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Nicotine may affect the secretion of adipokines leptin, resistin, and visfatin through activation of KATP channel

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ABSTRACT

Objective: It has been confirmed that adipokines are associated with atherosclerosis. Cigarette smoking was found to possibly influence adipokine secretion. However, the precise role of smoking in adipokine secretion and the underlying mechanisms are largely unknown. The aim of this study was to determine whether nicotine, the principal active ingredient of cigarettes, can influence adipokine secretion and its potential mechanism.

Methods: The present study consecutively enrolled 96 men, including 50 smokers with early atherosclerosis and 46 nonsmokers. Serum adipokines, including leptin, resistin, and visfatin, were determined with enzyme-linked immunosorbent assay in all participants. Furthermore, the effect of nicotine on secretion of these adipokines was examined in differentiated 3T3-L1 preadipocytes under the conditions of ATP-dependent potassium (KATP) channel blocked or unblocked.

Results: Compared with the control group, serum levels of leptin, resistin, and visfatin in smokers were significantly higher. In 3T3-L1 adipocytes, nicotine treatment significantly increased the levels of these adipokines ($P = 0.014$, 0.001, and 0.029, respectively). When the KATP channel was blocked, secretion of resistin and visfatin was reduced ($P < 0.001$), but no change was found in the leptin secretion ($P = 0.522$).

Conclusions: Nicotine may affect the secretion of adipokines leptin, resistin, and visfatin through activation of KATP channel.

Introduction

Cigarette smoking is a major risk factor for atherosclerosis and coronary diseases [1,2]. The underlying mechanisms are still not fully understood. Recently, adiponectin was determined to be associated with earlier atherosclerosis in men who smoke. Smoking might decrease adiponectin secretion through ATP-dependent potassium (KATP) channels in adipocytes [3]. These findings cigarette smoking leads to atherosclerosis.

Adiponectin is an important antiatherosclerosis adipokine. As a well-known endocrine organ, adipose tissue secretes a large number of cytokines, including adiponectin, leptin, resistin, and visfatin. These adipokines have a profound influence on the regulation of inflammation responses and atherosclerosis [4]. Leptin, visfatin, and resistin especially are considered proinflammatory and promote atherosclerosis in the most studied adipokines to date. According to findings from other studies, the present study provided a hypothesis that cigarette smoking may influence secretion of more adipokines.

The KATP channel played a critical role in the regulation of secretion of many biogenic hormones, such as insulin, growth hormone, rennin, and neurohumors. A previous study also found

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that nicotine altered adiponection secretion via KATP channels in 3T3-L1 adipocytes [3].

The present study was designed to evaluate serum levels of three critical cytokines—leptin, resistin, and visfatin—in men who smoke and who have early atherosclerosis, as well as the effects of nicotine on their secretion in 3T3-L1 preadipocytes in vitro. Moreover, the potential role of the KATP channel in the influence of smoking on secretion of these adipokines was also found.

Material and methods

Population investigated and ethics statement

The study population consisted of 96 men as described in previously [3]. The present study was approved by the Ethical Committee of Xi'an Jiaotong University, and all participants gave written informed consent. The participants were divided into a smoking group (n = 50; mean age 52 ± 11 y) and a control group of nonsmokers (n = 46, mean age 50 ± 12 y). All the smokers had a smoking history of >10 y, and their smoking frequency was at least twice a day. We excluded anyone who had a history of cancer, cardiovascular diseases, stroke, diabetes, renal failure, serious hepatic diseases, or hypertension. The study was carried out on the morning after admission into the study, before breakfast, and while abstaining from alcohol and caffeine. The values of carotid intima media thickness (IMT), large artery elasticity (C1), and small artery elasticity (C2), which have been regarded as valid markers of early, preclinical atherosclerosis, were measured as described previously [3]. Blood pressure was measured by well-trained physicians, and venous blood was collected from all the participants. Height and body weight were measured and body mass index (BMI) was calculated. Plasma samples for subsequent assay were stored at −80°C. Leptin, resistin, and visfatin were respectively determined using enzyme-linked immunoassay (ELISA) kits (Phoenix Pharmaceuticals, Burlingame, CA, USA) with sandwich ELISA method.

Cell culture and treatment

We cultured 3T3-L1 mouse preadipocytes to confluence and then induced to adipogenic differentiation as described previously [3]. The cells were divided into three groups. On day 4 of differentiation, 10 μmol/L nicotine (Sigma, Santa Clara, CA) was added to the medium for 26 h in the first group (nicotine group). The second group (Gli group) was incubated with nicotine for 2 h and then 20 μmol/L glibenclamide (Gli) the inhibitor of the KATP channel was added into the medium for 24 h. The third group (control group) was incubated with phosphate-buffered saline for 26 h. Six independent replicate experiments were performed for each group. An aliquot of the media from each group was collected and subjected to ELISA for the amount of leptin, resistin, and visfatin, respectively. Four replicates were performed for each ELISA analysis.

