

Insulin resistance is triggered by oxidative stress in mildly obese men

Salwa fayeze *Abd El Aziz GH .M** Mohamed Rehan***

*Department of Medical Biochemistry, Faculty of Medicine, Cairo University

**Department of Medical Biochemistry, Faculty of Medicine, Beni Suef University

*** Internal Medicine Department, Faculty of Medicine, Beni Suef University

salwafayeze@yahoo.com

Abstract: Recent reports indicate that obesity may induce systemic oxidative stress that plays a critical role in the pathogenesis of various diseases. Cumulative evidence suggests that increased oxidative stress may lead to insulin resistance in vivo or in vitro. The present study aimed to investigate the possible relationship of oxidative stress with mild obesity and insulin resistance in men. The serum levels malondialdehyde and total antioxidant capacity were measured in 31 mildly obese and 26 nonobese men and their relationship was evaluated with homeostasis model assessment of insulin resistance (HOMA-IR), body mass index and waist circumference. Obese men had significantly higher serum concentrations of malondialdehyde and lower levels of total antioxidant capacity than nonobese men ($P < 0.001$). The serum levels of malondialdehyde were significantly positive correlated with HOMA-IR, body mass index ($r = 0.78; 0.69; P < 0.001$ respectively), while total antioxidant capacity was significantly negative correlated with HOMA-IR, body mass index ($r = - 0.50; - 0.64 P < 0.001$) in all (obese and nonobese) men. Also HOMA-IR values were found to be significantly positive correlated with body mass index and waist circumference in all (obese and nonobese) men. The results of this study suggest that, increased oxidative stress together with the decreased antioxidative defence seems to contribute to decreased insulin sensitivity and related to adiposity and insulin resistance in men, and may be hypothesized to favor the development of insulin resistance in mildly obese men. [Journal of American Science 2010; 6(9):604-611]. (ISSN: 1545-1003).

Keywords: Insulin resistance, Oxidative stress, Obesity, Total antioxidant capacity, Malondialdehyde.

1. Introduction

Progressive development of insulin resistance (IR) is a prediabetic state which is today a widespread metabolic abnormality of adults and adolescents in industrialized societies (1).

Impaired insulin action is considered the first stage of type 2 diabetes mellitus (T2DM). The consequences of IR manifest at many levels and in many metabolic processes, producing a cluster of homeostatic abnormalities including glucose intolerance, overt hyperglycemia, hyperinsulinemia, and atherogenic dyslipidemia, collectively referred to as metabolic syndrome (MetS) (2-4). Insulin resistance is believed to have both genetic and environmental factors implicated in its etiology (5, 6). The genetic component seems to be polygenic in nature, and several genes have been suggested as potential candidates (5). However, several other factors can influence insulin sensitivity, such as obesity, ethnicity, gender, perinatal factors, puberty, sedentary lifestyle and diet (6).

Adipose tissue seems to play a key role in the pathogenesis of insulin resistance through several released metabolites, hormones and adipocytokines that can affect different steps in insulin action (7).

Obese individuals develop resistance to the cellular actions of insulin, characterized by an impaired ability of insulin to inhibit glucose output from the liver and to promote glucose uptake in fat and muscle (8, 9).

Systemic oxidative stress, defined as a persistent imbalance between the production of highly reactive molecular species (chiefly oxygen and nitrogen) and antioxidant defenses, correlates with fat accumulation in humans and mice (10-12). The hypothesis that oxidative stress is a causative factor in the development of insulin resistance has been supported by several studies that showed that reversal of the imbalance between reactive oxygen species (ROS) and antioxidants improves insulin resistance in mice and humans (13-15). Also, several clinical trials have demonstrated improvement of insulin sensitivity in insulin-resistant and diabetic patients treated with antioxidants, suggesting that Oxidative stress has been linked to insulin resistance (16-18).

