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Evaluation of insulin resistance, tumor necrosis factor alpha, and total antioxidant status in obese patients smoking cigarettes

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Abstract. – INTRODUCTION AND BACKGROUND: Obesity and smoking are leading causes of morbidity and mortality worldwide. Cross-sectional studies indicate that heavy smoking may be associated with a greater risk of obesity. While there are important unresolved issues in relation to the effect of smoking on body weight, there is increasing evidence that smoking is conducive to a greater accumulation of visceral fat and greater insulin resistance.

AIM: of this study was to determine the potential influences of obesity and smoking on tumor necrosis factor alpha (TNF-α), total antioxidant status (TAS), and insulin resistance.

SUBJECTS AND METHODS: 30 obese nonsmokers, 30 obese smokers, 30 normal-weight smokers, and 30 healthy volunteers (the control) were studied. In all subjects, assessments of TNF-α, TAS, and insulin were made. Insulin resistance was evaluated according to the homeostasis model assessment-insulin resistance (HOMA-IR) protocol.

RESULTS: TNF-α concentrations, as well as insulin resistance levels, in obese patients significantly exceeded those observed in the control. Compared to the control, obese patients presented significantly lower TAS levels. In the group of obese patients who actively smoked cigarettes, further increases in TNF-α and insulin resistance, as well as decreases in TAS level, were noticed. TNF-α concentration and insulin resistance levels were significantly higher, while TAS was lower in normal-weight smoking subjects, compared to the control. A positive correlation between TNF-α and HOMA-IR was found in the overall population.

CONCLUSIONS: Obesity may evoke inflammatory processes, oxidative stress, and insulin resistance, all of which are aggravated by cigarette smoking. TNF-α should be considered in the complex pathogenesis of insulin resistance in obese patients who actively smoke.

Key Words:
Obesity, Smoking, Inflammation, Insulin resistance.

Introduction

Obesity and smoking are leading causes of morbidity and mortality worldwide[1-3]. The co-occurrence of overweight and smoking has substantial consequences for health. According to the Framingham study[4], the life expectancy of obese smokers was 13 years shorter than that of normal-weight nonsmokers. In the same cohort, one-third to one-half of obese smokers died between the ages of 40 and 70 years, whereas only 10% of normal-weight nonsmokers did so.

Moreover, cross-sectional studies indicate that heavy smoking may be associated with a greater risk of obesity[5-7]. While there are important unresolved issues in relation to the effect of smoking on body weight, there is increasing evidence that smoking is conducive to a greater accumulation of visceral fat and greater insulin resistance, and that smoking also increases the risk of metabolic syndrome, type 2 diabetes, and other cardiovascular diseases[8,9].

In the context of the worldwide epidemic of obesity and the high prevalence of smoking, the relation between smoking, obesity, and associated conditions has major public health relevance, and in particular may lead to the progression of atherosclerosis. The ARICA (Atherosclerosis Risk in Communities) study[10] showed a significantly greater progression of atherosclerosis in patients who smoke, compared with those who

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had never smoked. The risk of progression of atherosclerosis was highest in smokers with additional risk factors, such as obesity and diabetes. However, the relation between smoking and obesity and mechanisms leading to increased cardiovascular risk are not fully understood.

Oxidative stress and inflammatory processes appear to be common denominators that underlie endothelial dysfunction in cardiovascular diseases, and which are involved in the progression of insulin resistance[11]. Insulin resistance has been shown to induce oxidative stress by generating excessive superoxide anion or H_2O_2 and decreasing catalase synthesis. This is part of a feed-forward mechanism that results in chronic conditions of oxidative stress and inflammation, leading to modulation of vascular endothelial function, smooth muscle contractility, and organ function[12,13].

The aim of this study was to determine the potential influence of obesity and smoking on TNF-α, TAS, and insulin resistance.

