The Levels of Protein Oxidation and Lipid Peroxidation in Sera of Obese Men

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Abstract

Background: Obesity is an important risk factor for the development of different type of degenerative diseases.

Objective: Investigate the relationship between oxidative stress and obesity.

Methods: 30 Obese (BMI 34-42 kg/m^2) subjects and 20 non-obese (BMI 23-24 kg/m^2)subjects were subject to the present study. Conjugated diene lipid hydroperoxides (CD), protein carbonyls, serum uric acid and total serum protein were determined spectrophotometrically.

Results: Results of present study show highly significant (P<0.001) increase in the levels of CDH and total carbonyl group and show increase in total protein whereas shows decrease in uric acid levels of obese men, when compared with control group.

Conclusions: Increase the levels of oxidation product and decrease the antioxidant levels indicate involvement of obesity in the conditions of oxidative stress.

الخلاصة

الخلفية: السمنة عامل خطر ومهم لتطور الأنواع المختلفة من الأمراض الانحلالية.

الأهداف: للتحري عن العلاقة بين الإجهاد التأكسدي والسمنة.

طرائق العمل:اخذت عين مكونة من 30 شخصا بدينا تتراوح قياسات كتلة الجسم لهم من 34 الى42 كيلوغرام /م^2 و20 شخصا غير بدين تتراوح قياسات كتلة الجسم لهم من 23 الى24 كيلوغرام /م^2.

وتم تقدير كل من بيرو كسيدات الدهون مقترنة الدايين و كاربونيل البروتين وحامض اليوريك في مصل الدم وبروتين مصل الدم الكلي بطرق لونية.

النتائج: أظهرت نتائج الدراسة ارتفاعا معنويا عاليا (P <0.001) في مستويات كل من بيرو كسيدات الدهون مقترنة الدايين و كاربونيل البروتين كما وأظهرت ارتفاعا غير معنويا في بروتين مصل الدم الكلي ،بينما أظهرت نقصاناً غير معنويا في مستويات حامض اليوريك في مصل الدم عند الرجال البدينين مقارنة بمجموعة السيطرة.

الاستنتاجات: إن الزيادة في مستويات نواتج التأكسد والنقصان في مستويات مضادات الأكسدة يشير إلى شمول السمنة في ظروف الإجهاد التأكسدي.

Introduction

obesity is a multifaceted metabolic disorder with strong genetic influence. [Rosenbaum *et al.*, 1997]. Active investigations for candidate genes responsible for obesity has been done. However, the exact genes have not been identified. [Eun Young *Oh et al.*,2000]. Also, obesity can define as a complex disorder, resulting from an imbalance in energy homeostasis, leading to accumulation of excess energy as fat. [Shanmugam *et al.*,2006,Huang *et al.*,2005].

In addition, obesity worsens numerous other health problems independently and in association with other diseases such as non insulin dependant diabetes mellitus (type 2 diabetes), coronary heart disease, respiratory complications, some kind of cancers, and osteoarthrities [Kopelman *et al.*, 2000, Montague *et al.*, 2000].

Other researchers described obesity as an important risk factor for the development of insulin resistance and type 2 diabetes.[Jurgen *et al.*,2002, Kahn and Flier 2000]. They attribute them finding to adipose tissue which is now recognized as an important source of many substances, such as leptin, tumor necrosis factor,

nonesterified fatty acids, or adiponectin, that may contribute to the development of insulin resistance in liver, adipose tissue, and skeletal muscle [Satiel, 2001].

Obese persons are frequently predisposes to many complications including hypertension, diabetes, hyperinsulinaemia and hypertriglyceridaemia. Together, these factors increase the mechanical and metabolic loads on the myocardium, thus increasing myocardial oxygen consumption.[Vincent *et al.*,1999].

A potentially negative consequence of elevated myocardial metabolism is the production of reactive oxygen species (ROS), such as superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH^{\cdot}$), during mitochondrial respiration. Production of ROS at high levels can exceed the antioxidant capacity of the cell, resulting in oxidative stress. Oxidative stress is associated with cellular damage including oxidation of cell membranes and proteins in conjunction with disturbances of cellular redox homeostasis.[Ji ,1995, Kukreja *et al.*,1992] .

Antioxidants act together in the cells of human blood against toxic ROS which are cause lipid peroxidation and oxidation of some specific proteins, thus affecting many intra- and intercellular systems.[Durdi *et al*.,2005]. Antioxidants are capable of stabilizing, or deactivating, ROS or free radicals before they attack cells. Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being.[Mark, 1998] .

