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Original Article

Association of body mass index with oxidative stress in patients with Type 2 diabetes: do apolipoprotein A-II -265T > C polymorphism alter this association?

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Background: To compare the oxidative stress (OS) factors in obese and non-obese subjects with Type 2 diabetes (T2D) and to evaluate the interaction between the apolipoprotein A-II (Apo A-II) - 265T > C polymorphism and body mass index (BMI) on OS factors.

Methods: In this comparative cross-sectional study, 21 diabetes centers in Tehran, Iran were studied. 180 patients with T2D were divided into two groups of 90 obese (BMI ≥ 30) and 90 non-obese (BMI < 30) with equal numbers of each genotypes of Apo A-II (TT = 30, CC = 30 and TC = 30). Superoxide dismutase (SOD) activity, total antioxidant capacity (TAC) and 8-isoprostane F2α concentration were compared between two groups obese and non-obese and between subjects with and without central obesity. Then, the interaction between the Apo A-II polymorphism and BMI on OS factors were analyzed by general linear model.

Results: After adjusting for confounding factors, it was observed that the mean of SOD activity and TAC were lower in the obese group than in the non-obese group ($p < 0.050$). The mean concentration of 8-isoprostane F2α was not statistically different between the two groups. Adjusting for BMI showed that the differences in the mean OS factors were not statistically significant between men and women with and without central obesity. It was also observed that the interaction between the Apo A-II polymorphism and BMI on OS factors were not significant.

Conclusion: OS level was the higher in obese subjects with T2D than non-obese one. The interaction between BMI and Apo A-II polymorphism had not a significant effect on OS factors.

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Introduction

The World Health Organization defines obesity as a body mass index (BMI) ≥ 30 [1]. Obesity results from an imbalance between energy intake and energy expenditure during an extended period of time [2]. The social, behavioral, psychological, metabolic, cellular, and molecular factors are involved in the development of obesity [3]. It also seems that some genetic variants modulate obesity risk [4, 5]. One of the possible factors contributing to obesity may be the apolipoprotein A-II (Apo A-II). Apo A-II is synthesized in the liver and is the second most abundant protein of high-density lipoprotein (HDL) particles [6]. The T > C transition at position -265 of Apo A-II gene promoter results in a reduction in the Apo A-II concentration [4]. This polymorphism is functional and some studies have shown that individuals homozygous for the allele C (CC) were more prone to obesity than T-allele carriers (TT/TC) [4, 6, 7]. On the other hand, it is known that Apo A-II concentration is associated with paraoxonase 1 activity which has antioxidant properties [8-10].

Diabetes mellitus is associated with long-term and debilitating complications and hyperglycemia can causes damage to major organs of the body such as the eyes, kidneys, heart, blood vessels, and nerves [11, 12]. The mechanisms by which diabetes causes these complications are complex and are not fully understood. Some evidence has been presented that oxidative stress (OS) plays a key role in the incidence and exacerbation of these complications [13, 14]. Studies have shown that reactive oxygen species (ROS) are important mediators in atherogenesis. ROS increases atherogenic process such as apoptosis of endothelial cells, the expression of endothelial adhesion molecule, inactivation of nitric oxide (NO), proliferation and migration of vascular smooth muscle cell and OS modification of lipoproteins [15]. Moreover, the adipose tissue produces hormones and pro-inflammatory cytokines, which can contribute to the OS [3].

Given the prevalence of obesity in Type 2 diabetes (T2D) and the possible relationship between obesity and genetic variants of Apo A-II in T2D outcomes, the current study was conducted to compare OS factors in obese and non-obese subjects with T2D. In addition, an interaction between the Apo A-II -265 T > C polymorphism and BMI on OS factors were studied in these patients groups.

Methods

In this comparative cross-sectional study, among 816 patients with T2D who had participated in the previous published study [16], 180 patients were selected using a table of random numbers. 816 patients had been gathered by random sampling from referral diabetics centers in Tehran. Subjects with fasting blood glucose ≥ 126 mg/dl or being treated by diabetic medicines were included. In present study, the target sample size was determined to allow detection of a 20% difference in mean serum concentration of at least one OS factor between the groups with an α error of 0.05 and a β power of 80%. This study was approved by the ethics committee of Tehran University of Medical Sciences.

Obesity was defined as BMI ≥ 30 kg/m^2 [1]. The subjects were divided into two groups of obese (BMI ≥ 30; n = 90) and non-obese (BMI < 30; n = 90) with equal numbers of each genotypes of Apo A-II (TT = 30, CC = 30 and TC = 30). Inclusion criteria were age 35-65 years and no use of insulin. All patients were non-smokers and did not consume narcotic and alcohol. None of the patients had any clinical symptoms of chronic diseases such as cardiovascular disease (CVD), stroke, liver disease, kidney failure, thyroid disorders, cancer, and inflammatory diseases. None of participants were receiving multivitamin-mineral supplement and anti-inflammatory medications in the past 3 months before start of the study.