Subjects and Methods

Participants

The protocol of the study was approved by the Research Ethics Committee of Poznan University of Medical Sciences. It conformed to all ethical issues included in the Helsinki declaration.

The study population consisted of 30 obese nonsmokers, 30 obese smokers, and 30 normal-weight smokers. The cigarette users have smoked an average of 10 pack-years. Thirty normal-weight healthy volunteers, who had never smoked but were otherwise matched for demographic characteristics, were used as the control. Obesity was defined as body mass index (BMI) ≥ 30 kg/m^2.

The exclusion criteria were: (1) arterial hypertension; (2) diabetes mellitus; (3) impaired glucose tolerance; (4) a history of coronary artery disease, stroke, congestive heart failure or peripheral arterial disease; (5) sleep apnea syndrome; (6) abnormal liver or renal function; (7) clinically significant inflammatory process within the respiratory, digestive, or genitourinary tract, as well as in the oral cavity, pharynx, or paranasal sinuses; (8) a history of infection within the month prior to the study; (9) a history of use of any pharmacological treatment or dietary supplements in the 3 months prior to the study; ([10]) unstable body weight (more than 3 kg of self-reported change during the previous 3 months).

Anthropometry

Anthropometric measurements of individuals wearing light clothing and no shoes were carried out. Weight was measured to the nearest 0.1 kg, and height was measured to the nearest 1 cm. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). Waist circumference (cm) was measured at the level of the iliac crest at the end of normal expiration. Waist circumference was measured to the nearest 0.5 cm.

Blood Pressure Measurement

Office blood pressure (BP) was measured using a digital electronic tensiometer (model 705IT, Omron Corporation, Kyoto, Japan). Regular or large adult cuffs were used, depending on patient arm circumference. Blood pressure measurements were performed in accordance with the guidelines of the European Society of Hypertension (2009).

Biochemical Measurements

Blood samples were taken following an overnight fast and after lying in the supine position for 30 minutes. Serum levels of lipids, including total cholesterol (TCH), high-density lipoprotein cholesterol (HDL-C), and triglycerides, were assayed by routine enzymatic methods. Low-density lipoprotein cholesterol (LDL-C) was calculated from Friedewald's formula. The level of blood glucose was determined by the routine enzymatic method. Plasma insulin was determined by immunoassay (DIA Source Immunoassays S.A., Nivelles, Belgium). Insulin resistance in the participants was evaluated according to the homeostasis model assessment-insulin resistance (HOMA-IR) protocol[14]:

$$\text{HOMA-IR index} = \frac{\text{Fasting insulin (mU/l)} \times \text{Fasting glucose (mmol/l)}}{22.5}$$

Serum TNF-α concentration was measured using enzyme immunoassay (ELISA) (R&D System, Inc., Minneapolis, MN, USA). Total antioxidant status (TAS) was measured using a TAS Randox kit (Randox Laboratories, Ltd Crumlin, UK) and spectrophotometry (SPECORD M40, Carl Zeiss, Jena, Germany).

Statistical Analysis

Data are shown as means ± SD. All calculations and statistics were performed using Statisti-

ca for Windows (version 6). The differences between groups were tested by one-way analysis of variance. With *p*-value less than 0.05, the groups were compared by the appropriate test (Student's *t* test for unpaired samples). Simple associations between variables were calculated as the Pearson coefficient of correlation. *p*-values of < 0.05 were considered significant. Logarithmic transformation was used to normalize nonnormally distributed dependent variables.

Results

The characteristics of all studied groups and the control group are shown in Table I.

Triglycerides concentrations revealed significant elevations in all studied groups, as compared to the control. The highest level was observed for obese smokers. HDL cholesterol levels showed significant reductions in the obese and obese smoker groups- see Table II. Fasting glucose level was significantly higher in all the studied groups, compared with the control.

