Impact of weight loss on oxidative stress and inflammatory cytokines in obese type 2 diabetic patients.

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Abstract

Background: Type 2 diabetes mellitus is associated with abnormal markers of inflammatory cytokines and oxidative stress markers. Although, these abnormalities could be modulated with weight reduction; there is limitation in clinical studies that have addressed the beneficial effects of weight reduction in modulating biomarkers of inflammatory cytokines and oxidative stress for obesity associated with type 2 diabetes mellitus.

Objective: This study was designed to detect the effects of weight loss on the inflammatory cytokines, oxidative stress markers in obese type 2 diabetic patients.

Material and Methods: Eighty obese patients with type 2 diabetes mellitus, their age ranged from 35-57 years and their body mass index ranged from 31-35 kg/m^2 were equally assigned into 2 groups: the weight reduction group received aerobic exercises, diet regimen, where as the control group received medical treatment only for 12 weeks.

Results: The mean values of body mass index (BMI), tumor necrosis factor–alpha (TNF-α), interleukin-6 (IL-6), C-reactive protein (sCRP), conjugated dienes (CD) and malondialdehyde (MDA) were significantly decreased, while the mean values of glutathione peroxidase (GPx), superoxide dismutase (SOD) and glutathione (GSH) were significantly increased in patients of group (A), while changes were not significant in group (B). Also, there were significant differences between mean levels of the investigated parameters in group (A) and group (B) at the end of the study.

Conclusion: Weight loss ameliorates inflammatory cytokines and oxidative stress markers in obese type 2 diabetic patients.

Keywords: Type 2 diabetes, weight reduction, oxidative stress, cytokines, obesity.

Introduction

Type 2 diabetes mellitus (T2DM) is considered nowadays as a worldwide epidemic and it is responsible for approximately 90% of all cases of diabetes in the world, also it is one of the most important chronic disturbances because of the significant number of people with diabetes and its severe chronic complications, responsible for elevated indexes of morbidity and mortality[1]. According to World Health Organization (WHO), the global forward estimation is that in the year 2030, 366 million people will present the disease[2]. Patients with T2DM have a two–four times increased cardiovascular disease (CVD) risk compared to healthy controls[3].

Adipose tissue represents an important source of cytokines, however excess body fat mass has been associated with increased levels of inflammatory cytokines such as TNF-α and IL-6[4,5]. Moreover, a decreased production of IL-10 by macrophages and lymphocytes has been associated with consequent increase of inflammatory responses in patients with Type 2 diabetes mellitus[6]. Low-grade systemic inflammation is characterized by a two to threefold increase in systemic plasma concentrations of cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-6 and C-reactive protein (CRP)[7]. However, diabetes impacts on the different steps in the pathogenesis of cardiovascular diseases (CVD)[8-10]. T2DM is often associated with a "low-grade inflammatory status" accompanied by insulin resistance[11]. Obesity and IR in patients suffering from diabetes are associated with a chronic systemic inflammation, characterized by an increased expression of

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inflammatory markers. Multiple stimuli are among the most common causes of myocardial inflammation in cardiomyopathies in diabetic patients[12-14]. Systemic inflammatory markers are risk factors for the development of type 2 diabetes and its macro-vascular complications[15]. Oxidative stress plays a key role in the pathogenesis of micro- and macro-vascular diabetic complications. The increased oxidative stress in subjects with T2DM is a consequence of several abnormalities, including hyperglycemia, IR, inflammation and dyslipidemia[16]. Oxidative stress causes insulin resistance, β-cell dysfunction and late diabetic complications[17]. It was demonstrated that markers of inflammation predict or/and are associated with T2DM and that inflammation is involved in the pathogenesis of atherosclerosis, a common feature of type 2 diabetes[18]. Free radicals are positively correlated with diabetic macro-vascular and micro-vascular complications[19].