Obesity is associated with enhanced lipid peroxidation, one of the most frequently used biomarkers providing an indication of lipid peroxidation level is the plasma concentration of

malondialdehyde (MDA), one of several by-products of lipid peroxidation processes (19). Total antioxidant capacity (TAC) assays have been designed to determine overall antioxidant power of samples contributed by antioxidant and their interactions. Evaluation of TAC in body fluid has been used as one of the biological markers for monitoring oxidative stress in humans (20-22).

The present study aims to investigate the implication of oxidative stress with obesity in insulin resistance in nondiabetic mildly obese men, and to assess the total antioxidant capacity and lipid peroxidation and correlate them with adiposity measures.

2. Patients and Methods

This study comprised 57 healthy volunteers, 31 men with mild obesity and 26 age-matched nonobese healthy men. The subjects were defined as obese if their BMI was ≥ 30 kg/m², as proposed by the National Institutes of Health (23). None of the subjects had diabetes mellitus, hypertension, or active smoking history. None of the subjects were receiving any medication that could affect insulin levels, insulin sensitivity, or oxidative stress, and they were not under any regular exercise or dietary therapy before the beginning of this study. All subjects sharing in this study were subjected to complete full history taking and clinical examination to exclude any disease affecting their oxidative stress status as renal and liver diseases or any inflammatory diseases.

Informed consent was obtained from all subjects before the beginning of the study. Anthropometric measurement was carried out including (weight, height and Waist circumference). The Waist circumference (WC) was measured in standing position, midway between the lowest rib and the iliac crest with a measuring tape. Increased WC was defined as a value >102 cm, as recommended by the National Cholesterol Education Programme-Adult Treatment Panel III (NCEP-ATP III) for men (24).

Sample collection

Venous blood samples (after fasting for about 12 hours) were collected in sterile tubes. Samples were immediately cooled to 4 °C and centrifuged at 3,000 rpm for 10 minutes. Serum was divided into two aliquots, one used immediately for measuring of fasting blood glucose, lipid profile (triacylglycerides, total cholesterol, HDL-c and LDL-c) to test for linear relations between variables. P-values less than or equal to 0.05 were considered statistically significant

c), using commercially available kits. The second aliquot was stored at -80°C for the assay of insulin level, total antioxidant capacity and malondialdehyde levels .

Subjects were asked to eat their ordinary breakfast meal, and another sample was drawn exactly 2 hours after the completion of their meal for measurement of the 2 hour post prandial blood sugar.

Human Insulin assays:

The concentration of serum insulin was measured with an enzyme-linked immunosorbent assay (BioSource Europe S.A.) (25).

Total antioxidant capacity assays:

The determination of the antioxidative capacity is performed by the reaction of antioxidants in the sample with a defined amount of exogenously provide hydrogen peroxide (H_2O_2) The antioxidants in the sample eliminate a certain amount of the provided hydrogen peroxide. The residual H_2O_2 is determined colorimetrically by an enzymatic reaction which involves the conversion of 3,5-dichloro -2- hydroxyl benzenesulphonate to a colored product (Biodiagnostic , Egypt) (26).

Malondialdehyde assays:

Thiobarbituric acid (TBA) reacts with malondialdehyde (MDA) in acidic medium at temperature of 95°C for 30 min to form thiobarbituric acid reactive product which can be detected colorimetrically (27).

Insulin resistance was assessed by using the homeostasis model assessment (HOMA). The HOMA-IR was derived as estimates of insulin sensitivity. HOMA-IR was calculated using the formula:

fasting insulin (U/mL) X fasting glucose (mmol/L)/22.5 . Subjects were considered as insulin resistant when $\text{HOMA} \geq 2.6$. (28, 29).

