The effect of smoking on peripheral insulin sensitivity and plasma endothelin level

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SUMMARY

Objectives: The aim of the present study was to investigate the effect of smoking on peripheral insulin effectiveness.

Methods: Seven healthy volunteers, nonsmokers, of mean age 39.6 ± 7.1 years and mean BMI 22.65 ± 11.98 kg/m², without family history of diabetes mellitus, with normal blood pressure participated in the study. All the parameters were studied twice — at baseline as well as after smoking (4 cigarettes per one hour). The study was performed in three days: at the first day we studied peripheral insulin effectiveness (M) in vivo by the artificial endocrine pancreas (Biostator), using the euglycaemic hyperinsulinaemic clamp technique, and insulin-receptor binding on circulating mononuclear blood cells; at the second day — the same parameters after one-hour smoking during the third hour of clamping; at the third day — plasma endothelin level, blood pressure and heart rate at baseline and after one-hour smoking.

Results: There was a significant decrease in glucose utilization during the second clamp test, when the volunteers smoked during the third hour as compared to the test at baseline (p = 0.04). This was accompanied by a significant decrease in insulin receptor affinity (p = 0.04). Systolic blood pressure and heart rate increased significantly after one-hour smoking (from 112.3 ± 15.37 mmHg to 123.6 ± 16.02 mmHg and from 64.65 ± 11.11 bpm to 70.8 ± 9.40 bpm, respectively). Plasma endothelin level increased significantly after smoking (from 0.62 ± 0.15 pg/ml to 2.05 ± 1.67 pg/ml, p = 0.03).

Conclusion: Our results demonstrate that smoking decreases peripheral insulin sensitivity reducing insulin receptor affinity. We have confirmed that smoking increases plasma endothelin level, which probably by causing vasoconstriction and consequent tissue hypoxaemia could decrease peripheral glucose utilization. We consider that smoking could also have a direct effect on insulin receptor affinity, thus leading to decreased peripheral insulin effectiveness.

Key-words: Smoking · Insulin sensitivity · Insulin receptors · Receptor affinity · Endothelin.

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RÉSUMÉ

Effet du tabagisme sur la sensibilité périphérique à l’insuline et le taux plasmatique d’endothél ine

Objectifs : Le but de la présente étude était d’investiguer les effets du tabagisme sur l’efficacité périphérique de l’insuline.

Méthodes : Sept volontaires sains, non fumeurs, d’âge moyen 39.6 ± 7.1 ans et de BMI moyen 22.65 ± 11.98 kg/m², sans antécédents familiaux de diabète, normotendus, ont participé à cette étude. Tous les paramètres ont été étudiés à deux reprises — à l’état basal puis après avoir fumé (4 cigarettes pendant une heure). L’étude a été réalisée en trois jours : le premier jour, nous avons étudié l’efficacité périphérique de l’insuline (M) in vivo par pancréas endocrine artificiel (Biostator), en utilisant la technique du clamp euglycémique hyperinsulémique, et la liaison du récepteur de l’insuline sur des cellules mononucléées circulantes ; le second jour, les mêmes paramètres ont été analysés après une heure de tabac au cours de la troisième heure du clamp ; le troisième jour a porté sur les taux plasmatiques d’endothél ine, la pression artérielle et la fréquence cardiaque à l’état basal et après une heure de tabac.

Résultats : On note une diminution significative de l’utilisation du glucose au cours du second test de clamp, lorsque les volontaires ont fumé pendant une heure lors de la troisième heure par rapport au test basal (p = 0.04). Cela s’accompagne d’une diminution significative de l’affinité du récepteur de l’insuline (p = 0.04). La pression artérielle systolique et la fréquence cardiaque augmentent significativement après une heure de tabagisme (p = 0.03 et p = 0.001, respectivement). Le taux plasmatique d’endothél ine augmente significativement après avoir fumé (de 0.62 ± 0.15 pg/ml à 2.05 ± 1.67 pg/ml, p = 0.03).

Conclusion : Nos résultats démontrent que le tabagisme diminue la sensibilité périphérique à l’insuline en réduisant l’affinité pour le récepteur de l’insuline. Nous avons confirmé que le tabagisme augmente le taux plasmatique d’endothél ine, ce qui probablement pourrait diminuer l’utilisation périphérique du glucose en provoquant une vasoconstriction et une hypoxie tissulaire. Nous considérons que le tabagisme pourrait aussi avoir un effet direct sur l’affinité du récepteur de l’insuline, conduisant ainsi à une diminution de l’efficacité périphérique de l’insuline.

Mots-clés : Tabagisme · Sensibilité à l’insuline · Récepteur de l’insuline · Affinité du récepteur · Endothél ine.

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smoking is considered one of the most powerful risk factors for the development of atherosclerosis and the vascular complications of diabetes mellitus [1]. Insulin resistance is known to correlate significantly with cardiovascular risk factors [2]. The data concerning the effect of smoking on peripheral insulin sensitivity are controversial. A number of studies support the view that smoking impairs insulin action and causes insulin resistance [1, 3-5]. Cigarette smokers have recently been shown to exhibit insulin resistance, dyslipidaemia and markers of the insulin resistance syndrome [6]. Ronnemaa T et al. have demonstrated that chronic smoking is associated with high age- and body mass index-adjusted plasma insulin levels, independent of other factors known to influence insulin sensitivity [7]. Insulin and C-peptide responses to oral glucose load have been reported to be significantly higher in smokers than non-smokers, whereas glucose levels were not substantially different, thus chronic cigarette smoking seems to markedly aggravate insulin resistance in type 2 diabetic patients [8]. There are also data that the effect of smoking on insulin sensitivity may be partially reversible after quitting [7, 9].