Obesity is associated with enhanced lipid peroxidation[20]. Oxidative stress is involved in pathological processes such as obesity, diabetes and cardiovascular diseases. Obese subjects have higher levels of oxidative stress biomarkers compared with control group[21]. Oxidative stress is known as the imbalance between oxidants and antioxidants in favor of the oxidants, potentially leading to cellular damage[22]. Free radicals are reactive oxygen species (ROS) having an unpaired electron, which are generated under physiological conditions during aerobic metabolism. These free radicals have the potential to trigger chain reactions when they react with proteins, lipids and other biological molecules, which are fatal to the cell. Under normal conditions, the free radicals which are produced are scavenged by a repertoire of enzymatic antioxidants like SOD, catalase, glutathione peroxidase (GPx), etc. and also by non-enzymatic antioxidants like vitamin C, a-tocopherol, cerulo-plasmin, GSH etc., thus preventing the oxidative stress[23,24].

Caloric restriction (CR), weight loss and exercise ameliorate the classic CVD risk factors hypertension and dyslipidemia among patients with T2DM[25]. In addition, weight loss and lifestyle interventions decrease plasma IL6 and TNFα levels in obese non-diabetic subjects[26]. Therefore, the aim of this study was to assess the effects of weight loss on the inflammatory cytokines, oxidative stress markers in obese type 2 diabetic patients.

Material and methods
Subjects
The subjects were consecutively recruited among patients referred from the outpatient clinics of the Internal medicine department at King Abdul Aziz University Hospital and other Hospitals in Jeddah, Saudi Arabia. Inclusion criteria were age (42-55 years), body mass index (BMI) 30-35 kg/m^2 and type 2 diabetes mellitus. We excluded patients undergoing any kind of active treatment that affect the endothelial function, as well as pregnant women, those with chronic kidney or liver disease and those with congestive heart failure; uncontrollable cardiac arrhythmias, hypertension, musculoskeletal disorders. The subjects were allocated randomly into two study groups: either to the lifestyle intervention group (A) who received treadmill aerobic exercise training and diet regimen or to the control group (B) who received no exercise training and no diet regimen. The original sample consisted of 236 participants who underwent the eligibility assessment. In the enrollment phase, 104 of them were excluded as they didn't meet inclusion criteria and 32 refused to participate, then the randomization was done. This substudy thus included 109 subjects (59 patients in the intervention group and 50 patients in the control group). During the follow up, in the intervention group 6 patients discontinued intervention (3 patients disliked the diet regimen, 2 patients had work related schedule problems and 1 patient discontinued due to unknown reasons) and in the control group 3 patients discontinued intervention (2 patients had work related schedule problems and 1 patient discontinued due to unknown reason). In addition, 5 patients in the intervention group and 4 patients in the control group were excluded from the analysis due to insufficient blood sample. This study was approved by the Ethical Committee for Scientific Research, Faculty of Applied Medical Sciences, King Abdulaziz University. All participants provided written informed consent.

Measurements
In all subjects, clinical and anthropometric data was collected at the time of enrollment. Clinical evaluations and

laboratory analysis were performed by independent assessors who were blinded to group assignment and not involved in the routine treatment of the patients. Body mass index (BMI) was calculated on the basis of weight (kilograms) and height (meters), and subjects were classified as normal weight (BMI 18.5–24.9 kg/m²), overweight (BMI 25–29.9 kg/m²), and obese (BMI ≥30 kg/m²). In addition, between 07:30 and 09:00, after an overnight fast of 12 h fasting blood sample was drawn. The plasma lipid profile (total cholesterol, total triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL), plasma glucose concentration and insulin were determined (Roche Diagnostics GmbH, Mannheim, Germany) using commercially available assay kits. Insulin resistance was assessed by homeostasis model assessment (HOMA-IR). HOMA-IR = fasting blood glucose (mmol/l) - fasting insulin (mIU/ml)/22.5[27].

A. Measurement of oxidative stress markers and anti-oxidant status:
For all participants serum (from 10 ml blood in plain vial) and plasma (from 5 ml blood in EDTA vial) were separated from the sample within 30 min of collection and stored in pyrogen free polypropylene cryo-tubes at (-80°C) until analysis. Assessment of lipid markers for peroxidation such as malondialdehyde (MDA) and conjugated dienes (CD) were determined according to Buege and Aust method.[28] However, Anti-oxidant status, glutathione (GSH) was determined by the method of Beutler and colleagues[29], on the other hand, glutathione peroxidase (GPx) and superoxide dismutase (SOD) were measured by the method of Nishikimi and colleagues[30].