Statistical Analysis

The data was coded and entered using the statistical package SPSS version 15. The data was summarized using descriptive statistics: mean, standard deviation, minimal and maximum values for quantitative variables and number and percentage for qualitative values. Statistical differences between groups were tested using independent sample t test, ANOVA (analysis of variance) for quantitative normally distributed variables and Nonparametric Mann Whitney test for quantitative variables which are not normally distributed. Correlations were done

3. Results

As expected, several differences were apparent between the two groups with regards to descriptive characteristics (Table 1).

BMI were significantly higher in obese men as compared to the controls. As it was expected, the obese group had significantly higher fasting serum

glucose, serum cholesterol, LDL-cholesterol values as compared to controls. The fasting HDLcholesterol and triglyceride level were not significantly different between obese patients and the controls.

Obese men showed significant increased values of HOMA-IR, serum MDA and TAC compared with nonobese men ($p < 0.001$) (Table 2).

Table (1) Descriptive characteristics of obese and non obese (control) men.

Variable	Non obese (n = 26)	Obese (n = 31)	P value
Age(years)	33.31±4.96	32.84± 4.99	0.72 (NS)
BMI (Kg/m ²)	23.00 ± 1.79	31.34± 0.84	<0.001*
Waist circumference(cm)	82.50±	106.29±3.29	<0.001*
Fasting glucose (mg/dl)	91.08±14.00	100.16±8.09	0.006*
Postprandial glucose(mg/dl)	118.73±17.17	123.55±8.97	0.20 (NS)
Total cholesterol (mg/dl)	184.73±43.38	222.55±39.01	0.001*
LDL- cholesterol (mg/dl)	102.73±21.41	130.81±26.65	<0.001*
HDL-cholesterol (mg/dl)	52.96±10.68	49.68±11.05	0.26 (NS)
Triglycerides (mg/dl)	123.38± 49.40	142.06± 77.46	0.42(NS)

(NS) means nonsignificant. *Means significant.

Table (2). Comparison of HOMA-IR, serum MDA and TAC between obese and non obese (control) men.

Variable	Non obese (n = 26)	Obese (n = 31)	P value
HOMA-IR	1.58 ± 0.51	5.03 ± 1.93	< 0.001*
Serum MDA (nmol/ml)	2.54 ± 0.88	6.10 ± 2.05	< 0.001*
Serum TAC (mM / L)	1.84 ± 0.53	1.10 ± 0.43	< 0.001*

HOMA-IR values were significantly positive correlated with serum levels of MDA in all (obese and nonobese) subjects ($r = 0.78$; $p < 0.001$), while were significantly negative correlated with serum TAC ($r = -0.50$; $p < 0.001$) (Table 3) (Fig. 1, 2). Also a significant positive correlation was found between serum MDA levels and BMI in all subjects

($r = 0.69$, $p < 0.001$), while serum TAC were significantly negative correlated with both serum MDA and BMI ($r = -0.43$, -0.64 , $p = 0.001$; < 0.001 respectively) (Table 3).

A positive correlations were also observed between HOMA-IR values and BMI in all subjects ($r = 0.70$, $p < 0.001$) (Table 3).

Table (3). Correlation coefficients (r) among HOMA-IR, serum MDA , serum TAC and BMI in all subjects.

	BMI(Kg/m2)	Serum MDA (nmol/ml)	Serum TAC (mM / L)
HOMA-IR	r = 0.70 p = <0.001*	r =0.78 p = <0.001*	r = -0.50 p = <0.001*
Serum MDA (nmol/ml)	r = 0.69 p = <0.001*		r = -0.43 p = 0.001*
Serum TAC (mM / L)	r = -0.64 p = <0.001*		