It is not easy to determine ROS or free radicals in a biological sample due to their short half-life. The estimation of radical damage is more easily achieve. Radicals rapidly react with macromolecules of different classes, whereby more stable molecules are formed, which can subsequently be determined. Oxidatively modified groups on lipids, proteins and DNA can be used to indirectly measure the damage occur by free radicals in biological samples.[Cedreberg, 2001] .

Oxidation of lipoproteins, for example, involves in peroxidation of their polyunsaturated fatty acid (PUFA) and yields large amounts of lipid peroxidation products such as conjugated diene hydroperoxides. Cleavage of these products generates aldehydes, such as malondialdehyde, which act as toxic messengers in the processes of lesion formation.[Bruno *et al.*, 2001] .

The levels of malondialdehyde or conjugated diene hydroperoxides and protein carbonyl, as markers of lipid and protein oxidation respectively, as well as uric acid concentration as an antioxidant was investigated in the present study to assess the oxidation status in obese man, and to investigate the relationship between oxidative stress and obesity; woman was excluded to avoid the effect of women's sex hormones.

This study has, to the best of our knowledge, never been tested in the Iraqi population.

Material and Methods

Reagents unless otherwise stated, were purchased from BDH Company. The present study was conducted on two groups;

Group 1 consists of 30 obese subjects with different grades of obesity (BMI 34-42 kg/m^2).

Group 2 included 20 non-obese subjects BMI 23-24 kg/m^2.

Quantitative Analysis of Conjugated Diene Lipid Hydroperoxides (CD)

Conjugated diene lipid hydroperoxides (CD) were extracted from 500 µL of serum by CH_3C1,: MeOH (2:1, v/v). The organic extract was dried under a nitrogen stream, resuspended in cyclohexane, and quantitated spectrophotometrically at 234 nm, using a molar absorption coefficient (ε) of 27,000 M^{-1} cm^{-1}.[Pryor and Castle, 1984] .

Quantitative Analysis of Protein Carbonyls or Oxidized Proteins

The quantitative analysis of protein carbonyls was performed using 2, 4-dinitrophenylhydrazine (DNPH) as described previously by Jain *et al*. (1989) with simple modification. Briefly, To 1 mL of serum, 4 mL of 12.5 mM DNPH in 2.5 M HCl was added and incubated at room temperature for 1 h. The protein was precipitated with 10% trichloroacetic acid. The pellet was washed 3 times by breaking the pellet with a glass rod to remove the free DNPH with 4 mL of ethanol:ethyl acetate (1:1,v/v). The pellets were dissolved in 6 M Guanidine-HCl at 37°C for 20 min with frequent vortexing. Insoluble materials were removed by centrifugation and absorbance was measured at 370 nm. The protein carbonyl content was calculated from the molar absorption coefficient (ε) of 22,000 M^{-1} cm^{-1}. [Jain *et al*.1989] .

Determination of Serum Uric Acid

Uric acid was determined enzymatically using Biomaghreb kit (Morocco). In which uric acid is oxidized by uricase to allantoine and H_2O_2, the later react with 4-aminophenazone in presence of peroxydase to form colored quinoneimine. Absorbance was measured at 550 nm

Determination of Total Serum Protein

Total serum protein was measured by using commercially available kits (Randox Laboratories Ltd., UK) based on biuret method, in which, peptide bonds of protein react with cupric ion (Cu^{2+}) in alkaline solution to form a blue colored product whose absorbance is measured spectrophotometrically at 540 nm.

Statistical Analysis

All values were expressed as mean ± standard deviation (SD) and ± standard error (SE). Student's t-test was used to estimate differences between the groups and differences were considered significant when the probability was ($p < 0.05$), and highly significant when the probability was ($p < 0.001$).

Results

Results of present study are listed in table1

Table 1 CDH, uric acid, total protein, and total carbonyl group in obese men and healthy control

	Group	Mean	SD	SE	P value	Significant
CDH (μ mole/L)	Control	8.5	2.3	0.51	<0.001	Sign.
	Obese	12.5	3.48	0.63		
Uric Acid (μ mole/L)	Control	6.05	0.48	0.10	>0.01	N.S.
	Obese	5.56	0.93	0.17		
Total Protein (gm/dl)	Control	5.89	0.42	0.094	>0.01	N.S.
	Obese	6.29	1.16	0.21		
Total Carbonyl Group (μ mole/L of serum)	Control	102.54	34.61	0.094	<0.001	Sign.
	Obese	148.4	49.15	0.21		
Total Carbonyl Group (μ mole/gm of protein)	Control	2.12	0.79	0.094	<0.001	Sign.
	Obese	3.19	1.17	0.21		

Sign. = significant, NS = not significant, SD = standard deviation, SE = standard error, P = probability

Table 1 shows highly significant (P<0.001) increase in the levels of CDH and total carbonyl group in both ways of presentation (μ mole/L of serum and μ mole/gm of protein) in obese men when compared with control group, and shows increase in

total protein in obese men, whereas shows decrease in serum uric acid levels of obese men, when compared with control group.