B. Measurement of inflammatory cytokines:
Venous blood samples after a 12-hours fasting were centrifuged at 4 °C (1000 X g for 10 min). Interleukin-6 (IL-6) levels were analyzed by "Immulite 2000" immune-assay analyzer (Siemens Healthcare Diagnostics, Deerfield, USA). However, (TNF-α), (sCRP) and interleukin-8 (IL-8) levels were measured by ELISA kits (R&D, USA) using ELISA technique (ELX 808; Bio-Tek Instruments, USA). All measurements of BMI, IL-6, TNF-α, sCRP, MDA, CD, CPX, GSH and SOD were taken before the start of the study (pre-test) and after three months at the end of the study (post-test).

Procedures
We hypothesized that weight reducing program included treadmill aerobic exercise and diet regimen for 12 weeks would result in modulation in inflammatory cytokines and oxidative stress markers in obese patients with type 2 diabetes mellitus. As such, we randomized our subjects into either a training group(A) or a control group (B). As All patients were divided randomly into the following groups:

1. The training group (Group A):
Patients were submitted to the aerobic exercise training to complete a 12-week aerobic exercise-training program on a treadmill aerobic exercise (Enraf Nonium, Model display panel Standard, NR 1475.801, Holland). Each session of physical exercise was divided in: 5 min of warm up, with stretching exercises and circling of members and body; 30 min of aerobic exercise divided into row ergometer (15 min) and bicycle ergometer (15 min).; and 5 min of cold down at the end, with stretching, flexibility and relaxation exercises, consisting of five sessions per week. The training program was performed at 70% of the individual age-predicted HRmax according to Tanaka et al., [31]. In addition, a dietician performed an interview-based food survey for all participants of group (A) for detection of feeding habits, abnormal dietary behavior and to prescribe the balanced low calorie that provide 1200 Kilo calories/day for 12 weeks.

2. The control group (Group B):
Patients maintained their ordinary life style and received no exercise or diet regimen training.

Statistical analysis
All results are presented as means ± SD. The mean values of the investigated parameters obtained before and after the study. Independent "t" test was used for the comparison between the two groups (P<0.05).

Results
The two groups considered homogeneous regarding the baseline clinical variables (Table 1).

The mean values of body mass index (BMI), (TNF-α), (IL-6), (sCRP), conjugated dienes (CD) and malondialde-hyde (MDA) were significantly decreased, while the mean

Table (1): Baseline clinical participants' characteristics in both groups

	Mean ±SD		Significance
	Intervention group (n:40)	**Control group (n:40)**	
Age (year)	48.36 ± 5.12	47.17 ± 5.63	P >0.05
Gender (Male/Female)	23/17	21/19	P >0.05
Weight (kg)	88.92 ± 5.76	90.11± 6.24	P >0.05
Height (cm)	169.17 ± 8.22	171.37 ± 7.81	P >0.05
Waist-hip ratio	0.86 ± 0.05	0.88 ± 0.07	P >0.05
BMI (kg/m^2)	31.85 ± 3.46	32.24 ± 3.62	P >0.05
SBP (mm Hg)	123.41 ± 7.23	125.16 ± 8.27	P >0.05
DBP (mm Hg)	82.65 ± 6.81	84.12 ± 5.78	P >0.05
TC (mg/dl)	193.14 ± 9.55	195.26 ±10.17	P >0.05
LDL-c (mg/dl)	132.53 ± 7.11	135.16 ± 8.26	P >0.05
TG (mg/dl)	154.05 ± 9.25	156.49 ±8.77	P >0.05
HDL-c (mg/dl)	35.21 ± 2.15	33.85 ±2.36	P >0.05
HOMA-IR	4.82 ± 1.67	4.96 ± 1.44	P >0.05
HbA1C (%)	7.66±1.58	7.83±1.73	P >0.05
Diabetes duration (year)	11.16 ± 2.11	12.24 ± 1.88	P >0.05

BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; TC: Total cholesterol; LDL-c: Low-density lipoprotein cholesterol; TG: Triglyceride; HDL-c: High-density lipoprotein cholesterol; HOMA-IR: Homeostasis Model Assessment-Insulin Resistance Index; HbA1C: Glycosylated hemoglobin; * Significant level (p<0.05).

values of glutathione peroxidase (GPx), superoxide dismutase (SOD) and glutathione (GSH) were significantly increased in patients of group (A) (Table 2), while changes were not significant in group (B) (Table 3).
Also, there were significant differences between mean levels of the investigated parameters in group (A) and group (B) at the end of the study (Table 4).