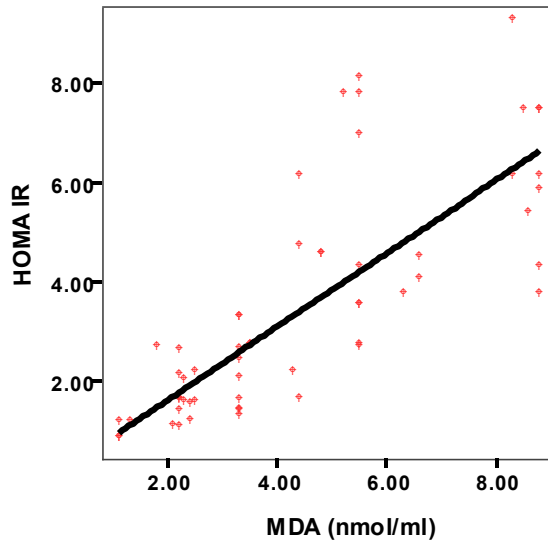


Fig. (1) Correlation between the serum levels of MDA and HOMA-IR in all (obese and nonobese) subjects.

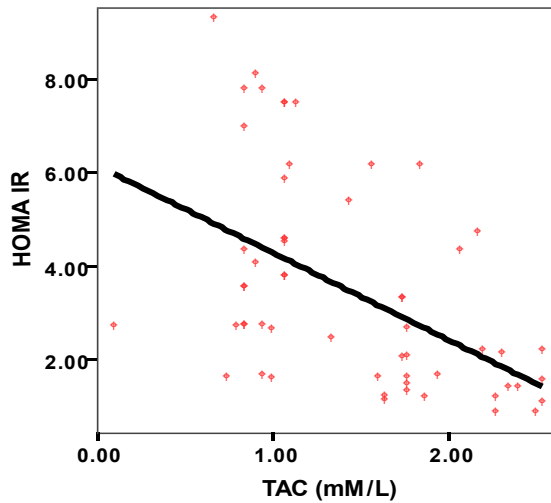


Fig. (2) Correlation between the serum levels of TAC and HOMA-IR in all (obese and nonobese) subjects.

In obese and non-obese men, serum MDA levels were found to be significantly positive correlated with waist circumference (WC) and serum fasting glucose, cholesterol, LDL-cholesterol, while TAC serum levels significantly negative correlated with (WC) and LDL- cholesterol (table 4).

A significant positive correlations were observed between HOMA-IR values and (WC) and levels of cholesterol and LDL-cholesterol in all subjects (Table 4).

Table (4). Correlation coefficients (r) among HOMA-IR, serum MDA , serum TAC and other parameters in all subjects.

	Fasting glucose(mg/dl)	Total cholesterol(mg/dl)	LDL-cholesterol(mg/dl)	Waist circumference(cm)
HOMA-IR	r = 0.313 p = 0.01*	r = 0.422 p = 0.001*	r = 0.420 p = 0.001*	r = 0.692 p = <0.001*
Serum MDA (nmol/ml)	r = 0.361 p = 0.006*	r = 0.462 p = <0.001*	r = 0.348 p = 0.008*	r = 0.665 p = <0.001*
Serum TAC (mM / L)	r = -0.258 p = 0.052*	r = -0.244 p = 0.067	r = -0.366 p = 0.005*	r = -0.598 p = <0.001*

4. Discussion:

A large number of endocrine, inflammatory, neural, and cell-intrinsic pathways have been shown to be dysregulated in obesity. Although it is possible that one of these factors plays a dominant role, many of these factors are interdependent, and it is likely that their dynamic interplay underlies the pathophysiology of insulin resistance.

The present results provide considerable insight into the relationship between obesity, oxidative stress by the significant higher levels of MDA in obese compared to nonobese men and at the same time, there was a significant decrease in serum TAC in obese as compared to nonobese. These results are consistent with *Araki et al., 2010* (30) who suggested that oxidative stress is enhanced in relation to visceral fat accumulation in childhood obesity and proposed that Oxidative stress may be associated with the development of obesity-related complications. Also, *Franch et al., 2010* (31) evaluated the presence of oxidative stress in obese children by measuring the levels of markers of oxidative damage (malondialdehyde [MDA], and plasma carbonyl groups [CG]) and measures of antioxidant defense, such as the enzyme glutathione peroxidase (GPx) and low molecular scavengers (erythrocyte-reduced glutathione [GSH], α -tocopherol and β -carotene), they founded that severe childhood obesity is associated with oxidative stress. Thus, recommended that, providing foods with high antioxidant capacity in addition to a hypocaloric diet is crucial for the treatment of obese children. Several studies supported the results of the present study and

concluded also that larger indices of adiposity were found to be associated with increased levels of oxidative stress and decreased levels of antioxidant defense (32-34).