Discussion

Shanmugam *et al.* (2006) suggest that the development of obesity occur as a result of genetic and/or environmental changes, which ultimately impact three important biochemical processes. These are:

1. Food intake regulation, which determines the satiety or hunger sensation that depends on interplay among central signaling molecules (leptin, neuropeptide Y, etc.).
2. Energy regulation, by a burning mechanism called thermogenesis, which is mediated by uncoupling proteins which dissipate part of food derived calories or stored energy as heat instead of generating ATP and subsequent storage of energy as fat.
3. Adipogenesis, the process of formation and differentiation of preadipocytes into mature adipocytes, which accumulate the excess energy as fat. Recent advances in the understanding of molecular aspects of these processes have enabled us to develop novel methods of controlling obesity by a variety of pharmacological, nutritional, and, possibly, genetic interventions.

For these reasons we try to investigate the correlation between oxidative stress and obesity to aid in the understanding of molecular aspects of obesity problems.

The elevation of Conjugated Diene Lipid Hydroperoxides (CD) and Protein Carbonyls could beyond to several causes; the first, decrease Gpx activity in obese [Hadwan, 2009] which due to increased H_2O_2 production and consume antioxidants in adipose tissue of obese. The second cause beyond to the high percentage of polyunsaturated fatty acids (PUFA) in the obese tissues; polyunsaturated fatty acids (PUFA) are highly susceptible to ROS-induced damage in tissues.

A study preformed on animal models suggest that chronic dietary vitamin A supplementation at high doses effectively regulates obesity and significantly reduced body weight in rats.[Shanmugam *et al*.,2006]

Other study carried out on rats show that obese rats receiving normal levels of vitamin A diet showed high serum HDL-C and lower hepatic SR-BI expression levels compared with lean counterparts. The results emphasize the importance of retinoids as physiological regulators of adipose tissue development and function in intact animals.[Shanmugam *et al*.,2007]

A study conducted on human in USA show that obese participants had serum retinol (vitamin A), α-tocopherol (vitamin E) and the carotenoids concentrations lower than normal weight participants.[Marian *et al*.,2001]

All of these studies are emphasized our findings, that clearly reveal an increases in the parameters of oxidative stress (CDH and total carbonyl) and decreases in the parameters of antioxidants (uric acid), as shown in table1.

A possible mechanism to explain our observations is that an increased PUFA in tissues of obese men promotes lipid oxidation by increasing the amount of substrate available for peroxidation, leading to elevation CDH levels.

Human studies investigating the oxidation of serum lipoproteins have shown similar results. That is, lipid peroxidation is positively correlated with serum triglyceride levels and body mass index (BMI). Hence, it seems likely that increased fat deposition may have contributed to the observed increase in lipid peroxidation in the hearts of the obese animals.[Vincent *et al*.,1999]

In the present study, we express our data of total carbonyl group in μ mole/gm of serum protein and in μ mole/L of serum, but we thought that the former way is more conventional due to change in total serum protein in each individual.

The decreases of mitochondrial functions are commonly described in skeletal muscle of obese and insulin resistant patients. Impairment of mitochondrial protein synthesis might be related to mitochondrial dysfunction.[Emilie *et al*.,2007] Mitochondrial dysfunction leads to increased oxidative stress. Mitochondrial dysfunction promotes generation of ROS, as a result, total carbonyl group (a marker of protein oxidation) increase in degenerative conditions. [Zhang *et al*.,1999] This may explain the significant increase in protein oxidation products in the present study.

Uric acid is one of the major endogenous water-soluble antioxidants of the body. Urate (the soluble form of uric acid in the blood) can scavenge ROS [Becker *et al*.,1993, Simie *et al*.,1989] Thus, low circulating uric acid levels may be an indicator that the body is trying to protect itself from ROS, this may elucidate the decrease in serum uric acid of obese men in the present study.

Conclusions

The marked elevation in the levels of oxidation product (Conjugated diene lipid hydroperoxides and protein carbonyls), and the depletion in the levels of antioxidant (serum uric acid) indicate involvement of obesity in the conditions of oxidative stress.

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