Table (2): Mean value and significance of different variables such as (IL-6, TNF-α, sCRP, ICAM-1, VCAM-1and PAI-1: Ac) in-group (A) before and at the end of the study.

	Mean ±SD		Significance
	Before	After	
BMI (kg/m^2)	31.85 ± 3.46	25.47 ± 2.65*	P<0.05
TNF- α (pg/mL)	11.94 ± 2.63	8.23 ± 2.48*	P<0.05
IL-6 (pg/mL)	5.87 ± 1.34	3.41 ± 1.25*	P<0.05
sCRP (mg/L)	4.26 ± 1.22	2.12 ± 0.91*	P<0.05
CD (mmol/L)	24.66 ± 5.14	18.72 ± 4.15*	P<0.05
MDA (mmol/L)	25.27 ± 5.26	19.13 ± 4.32*	P<0.05
GPx (units/gHb)	21.33 ± 4.11	26.22 ± 3.73*	P<0.05
SOD (units/mL)	43.51 ± 5.57	*6.28 ± 52.87	P<0.05
GSH (mmol/gHb)	2134.11 ± 173.18	2588.25 ± 191.17*	P<0.05

BMI: Body mass index; TNF- α: tumor necrosis factor – alpha; IL-6: Interleukin-6; sCRP: C- reactive protein; CD: conjugated dienes; MDA: Malondialdehyde; GPx: Glutathione peroxidase; SOD: Superoxide dismutase; GSH: Glutathione; * Significant level (p<0.05).

Table (3): Mean value and significance of different variables such as (IL-6, TNF-α, sCRP, ICAM-1, VCAM-1and PAI-1: Ac) in-group (B) before and at the end of the study.

	Mean +SD		Significance
	Before	After	
BMI (kg/m^2)	32.24 ± 3.62	32.41 ± 3.68	P >0.05
TNF- α (pg/mL)	12.13 ± 2.54	12.26 ± 2.56	P >0.05
IL-6 (pg/mL)	5.91 ± 1.43	5.95 ± 1.61	P >0.05
sCRP(mg/L)	4.43 ± 1.29	4.51± 1.32	P >0.05
CD (mmol/L)	25.17 ± 4.76	25.68 ± 4.79	P >0.05
MDA (mmol/L)	25.36 ± 5.12	25.72 ± 5.26	P >0.05
GPx (units/gHb)	20.98 ± 3.75	20.63 ± 3.41	P >0.05
SOD (units/mL)	43.16 ± 5.14	42.77 ± 4.96	P >0.05
GSH (mmol/gHb)	2091.30 ± 162.72	2064.83± 151.41	P >0.05

BMI: Body mass index; TNF- α: tumor necrosis factor – alpha; IL-6: Interleukin-6; sCRP: C-reactive protein; CD: conjugated dienes; MDA: Malondialdehyde; GPx: Glutathione peroxidase; SOD: Superoxide dismutase; GSH: Glutathione.

Discussion

Type 2 diabetes mellitus is the most prevalent metabolic disease in the world[32], which is related to vascular prob-

Table (4): Mean value and significance of (IL-6, TNF-α, sCRP, ICAM-1, VCAM-1 and PAI-1: Ac) in group (A) and group (B) at the end of the study.