In all the studied groups, TNF-α and insulin concentrations, as well as insulin resistance levels, significantly exceeded those observed in the control. Compared to the control, all the studied groups presented significantly lower TAS levels. The highest TNF-α and insulin concentrations, as well as the highest insulin resistance levels, were found in obese smokers. Moreover, this group of patients was characterized by the lowest TAS level- see Table II.

A positive correlation between TNF-α and HOMA-IR in all smokers (both obese and normal-weight) was found (Figure 1).

TNF-α concentration correlated positively with HOMA-IR in all studied subjects (taking obese nonsmokers, obese smokers, normal-weight smokers, and the control together) (Figure 2).

Discussion

It is widely known that damage to the endothelium and vascular wall contribute to the development of the complications observed with obesity and smoking. It is extremely important from a clinical point of view to understanding the mechanisms involved in the development of cardiovascular complications associated with obesity, and their interrelationships with smoking, in order to identify new therapeutic options.

Our results demonstrated a significant influence of smoking and obesity on insulin resistances level, TNF-α concentration, and TAS. Moreover, the coexistence of smoking and obesity significantly aggravates the abnormalities observed.

Insulin concentrations and insulin resistance levels in the studied groups significantly exceeded those observed in the control. Our research confirms the insulin resistance increases in patients with obesity. Similar correlations have been shown by other Authors[15]. The evidence confirms the independent contribution of hyperinsulinemia and insulin resistance in the development of cardiovascular complications[16,17]. The results of the *Insulin Resistance Atherosclerosis Study* (IRAS) demonstrated an independent relationship (unrelated to the traditional risk factors for cardiovascular disease) between the thickness of the intima-media in the carotid artery and de-

Table I. Subject characteristic.

Characteristic	Obese non-smokers n = 30	Obese smokers n = 30	Normal-weight smokers n = 30	Normal-weight non-smokers n = 30
Age (years)	43.9 ± 9.3	48.9 ± 14.8	46.7 ± 15.4	44.0 ± 10.0
Gender (female/male)	14/16	15/15	16/14	16/14
BMI (kg/m^2)	37.9 ± 5.3	38.9 ± 5.5	24.6 ± 2.9*$^{\#}$	23.4 ± 2.0*$^{\#}$
Waist c ircumference (cm)	112.9 ± 7.5	111.8 ± 11.2	87.3 ± 9.4*$^{\#}$	82.6 ± 10.6*$^{\#}$
SBP (mmHg)	129 ± 6	131 ± 5	131 ± 5	132 ± 7
DBP (mmHg)	82 ± 5	84 ± 3	83 ± 3	80 ± 5
Indicator of the intensity of smoking (packyear)	–	8.8 ± 2.3	8.7 ± 2.4	–

*$p < 0.05$ vs obese non-smokers; $^{\#}p < 0.05$ vs obese smokers. BMI: indicates body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure.

Table II. Lipids profile and glucose concentration in the studied groups.

Characteristic	Obese non-smokers n = 30	Obese smokers n = 30	Normal-weight smokers n = 30	Control n = 30
TCH (mmol/L)	5.8 ± 1.2	5.6 ± 1.1	5.5 ± 1.3	4.2 ± 1.0*,#
LDL-C (mmol/L)	3.8 ± 1.0	3.5 ± 1.2	3.4 ± 1.1	2.4 ± 0.9*,#
HDL-C (mmol/L)	1.1 ± 0.3	1.0 ± 0.2	1.3 ± 0.4*,#	1.5 ± 0.3*,#
TG (mmol/L)	2.0 ± 1.2	2.5 ± 0.9*	1.9 ± 1.3#	0.9 ± 0.4*,#
Fasting glucose (mmol/L)	5.0 ± 0.5	5.1 ± 0.7	4.9 ± 0.5	4.4 ± 0.4*,#

*$p < 0.05$ vs obese non-smokers; #$p < 0.05$ vs obese smokers. TCH: indicates total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triglycerides.

creases in insulin sensitivity[18]. We found abnormal lipid profiles in the patients under study to be a typical consequence of obesity and insulin resistance. The critical role of insulin resistance in the pathogenesis of lipid disorders is well-documented[19].