Statistical analysis

Data are expressed as mean ± SE. Analysis of all the data was carried out using SPSS 13.0 (International Business Machines Corporation (IBM), Armonk, NY) statistical analysis software. In the in vitro study, differences were analyzed by one-way analysis of variance. The level of significance was set at $P < 0.05$.

Results

Serum contents of leptin, resistin, and visfatin were associated with smoking

The clinical and biochemical characteristics of the study participants are shown in Supplementary Table 1. There was no remarkable difference in the mean ages, BMI, and contents of triacylglycerol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, or fasting glucose between the two groups. Diastolic blood pressure and total cholesterol in smokers were significantly higher than in the control group ($P < 0.05$). The C1 and C2 of the smoking group were significantly lower than the control group, whereas IMT was markedly higher for smokers compared with nonsmokers.

As shown in Table 1, levels of leptin, resistin, and visfatin in the smoking group were significantly higher than those of the nonsmokers.

Nicotine affected secretion of the adipokines in 3T3-l1 adipocytes

Cigarettes consist of >4000 chemicals, but nicotine is the principal active ingredient for cigarette addiction. Compared with control participants, 10 μmol/L nicotine treatment significantly increased leptin, resistin, and visfatin secretion into the media of the adipocytes (leptin: 0.105 ± 0.039 versus 0.052 ± 0.027 ng/mL, $P = 0.042$; resistin: 2.121 ± 0.380 versus 0.076 ± 0.045 ng/mL, $P < 0.001$; visfatin: 17.048 ± 4.580 versus 4.555 ± 0.214 ng/mL, $P < 0.001$; Fig. 1).

Nicotine might affect the adipokines secretion through the KATP channel in 3T3-l1 adipocytes

Furthermore, function of the KATP channel in adipocytes was investigated in the adipokine secretion. Glibenclamide, a specific inhibitor of KATP channel, was added to the media, together with nicotine. Gli treatment markedly reduced the resistin and visfatin secretion compared with nicotine treatment alone (resistin: 0.504 ± 0.201 versus 2.121 ± 0.380 ng/mL, $P < 0.001$; visfatin: 6.560 ± 0.272 versus 17.048 ± 4.580 ng/mL, $P < 0.001$; Fig. 1). However, the concentrations of resistin and visfatin secreted by adipocytes treated by Gli plus nicotine were still higher than in the control group (resistin: 0.504 ± 0.201 versus 0.076 ± 0.045 ng/mL, $P < 0.001$; visfatin: 6.560 ± 0.272 versus 4.555 ± 0.214 ng/mL, $P < 0.001$). The results suggested that nicotine decreased adiponectin secretion through the KATP channels in adipocytes, whereas Gli treatment reduced the leptin secretion compared with nicotine treatment alone (0.091 ± 0.019 versus 0.105 ± 0.039 ng/mL, $P = 0.522$; Fig. 1). The KATP channel in adipocytes might not be associated with leptin secretion.

Discussion

Although there have been a number of studies that focused on the association between adipokines and atherosclerosis, it is rare that researchers directly study the effect of smoking on secretion of adipokines. The present study explored the effect of smoking on the other three major adipokines—leptin, resistin, and visfatin. To eliminate any influence by drugs, obesity, or other illnesses, we selected male smokers who were free from any other cardiovascular diseases, diabetes, and hypertension. Moreover, we performed an in vitro study in 3T3-L1 adipocytes to investigate direct effects of nicotine on secretion of these hormones. This was a direct and comprehensive study on the effect and potential mechanism of smoking on adipokine secretion.

Data from the present study indicated that serum concentrations of leptin, resistin, and visfatin were significantly higher in male smokers than in nonsmokers. We also found that nicotine increased the secretion of leptin, resistin, and visfatin

Table 1
Serum levels of leptin, resistin, and visfatin in smokers

	Smokers (n = 50)	Control (n = 46)	*P* value
Leptin (ng/mL)	10.611 ± 3.033	6.682 ± 1.208	0.014*
Resistin (ng/mL)	19.443 ± 5.251	10.210 ± 3.997	0.001*
Visfatin (ng/mL)	4.842 ± 1.768	2.434 ± 1.511	0.029*

Data presented as mean ± SE. Serum levels of leptin, resistin, and visfatin were significantly higher than the control ($P < 0.005$)

* $P < 0.05$.