	Mean +SD		Significance
	Group (A)	Group (B)	
BMI (kg/m^2)	25.47 ± 2.65*	32.41 ± 3.68	P<0.05
TNF- α (pg/mL)	*8.23 ± 2.48	12.26 ± 2.56	P<0.05
IL-6 (pg/mL)	3.41 ± 1.25*	5.95 ± 1.61	P<0.05
sCRP(mg/L)	2.12 ± 0.91*	4.51± 1.32	P<0.05
CD (mmol/L)	18.72 ± 4.15*	25.68 ± 4.79	P<0.05
MDA (mmol/L)	*19.13 ± 4.32*	25.72 ± 5.26	P<0.05
GPx (units/gHb)	26.22 ± 3.93*	20.63 ± 3.41	P<0.05
SOD (units/mL)	52.87 ± 6.28*	42.77 ± 4.96	P<0.05
GSH (mmol/gHb)	2588.25 ± 191.17*	2064.83 ± 151.41	P<0.05

BMI: Body mass index; TNF- α: tumor necrosis factor – alpha; IL-6: Interleukin-6; sCRP: C- reactive protein; CD: conjugated dienes; MDA: Malondialdehyde; GPx: Glutathione peroxidase; SOD: Superoxide dismutase; GSH: Glutathione; * Significant level (p<0.05).

lems[33,34]. The American Heart Association recommended weight loss to reduce the severity of cardiovascular risk factors in overweight and obese patients[35]. The main finding of the present study was that weight reducing program ameliorated inflammatory cytokines (TNF-α, IL-6, sCRP) and markers of oxidative and anti-oxidative stress (MDA, CD, CPX, GSH and SOD) in obese patients with type 2 diabetes mellitus as a result of weight loss, these results are in line with many previous studies.

The effect of dietary interventions on sCRP levels has been studied in obese non-diabetic subjects. Weight loss was clearly associated with a decrease in sCRP in these subjects and related to the amount of weight loss[36]. In obese non-diabetic patients, diet, exercise or combined interventions have controversial effects on plasma IL-6 and TNF-α level[37-43]. Sheu and colleagues reported that 5% of body weight loss obtained after 12 weeks of caloric restriction and exercises resulted in significant reduction in TNF-α and IL-6 of obese women[44]. Lang and colleagues established that a weight-reducing program had anti-atherogenic and inflammatory effects in their study on three obese men and eleven obese women for eight weeks[45]. Choo and colleagues proved that weight-reducing program in the form of diet regimen for three

months followed by diet regimen added to exercise intervention for nine months had a remarkable reduction in the risk of cardio-metabolic and subclinical atherosclerosis[46]. Madsen and colleagues stated that inflammatory markers were reduced significantly if body weight was reduced by 10% in obese subjects[47]. Esposito e al. suggested that weight-reducing program for 2 years significantly reduced C-reactive protein[48]. In addition, Nicklas and colleagues stated that 12 months life style modification program significantly reduced TNF-α level in obese individuals[49]. Reductions in pro-inflammatory cytokines concentrations after weight loss is explained by reduction in fat mass[50].

Concerning the markers of oxidative and anti-oxidative stress, the observation in this study indicated a significant reduction in MDA & CD and increased in CPX, GSH and SOD as a result of weight loss at the end of the study. Nevertheless, the current data is in line with one previous study by Roberts et al. Proved that after three weeks of combination between diet and exercise there was a significant reduction in BMI, lipid profile, fasting blood sugar, sCRP and insulin homeostasis which ameliorates oxidative stress, inflammation and monocytes-endothelial interaction among diabetic patients[51].

The possible mechanism for modulation of the oxidative stress markers induced by weight reduction could be due to reverse the mechanism by which obesity produces oxidative stress. The first of these is the mitochondrial and peroxisomal oxidation of fatty acids, which can produce ROS in oxidation reactions, while another mechanism is over-consumption of oxygen, which generates free radicals in the mitochondrial respiratory chain that is found coupled with oxidative phosphorylation in mitochondria. Lipid-rich diets are also capable of generating ROS because they can alter oxygen metabolism[52]. So that upon reduction of adipose tissue, the activity of antioxidant enzymes such as SOD, catalase (CAT), and GPx, was found to be significantly increased.

Conclusion
Weight loss ameliorates inflammatory cytokines and oxidative stress markers in obese type 2 diabetic patients.

Acknowledgment

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