In the previous published study, after obtaining informed consent from the patients, the information of age, sex, duration of diabetes, taking oral hyperglycemic agents, and lipid-lowering medications had been obtained using questionnaire and by interview. Height, weight and waist circumference (WC) of subjects were measured according to standard protocols [12]. Fasting weight to the nearest 100 g with minimal clothing, height to the nearest 0.5 cm and WC to the nearest 0.5 cm, using a non-stretching meter, were measured. BMI had been calculated. WC ≥ 102 cm for men and WC ≥ 88 cm for women was considered as central obesity [17]. The information of physical activity using validated self-administered questionnaire (MET) [18], and dietary intakes during the past year using a validated semi-quantitative food frequency questionnaire (FFQ) [19] were obtained.

After 12 hours fasting, blood sample was collected from each subject for biochemical and genetic experiments. Genomic DNA had been extracted using salting out method. Apo A-II

genotypes had been determined by TaqMan assay method and using real time-polymerase chain reaction [20]. Serum and plasma had been separated and stored at −80°C until analysis. The levels of triglycerides, total cholesterol, HDL-cholesterol, and low-density lipoprotein-cholesterol were determined by enzymatic method, using Pars Azmun Company kit. In the present study, total antioxidant capacity (TAC) was measured by spectrophotometry method [21]. Serum superoxide dismutase (SOD) activity was measured by colorimetric method, using the SOD assay kit (Cayman Chemical Company, USA), and serum 8-isoprostane F2α concentration was measured using the human 8-iso-Prostaglandin F2α ELISA Kit (Shanghai Crystal Day Biotech Co., Ltd.).

Nutritionist III software (version 7.0; N Squared Computing) was used to analyze the FFQ. Analyses were performed using Statistical Package for the Social Science (SPSS), version 20 (SPSS Inc., Chicago, IL, USA). The normal distribution of variables was assessed using the Kolmongorov–Smirnov test. Chi-square test was used to compare qualitative variables and independent t-test were performed for comparing mean quantitative variables.

According to the previous studies [4, 6, 22], TT and TC subjects were grouped and compared with CC subjects. Interaction between the genetic variants of Apo A-II and BMI on OS factors were analyzed by general linear model.

The analysis of covariance was applied to adjust the effect of confounding factors. Results were considered as statistically significant for a value of $p < 0.005$.

Results

In this study, 180 obese and non-obese patients with T2D, including 64 men and 116 women were studied (mean age 53.98 ± 0.49 years). The results including personal information, dietary intakes, and lipid profile in obese and non-obese groups have shown in Table 1. The means of age, WC and intake of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were significantly different between obese and non-obese subjects ($p \leq 0.001$). Correlation between MUFA and PUFA intake was > 0/2 in the collinearity test. Therefore, only the effect of MUFA was adjusted.

Table 1. Characteristics of study participants

Characteristics	Obese	non-Obese	p value
Study population (n)	90	90	-
Lipid lowering medications (n)	44	52	0.23*
Oral hyperglycemic agents (n)	86	81	0.15*
Age (years)	52.87 ± 0.74	55.10 ± 0.63	0.02†
BMI (kg/m^2)	33.02 ± 0.34	25.99 ± 0.23	<0.001†
WC (cm)	98.88 ± 0.95	85.62 ± 0.83	<0.001†
Physical activity, (Met.hour/day)	37.74 ± 0.51	39.10 ± 0.63	0.09†
Food intake			
Energy (Kcal/day)	2576.83 ± 95.16	2643.64 ± 130.36	0.68†
Carbohydrate (g/day)	347.44 ± 8.14	363.22 ± 6.69	0.25‡
Protein (g/day)	80.64 ± 1.88	84.04 ± 2.04	0.17‡
Fat (g/day)	110.11 ± 3.18	100.97 ± 3.44	0.12‡
PUFA (g/day)	26.83 ± 1.17	22.15 ± 1.19	0.02‡
MUFA (g/day)	28.76 ± 1.37	23.82 ± 1.29	0.01‡
SFA (g/day)	27.06 ± 1.18	26.58 ± 1.09	0.37‡
Vitamin E (mg/day)	24.17 ± 1.56	19.92 ± 1.37	0.17‡
Vitamin C (mg/day)	182.95 ± 10.26	180.73 ± 11.83	0.49‡
Vitamin A (µg/day)	715.73 ± 37.13	716.46 ± 44.26	0.70‡
Fiber (g/day)	39.85 ± 1.80	45.32 ± 3.41	0.11‡
Lipid profile			
Triglycerides (mg/dl)	190.75 ± 13.39	171.52 ± 11.85	0.28†
Total cholesterol (mg/dl)	200.40 ± 7.78	194.76 ± 9.79	0.65†
HDL-c (mg/dl)	53.48 ± 1.36	53.24 ± 1.25	0.89†
LDL-c (mg/dl)	112.75 ± 4.13	106.82 ± 3.91	0.29†