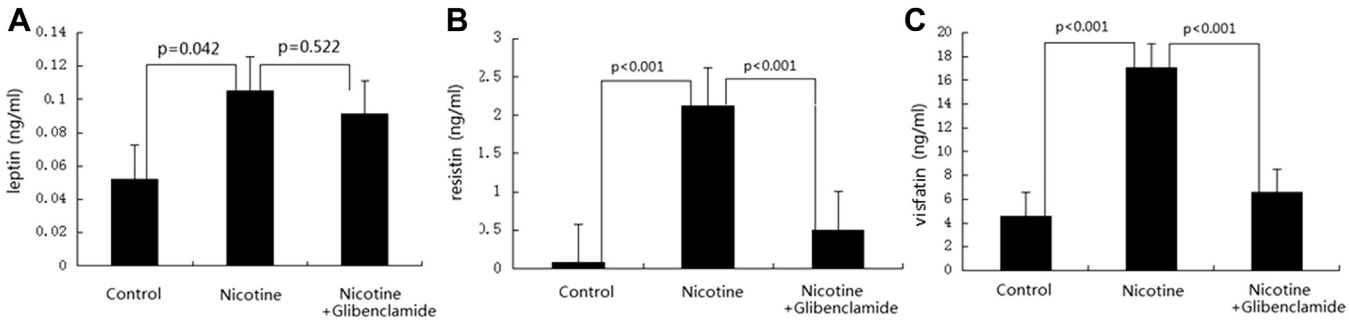

Fig. 1. Effects of nicotine and Gli treatment on secretion of leptin, resistin, and visfatin in 3T3-L1 adipocytes. 10 μmol/L nicotine treatment significantly increased (A) leptin ($P = 0.014$), (B) resistin ($P = 0.001$), and (C) visfatin ($P = 0.029$) secretion. 20 μmol/L Gli (inhibitor of KATP channel) treatment markedly blocked the resistin and visfatin secretion ($P < 0.001$), whereas leptin secretion was not changed by Gli ($P = 0.052$). For each group, n = 6. Gli, glibenclamide.

in vitro. Inhibition of the KATP channels partially resisted the increase of resistin and visfatin, but had no effect on leptin increase. These results suggested that cigarette smoking influenced adipokine secretion and the KATP channel might partly mediate the influence. This may be a potential pathway through which cigarette smoking induces atherosclerosis.

It is well known that adipose tissue is not only a fat storage organ, but also an important endocrine organ that secretes a large number of cytokines under both physiological and pathologic conditions [5]. It plays a key role in atherosclerosis through secretion of various adipokines. Adiponectin, leptin, resistin, and visfatin are main factors secreted by adipose tissue and also most closely associated with atherosclerosis [6].

The adipokine leptin is known as a hypothalamic modulator of food intake, body weight, and energy stores [7–9]. However, high-circulating leptin levels could result in leptin resistance similar to the insulin resistance found in type 2 diabetes [10]. Hyperleptinemia in the general population is associated with atherosclerosis and the metabolic syndrome [11]. Results from the present study showed that high serum leptin levels induced by smoking in male smokers probably contributed to early atherosclerosis.

Resistin is another important adipokine and is considered an independent marker of inflammation [12,13]. It has been confirmed that resistin plays a role in the pathogenesis of atherosclerosis [14–17]. Experimental and clinical studies have demonstrated that the plasma concentrations of resistin correlated directly with atherosclerosis and may have a proinflammatory effect on endothelial cells [18–22]. Although increased serum concentrations of resistin were found in patients with diabetes who smoked compared with nonsmokers and former smokers, few studies explored the association between smoking and resistin in the general population. The present study determined the serum resistin levels in male smokers [23].

Visfatin is a ubiquitous adipokine that was first identified to be closely associated with atherosclerosis [24–26]. Previous studies suggested that visfatin might be correlated with inflammatory states, endothelial dysfunction [27], and vascular pathology [28–30]. Reports about the effect of cigarette smoking on visfatin are rare. To our knowledge, the present study is the first to determine the association between smoking and visfatin.

Furthermore, to determine the effect of cigarette smoking on those adipokines, an experiment was performed in adipocytes in vitro. The results also supported that cigarette smoking increased the secretion of leptin, resistin, and visfatin. For the important roles of leptin, resistin, and visfatin in atherogenesis, the present study implied that smoking may induce atherosclerosis through affecting secretion of the adipokines. It provided a novel insight to the exploration for the mechanism of cigarette-inducing atherosclerosis. Our next study will focus on the association between these adipokines and atherosclerosis in smoking men.

The adipokines leptin, resistin, and visfatin play an important role in atherogenesis and are upregulated in smokers; however, the underlying mechanism is unclear. The KATP channel was considered a crucial mediator in secretion of many cytokines and hormones in many types of cells including adipocytes. Nicotine is the most important active ingredient of cigarette and may have an effect on the KATP channel [6]. The hypothesis that nicotine may influence these adipokines via the KATP channel was verified in the present study. The results showed that nicotine increased secretion of leptin, resistin, and visfatin in adipocytes, whereas the increasing of resistin and visfatin was resisted when the KATP channel was blocked by its specific inhibitor Gli. Study results suggested that nicotine might increase secretion of resistin and visfatin via the KATP channel in adipocytes. These findings are consistent with the results of a previous study on adiponectin [3]. These clues indicated that KATP is an important modulator of adipokine secretion and also a topic for further study. Although the present study was simple and preliminary, the findings provided a novel insight to study the mechanism of nicotine affecting secretion of adipokines.

Conclusion

Serum levels of leptin, resistin, and visfatin were influenced by nicotine, the main component of smoke. The KATP channels in adipocytes may partly mediate the effect of smoking on resistin and visfatin.

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.nut.2015.12.001.

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