Higher insulin concentration and higher HOMA-IR were both observed in the normal-weight smokers. Weizman et al[20] have shown that insulin response to an oral glucose load was more pronounced in smokers than in nonsmokers. Insulin resistance was dose-dependently related to smoking. In healthy men, chronic smoking was associated with high plasma insulin concentrations, independently of other factors known to influence insulin sensitivity. In summary, the long-term use of nicotine gum was associated with hyperinsulinemia and insulin resistance[21].

It is postulated that the increased insulin resistance in smokers may be related to their tendency towards increased abdominal fat accumulation. Cross-sectional studies indicate that smokers tend to have both a larger waist circumference and a smaller hip circumference, compared to nonsmokers[22-24]. Waist-to-hip ratio (WHR) has been positively associated with the number of pack-years of smoking, and there is a dose-response relation between WHR and the number of cigarettes smoked . In former smokers, WHR has been negatively associated with the time since the cessation of smoking[22]. Adipocytokines produced by adipose tissue, such as leptin, TNF-α, interleukin-6, resistin, and adiponectin, play a role in the development of insulin resistance[25]. However, in our study, waist circumference in normal weight smokers was comparable to the control.

Furthermore, smokers have been shown to have higher fasting plasma cortisol concentrations than do nonsmokers[22]. Higher cortisol concentrations may be a consequence of the stimulation of sympathetic nervous system activity that is induced by smoking[23], and higher cortisol may lead to hyperinsulinemia[25].

We found higher concentration of TNF-α in all the studied groups, with the highest level in the obese smokers. Numerous studies show that levels of TNF-α increase with increasing body weight[26]. Hotamisligil et al[27] have shown increased expression of the TNFR2 receptor in adipose tissue in patients with excessive body weight; they also have also demonstrated that the level of the sTNFR2 receptor is six times higher in obese people than among their control group.

Table III. Insulin concentration, HOMA-IR, TNF-α and TAS level in the studied groups.

Characteristic	Obese non-smokers n = 30	Obese smokers n = 30	Normal-weight smokers n = 30	Control n = 30
Insulin (μUI/mL)	36.1 ± 19.3	45.7 ± 15.5*	11.2 ± 3.6#	7.5 ± 2.8*,#
HOMA-IR	7.9 ± 3.7	10.5 ± 4.3*	2.5 ± 0.9#	1.9 ± 0.6*,#
TNF (ng/L)	6.4 ± 1.2	7.4 ± 2.4*	4.1 ± 1.3#	2.5 ± 0.8*,#
TAS (mmol/L)	1.84 ± 0.16	1.64 ± 0.23*	1.84 ± 0.19#	2.08 ± 0.18*,#

*$p < 0.05$ vs obese non-smokers; #$p < 0.05$ vs obese smokers. HOMA-IR indicates insulin resistance index; TNF-α, tumor necrosis factor alpha; TAS, total antioxidant status.

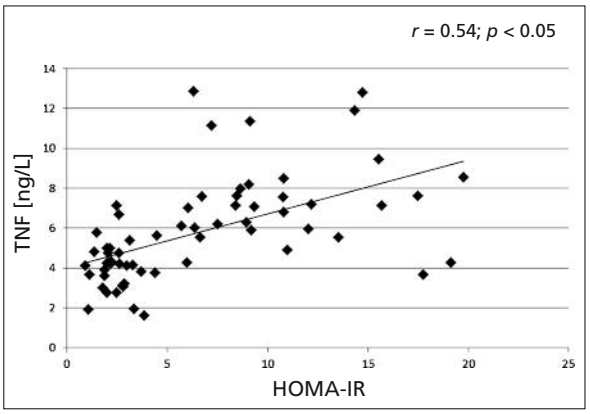

Figure 1. Plasma TNF-α correlates with HOMA in smoking participants.