Data are presented as mean ± SE or number of subjects. *p value was calculated from Chi-square. †p value was calculated from independent t-test. ‡p value was calculated from ANCOVA and adjusted for energy by ANCOVA. PUFA = Poly unsaturated fatty acid; MUFA = Mono unsaturated fatty acid; SFA = Saturated fatty acid; HDL-c = High-density lipoprotein-cholesterol; LDL-c = Low-density lipoprotein-cholesterol

Table 2. Comparison of OS factors between obese and non-obese groups

Oxidative stress factors	Obese (n = 90)	Non-obese (n = 90)	p value*	p value†
SOD, U/ml	0.13 ± 0.009	0.15 ± 0.005	0.04	0.04
TAC, g/dl BSA	2.37 ± 0.04	2.58 ± 0.06	0.01	0.008
8-isoprostane F2α, pg/ml	73.90 ± 0.63	71.04 ± 0.62	0.002	0.12

Data are presented as mean ± SE. *p value was calculated from independent t-test. †Adjusted for WC, age, sex, MUFA by ANCOVA. BSA = Bovine serum albumin; SOD = Superoxide dismutase; TAC = Total antioxidant capacity, SE = Standard error, OS = Oxidative stress

Table 3. Comparison of OS factors between men and women with and without central obesity

Waist circumference	SOD, U/ml	TAC, g/dL BSA	8-isoprostane F2α, pg/ml
Men			
WC < 102 cm	0.15 ± 0.006	2.43 ± 0.07	70.37 ± 0.92
WC ≥ 102 cm	0.12 ± 0.011	2.36 ± 0.12	72.47 ± 1.40
p value*	0.04	0.61	0.23
p value†	0.36	0.72	0.76
Women			
WC < 88 cm	0.15 ± 0.005	2.65 ± 0.08	73.01 ± 0.72
WC ≥ 88 cm	0.14 ± 0.013	2.40 ± 0.06	73.70 ± 0.80
p value*	0.59	0.02	0.53
p value†	0.7	0.5	0.28

Data are presented as mean ± SE. *p value was calculated from independent t-test. †Adjusted for BMI by ANCOVA. BSA = Bovine serum albumin; SOD = Superoxide dismutase; TAC = Total antioxidant capacity; WC = Waist circumference; OS = Oxidative stress, SE = Standard error

Table 4. Interaction between the genetic variants of Apo A-II and BMI on OS factors

Oxidative stress factor	Obese		Non-obese		p value*	p value†
	CC (n = 30)	TT/TC (n = 60)	CC (n = 30)	TT/TC (n = 60)		
SOD, U/ml	0.10 ± 0.003	0.15 ± 0.013	0.13 ± 0.006	0.17 ± 0.007	0.28	0.32
TAC, g/dL BSA	2.27 ± 0.07	2.42 ± 0.06	2.45 ± 0.11	2.65 ± 0.07	0.77	0.71
8-isoprostane F2α, pg/ml	76.35 ± 1.21	72.68 ± 0.68	73.45 ± 1.10	69.84 ± 0.71	0.97	0.88

Data are presented as mean ± SE. *p value was calculated from general linear model. †Adjusted for WC, age, sex and MUFA by ANCOVA. BSA = Bovine serum albumin; SOD = Superoxide dismutase; TAC = Total antioxidant capacity; WC = Waist circumference, Apo A-II = Apolipoprotein A-II; BMI = Body mass index; OS = Oxidative stress, MUFA = Monounsaturated fatty acids, SE = Standard error

The results regarding OS factors in the two groups of obese and non-obese have shown in table 2. Serum SOD activity and TAC level were significantly lower and mean concentrations of 8-isoprostane F2α was significantly higher in the obese subjects than in the non-obese subjects ($p < 0.050$). After adjusting for confounding factors including WC, age, sex and MUFA, the mean of SOD activity and TAC were significantly lower in obese group in comparison to non-obese group ($p < 0.050$).

Table 3 shows mean OS factors in men and women according to WC. Then, the analysis was performed separately for each sex. The mean SOD activity was significantly lower in men with WC ≥ 102 cm than in men with WC < 102 cm ($p < 0.050$). Furthermore, mean TAC was significantly lower in women with WC ≥ 88 cm to compared women with WC < 102 cm ($p < 0.050$). After adjusting for BMI, it was found that OS levels did not differ between men and women with and without central obesity.

The interaction between the Apo A-II polymorphism and BMI on OS factors are shown in table 4. According to the previous studies [4, 6, 22], TT and TC subjects were grouped and compared with CC subjects. After adjusting for confounding factors, including WC, age, sex, and MUFA, we were observed that results were not significant.