The results of this study provide also relevant information concerning the link between obesity and oxidative stress and the development of insulin resistance. HOMA-IR was significantly positive correlated with serum levels of MDA, while significantly negative correlated with serum TAC. Increased oxidative stress together with the decreased antioxidative defence seems to contribute to decreased insulin sensitivity and impaired insulin secretory response in obese diabetics, and may be hypothesized to favour the development of diabetes during obesity (35). Oxidative stress leads to the activation of multiple serine kinase cascades (36).

There are a number of potential targets of these kinases in the insulin-signaling pathway, including the insulin receptor (IR) and the insulin receptor substrate (IRS) family of proteins (36, 37,12). Increased phosphorylation of the IR or IRS on discrete serine or threonine sites decreases the extent of their tyrosine phosphorylation, and is consistent with impaired insulin action (37). The activation of each pathway (nuclear factor-kB [NF-kB], p38 map kinase [MAPK], and N-terminal JUN kinase [JNK]), which can affect insulin action, is sensitive to oxidative stress (12). The present results are supported by *Koca et al., 2009* who found that, Insulin resistance is related with oxidative stress in systemic lupus erythematosus and stated that, In inflammatory diseases, relations between oxidative

stress and insulin resistance, each of them triggers or enhances the other one.

Also several studies have shown correlation between hyperinsulinemia and free radical production in human fat cells and rats (38, 39,40). Urakawa *et al.*, 2003 (41) suggested that hyperinsulinemia and insulin resistance may play a role in the pathogenesis of oxidative stress. On the other hand, it was previously reported that insulin exerts a potent anti-inflammatory effect and that it reduces ROS generation by mononuclear cells in obese subjects (42). Shamir *et al.* (43) reported insulin-mediated reduction of oxidative stress in apolipoprotein E-deficient mice. These observations suggest that insulin may have a protective role against increased oxidative stress.

By contrast, studies discovered that acute oxidative stress not only did not impair insulin signaling, but increased insulin dependent IRS1 and AKT phosphorylation and reversed hyperglycemia-induced insulin resistance, restoring insulin stimulation of glucose uptake (44). Several reports also indicate positive effects of oxidative stress on glucose uptake in adipose cells and in isolated muscles. For example, oxidative stress induced by either peroxide or xanthine oxidase increases glucose uptake in isolated human muscle, in a PI3K-dependent manner (45).

Another example of a positive effect of oxidative stress on insulin sensitivity is aerobic exercise. Muscle contraction, which is accompanied by increased oxidative phosphorylation, was shown to significantly increase oxidative stress in human and rodent muscle, followed by activation of MAPK signaling pathways, including JNK (46, 47).

Studies that oppose the present results were related to acute status of oxidative stress while this study included obese men which represent a chronic oxidative stress state (48).

In brief, the present study showed that the decrease in TAC and increase MDA (indicators of oxidative stress) are associated with mild obesity and insulin resistance in men and these findings suggest that obesity is an important factor for enhanced oxidative stress and that this oxidative stress triggers the development of insulin resistance in men. This may be of value in measures should be followed to avoid development of type 2 DM in mild obese men. Further studies are recommended including more sample size and broader classes of obesity to assess the degree of insulin resistance relative to the severity of oxidative stress due to obesity, to clarify the mechanisms underlying this association and elucidate the effect of supplementation of antioxidant vitamins to obese subjects on insulin resistance.

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