The pathogenesis of the increased concentration of TNF-α in smokers has not been definitively explained. The results of our study showing increased concentrations of TNF-α have confirmed that smoking induces systemic inflammation. In our previous study, we demonstrated the increased concentration of acute-phase proteins in smokers[28]. One large prospective study with an 18-year follow-up has revealed that all the measured acute-phase protein levels (fibrinogen, α1-antitrypsin, haptoglobin, α1-acid glycoprotein, and ceruloplasmine) increased significantly with increasing cigarette consumption in healthy men, independent of other known cardiovascular risk factors[29]. Some Authors have suggested that an important source of proinflammatory cytokines in smokers may result from the excessive accumulation of abdominal fat[30,31]. The excessive activity of TNF-α increases impairs endothelial function in obese and smoking subjects, reduces the activity of nitric oxide synthase[10], and stimulates the chronic intravascular inflammatory processes – which may explain the accelerated progression of atherosclerosis in this group of patients[32,33]. Moreover, the significant role of TNF-α in the complex pathogenesis of insulin resistance has been shown in animal models[34] and humans[35,36]. Our findings of a significant correlation between TNF-α and HOMA-IR are agree with the results of other investigators.

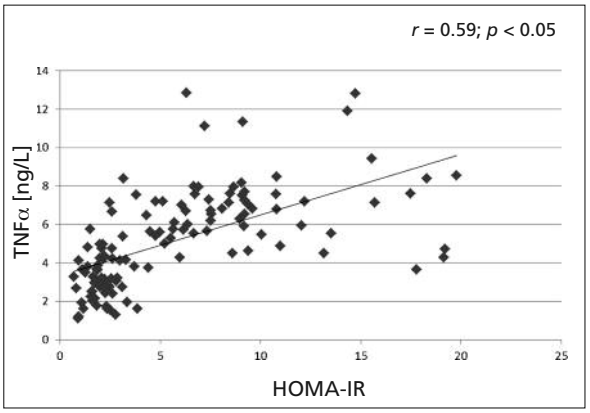

Figure 2. Plasma TNF-α correlates with HOMA in the overall studied population (including smoking and nonsmoking subjects).

An imbalance between antioxidants and the oxidant-generating systems, leading to oxidative stress, has been proposed as contributing to the pathogenesis of atherosclerosis. A strong inverse correlation between plasma antioxidants levels and the prevalence of atherosclerosis in peripheral arteries demonstrates that subjects with low antioxidant capacities are at increased risk for atherosclerosis[37]. Because the effects of plasma antioxidant components are additive, the measurements of TAS reflect the antioxidant status of plasma[38].

In our study, all the groups presented significantly lower TAS levels, compared to the control. Both obesity and smoking predispose to an increase in reactive oxygen species (ROS)[39]. Oxidative stress appears to be the common denominator underlying the endothelial dysfunction that promotes the development of atherosclerosis[40].

The coexistence of obesity and cigarette smoking in the development of atherosclerosis is most likely a synergistic effect. Our study has shown further increases in TNF-α and HOMA-IR, as well as decreases in TAS in the group of obese smokers, as compared to obese nonsmokers and normal-weight smokers.

The results of the Pooling Project[41] also provide evidence for a synergistic effect of cigarette smoking on hypertension and elevated cholesterol levels. Also other studies have confirmed this synergy[42]. In the broader perspective, considering that obesity is epidemic and that the prevalence of smoking is high and increasing in many parts of the world (especially in developing countries[43], it is clear that the co-occurrence of both conditions will increase with devastating effects on the health of the world's populations.

Conclusions

Both obesity and smoking may contribute to the development of insulin resistance, which has

been demonstrated to be a significant predictive factor for cardiovascular disease. Moreover, our findings indicate that obesity and smoking predispose to a chronic elevation of TNF-α activity and oxidative stress.

Conflict of Interest

None.

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