Ethylenediaminetetraacetic acid (tris-EDTA) buffer pH 8.2 and 1000 µL of 0.2 mM of pyrogallol were added to the volume of 50 µL of serum. A change in absorption was traced at 420 nm for 30 minutes. The activity of SOD is expressed as U\l of serum (10).

Determination of CAT activity

Catalase activity was determined spectrophotometrically at 570 nm based on the formation of chromic acetate by reduction of dichromate dissolved in acetic acid when boiled in the presence of H_2O_2 and expressed in mmoles per liter per second (Katal) (11) (*Sinha, 1972*). The reaction mixture contained 1.0 ml of 0.01M phosphate buffer (pH 7.4) with 0.1 ml of serum and 0.4 ml of 200mM H_2O_2.

Determination of TTG

The TTGs of serum were determined spectrophotometrically at 412 nm using Ellman's reagent 5,5'- dithiobis (2-nitrobenzoic acid) (DTNB) as previously described (12).

Determination of MDA

Serum lipid peroxidation product MDA was determined by the colorimetric thiobarbituric acid (TBA) method. Lipid peroxides break down to form MDA under acidic and heating conditions. The latter compound reacts with TBA to form pink MDA-TBA adduct that absorbs at 532 nm (13).

Statistical analysis

The results of the present study were expressed as a mean ± SD. The difference between groups was determined using student's t- test, and value of $p \leq 0.05$ was considered statistically significant. Person's correlations were used to determine the relationship between the parameters being studied.

Results

The SOD and CAT activities of the blood serum of obese men (431.1±15.5 U/L and 0.54±0.08 kat/ml) were significantly higher (P=0.03) (P=0.048) than those of the control group (402±23 U/L and 0.35±0.095 kat/ml). They are shown in Figures 1 and 2.

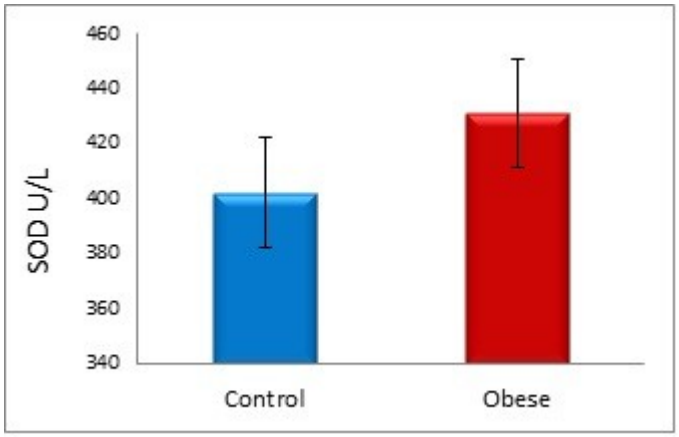

Figure 1. The SOD activity U/L of the serum of obese men and of the control group.

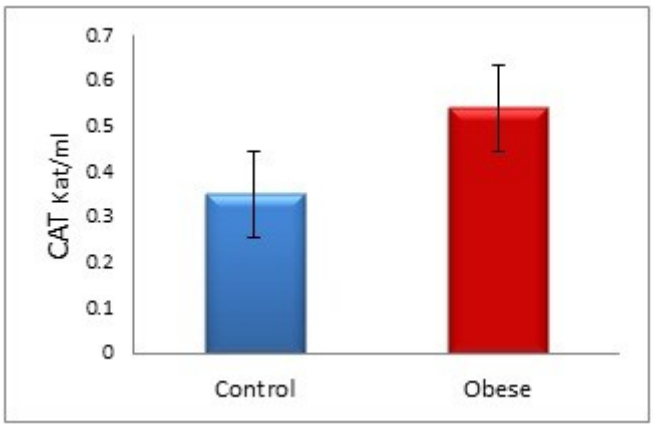

Figure 2. The CAT activity Katal/ml of the serum of obese men and of the control group.

There was also a significant increase in MDA concentration of the serum of obese men (1.42±0.07 µmol/L) when compared with that of control group (0.54±0.03 µmol/L) with (P= 0.0001) as shown in Figure 3.
There was, however, a significant decrease in TTG level of the serum of obese men (197.6±48 µmol/L) when compared with that of control group (289.5±51 µmol/L) with (P=0.008) as shown in Figure 4.

Discussion

Obesity is a major problem in developing and developed countries (14, 15). Two mechanisms have previously been proposed to explain the morbidity of obesity: inflammation and oxidative stress (6). The mechanism of inflamation is based upon the production of the adipocytes, one variety of which are molecules called adipocytokines. Abnormality in the production of these adipocytokines contributes to the obesity pathogenesis with thrombosis development and insulin resistance (16). The other mechanism, oxidative stress, is based upon increased reactive oxygen species in accumulated fat of obese humans and mice. This mechanism suggests that the generation of ROS is increased dramatically in adipocytes of obese mice, and associated with increased expression of NADPH oxidase, which results in elevated levels of ROS (16).
The elevated levels of ROS can overwhelm the antioxidant defense system of the body, and as a result, generate oxidative stress. Results of this study clearly suggest that obese men have an increased ROS. While ROS are potentially harmful to cells, cells do have an endogenous defense system against these species. They contain both enzymatic and non-enzymatic antioxidants that form the first line of defense against ROS (17) as shown in Figure 5.
The conversion of superoxide radical $O_2^{\cdot -}$ to hydrogen peroxide is catalyzed by SOD. Vincent *et al*., (18) reported that high concentrations of hydrogen peroxide act to stimulate the increment of cellular levels of CAT. The CAT activity falls under another type of harmful species called