Discussion

The aim of the present study was to compare the OS factors in obese and non-obese subjects with T2D and to evaluate interaction between Apo A-II -265 T > C polymorphism and BMI on OS factors. The results of present study showed that the mean SOD activity and TAC were lower and 8-isoprostane F2α concentration was higher in the obese group than in the non-obese group. After adjusting for confounding factors, there was significance difference in the mean SOD

activity and TAC between two groups.

The several studies have compared level of OS between obese and non-obese in healthy individuals [15, 23-27] and few studies have investigated OS in obese and non-obese patients with diabetes [28]. The prevalence of diseases associated with OS such as CVD is higher in patients with diabetes compared with healthy subjects [29]. Therefore, it is important to identify possible factors which may increase level of OS in these patients.

On the other hand, the results of studies on antioxidant enzymes activity are inconsistent in obese and non-obese subjects. Some studies have shown that the antioxidant enzymes activity such as SOD was higher in obese subjects than non-obese subjects [24, 28], but according to the results of the present study and some other studies, the SOD activity is lower in obese subjects [15, 26]. It seems that the OS initially increases levels and activities of antioxidants in the body to fight against free radicals but progress and continuity in OS gradually causes impaired antioxidant system and reduced antioxidant enzymes activities [30, 31].

Most studies have measured the SOD activity in erythrocytes. In the present study, serum SOD activity was investigated, which is the extracellular SOD, and it plays a key role in maintaining biological life of NO in endothelial tissue [32]. According to our knowledge, the present study is the first study that was compared serum SOD activity, TAC and 8-isoprostane F2α concentration between obese and non-obese subjects with diabetes.

There are several hypotheses for the possible mechanisms leading to increased ROS production in the obese subjects. The dipocytes and pre-adipocytes in adipose tissue are known as a source of proinflammatory cytokines such as tumor necrosis factor alpha, interleukin-1 (IL) and IL-6. These cytokines are strong stimulators for the production of reactive oxygen and nitrogen by macrophages and monocytes [33]. Angiotensin II is also secreted by adipose tissue, which stimulates activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. NADPH oxidase induces ROS production in adipocytes [34]. The hormone leptin is also produced by adipose tissue [35] and can directly stimulates the ROS production in endothelial cells [36]. Leptin also induces OS by increased proliferation of monocytes and macrophages and production of inflammatory cytokines [37]. On the other hand, obesity increases myocardial metabolism, which increase oxygen consumption. Increased oxygen consumption lead to production of ROS [23, 38].

In the present study, after adjusting for BMI, it was observed that mean TAC and SOD activity were lower and mean concentration of 8-isoprostane F2α was higher in men and women with central obesity compared to men and women with normal WC, but the differences were not statistically significant. The results of most studies show a significant increase in OS in subjects with central obesity compared to controls [23, 25, 27]. In addition, in some studies have not been observed a significant association between WC and OS factors [24]. The difference in studied population is probably responsible for inconsistent results. Participants in our study were T2D patients, but the above-mentioned studies have been conducted on healthy subjects. Hyperglycemia in diabetes can lead to OS by glucose auto-oxidation, non-enzymatic protein glycation and production of advanced glycation end products [39]. Therefore, in our study ROS production might be high in patients without central obesity which lead to the lack of the significant difference in OS levels between patients with and without central obesity.

The interaction between the Apo A-II polymorphism and BMI on OS factors also indicated that mean SOD activity and TAC were lower and mean 8-isoprostane F2α concentration was higher in the CC group compared to the TT/TC group. According to result of some studies, a T > C transition at position -265 results in decreased Apo A-II expression in hepatocytes and consequently decreased Apo A-II concentration in plasma [40, 41]. The results of animal studies have shown that decrease in Apo A-II concentration is associated with lower levels of blood leukocytes and inflammatory factors such as C-reactive protein [10, 42]. The inflammatory factors concentration is positively associated with OS level [3]. However, the interaction between the Apo A-II polymorphism and BMI on OS factors indicated no significant effect and these two variables did not change the effect of each other on OS level. According to our knowledge, no study has investigated the interaction between the two factors on OS factors. Therefore, more researches need to be conducted to further explore the relationships between the Apo A-II polymorphism and OS.

In our study, we did not evaluate leptin level which can be a limitation of the current study.

Conclusion

OS level was higher in obese subjects with T2D compared to non-obese one. Therefore, maintaining normal weight and weight loss through the changing lifestyle, improvement in nutrition and increasing physical activity may have an important role in the prevention of diabetes complications and improving the quality of life and increasing longevity in these patients. The interaction between BMI and Apo A-II polymorphism had not a significant effect on OS factors.

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Conflict of interest

None of the authors had any personal or financial conflicts of interest.

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