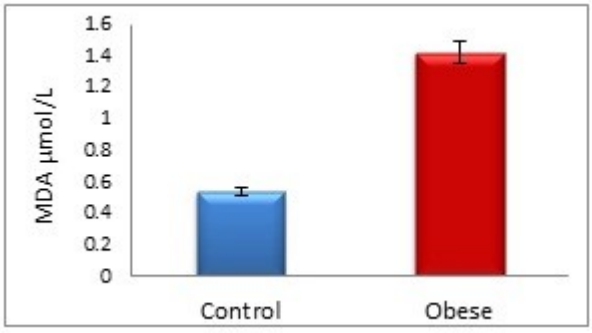

Figure 3. The MDA µmole/L levels of the serum of obese men and of the control

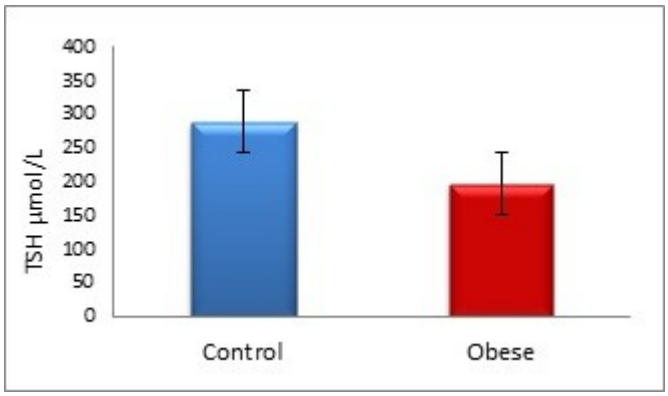

Figure 4. The total thiol µmol/L of the serum of obese men and of the control group.

polyunsaturated fatty acids hydroxyl peroxide that results from the reactions of free radicals. This matter is confirmed by high levels of the end product of lipid peroxidation (MDA) in this study (19).

Lastly, total thiol concentrations decreased for obese men. This is important because total thiol groups (including glutathione) play a major role in the maintenance of intracellular redox status and antioxidant vitamin levels. Therefore, the failure to increase total thiol levels may have been a factor in the observed increase of lipid peroxidation of obese men.

The present study suggests that superoxide radical $O_2^{\cdot -}$ and polyunsaturated fatty acid hydroperoxide collectively compete on the CAT active site. There is a significant increase in CAT activity, although it is insufficient to convert the two substrates into harmless species.

In conclusion, there is a balance between CAT activity on one side and the SOD activity and polyunsaturated fatty acids hydroxyl peroxide on the other side. This balance plays a key role in the regulation of ROS and the defense mechanism in obese men as shown in Figure 6.

Abbreviations

BMI: Body mass index; CAT: Catalase; GPx: Glutathione peroxidase; GSH: Reduced glutathione; GSSG: Oxidized glutathione; MDA: Malondialdehyde; PUFA: Polyunsaturated fatty acid hydroperoxide; ROS: Reactive oxygen species; SOD: Superoxide dismutase; TTG: Total thiol

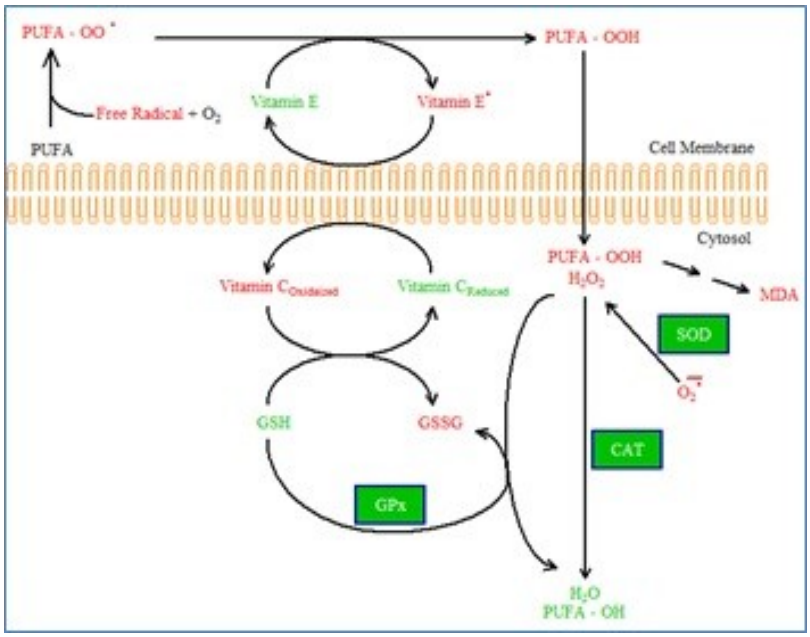

Figure 5 Mechanism of antioxidant defense system.

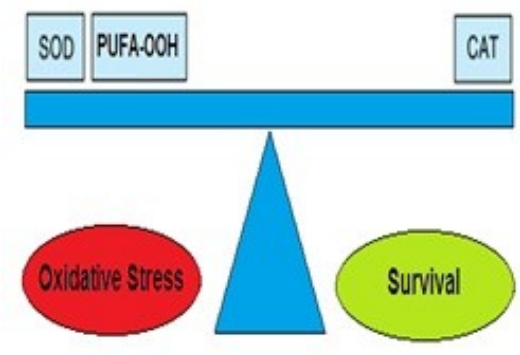

Figure 6 Balance of oxidative stress and antioxidants.

group.

Competing interests

The authors declare that they have no competing interests.

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