Still there are data from other studies, which have not found an association between active smoking and insulin sensitivity [10].

Although cigarette smoking is an established risk factor for atherosclerosis, the true mediator of vessel diseases associated with smoking is not known. Cigarette smoking is known to impair endothelial function [11]. Elevated plasma levels of endothelin, a peptide derived from endothelium, and a disturbed vascular reactivity have been described in insulin-resistant states such as syndrome X, obesity, non-insulin-dependent diabetes mellitus, and in some studies in essential hypertension [12-14]. However, whether endothelin has a direct effect on insulin-mediated glucose uptake is still unclear [15]. Endothelin is supposed to play a role in vessel tone regulation, being itself a vasoconstrictor [16-19]. Several studies have demonstrated an increase in plasma endothelin-1 after cigarette smoking [20, 21]. It has been reported that heavy and light cigarette smokers have similar dysfunction of endothelial vasoregulatory activity [22, 23].

The aim of the present study was to investigate the effect of smoking on peripheral insulin effectiveness and on insulin-receptor binding in healthy volunteers and try to find the possible mechanism of this effect. We have also studied the effect of smoking on plasma endothelin level.

Material and methods

Subjects

Seven healthy volunteers, nonsmokers (4 females and 3 males), of mean age 39.6 ± 7.1 years (from 26 to 56 years) and mean body mass index (BMI) 22.65 ± 11.98 kg/m², without family history of diabetes mellitus and with normal blood pressure (mean systolic blood pressure 107.8 ± 9.1 mmHg; mean diastolic pressure 75 ± 7 mmHg) participated in the study.

The healthy volunteers were informed about the aim of the study and the nature of the methods used according to the Helsinki Declaration and they gave their written consent to participate in the study. The study design was approved by the local Ethics Committee.

Methods

All the parameters were studied twice — at baseline as well as after smoking (4 cigarettes per one hour). The study was performed in three days: at the first day we studied peripheral insulin effectiveness (M) and insulin-receptor binding (total number of insulin receptors and number of high affinity receptors per cell; insulin receptor affinity) at baseline without smoking; at the second day — the same parameters after one-hour smoking during the third hour of clamp technique; and at the third day — plasma endothelin level, arterial blood pressure and heart rate at baseline and after one-hour smoking.

Peripheral insulin effectiveness (M) was studied in vivo by means of the artificial endocrine pancreas (Biostator), using the euglycaemic hyperinsulinaemic clamp technique [24]. The study was initiated at 8 a.m. after an overnight (12-hour) fast. Insulin (in 0.9% saline) was infused at a constant rate (1 mU/kg/min) for three hours, yielding an increase of plasma insulin level to 79.1 ± 13.2 mIU/L (coefficient of variation 19%). Euglycaemia was maintained during the three-hour clamping by the infusion of varying amounts of glucose. Mean plasma glucose level was 86 ± 2 mg/dl (coefficient of variation 3.3%). Peripheral insulin sensitivity was estimated by the amount of glucose, infused during the second hour of clamping. The healthy volunteers underwent the three-hour clamping twice — once without smoking and a second time, when they smoked four cigarettes during the third hour of clamping.

Blood for the study of immunoreactive insulin (IRI) and insulin-receptor binding during the two clamping tests was taken from a third contralateral vein at baseline as well as after the second and the third hour of clamping.

Insulin-receptor binding was studied by incubating circulating mononuclear blood cells, isolated according to the method of Boyum [25] with Tyr¹⁴ monoiod/¹²⁵I-insulin (Amersham) — 0.2 ng/ml and increasing concentrations of native, unlabelled insulin (NovoNordisk) — from 0 to 10⁷ ng/ml in Hepes (pH 7.8) [26]. Data are corrected for non-specific hormonal binding (in the presence of 10⁷ ng/ml native insulin) and are analysed by a computer-fitted programme. Results are presented as number of receptor sites per cell (total and of the high affinity ones) and insulin-receptor affinity.

Blood samples for plasma endothelin level were taken at baseline as well as after one hour smoking during the third
day of the study. Plasma level of endothelin was measured after being extracted with Sep-pak C-18 cartridges by radio-immunoassay (BIOMEDICA, Austria); test sensitivity 0.25 pg/tube (95% B/Bo), cross reaction with big endothelin being < 1%; inter-, intra-assay variations – 10% and 6%, respectively.

Statistical analysis was performed using SPSS 9.1 statistical package. Non-parametric analysis after logarithmic transformation of the data was used for statistical evaluation of experimental data. Data are presented as mean ± SD.

Results

The volunteers demonstrated an increase of 21.16% in the amount of glucose infused during the third hour of clamping as compared to the second (8.638 ± 2.125 mg/kg/min vs 7.129 ± 2.280 mg/kg/min). In the second clamp test, when the healthy volunteers smoked during the third hour, peripheral insulin effectiveness did not increase and even showed a decrease of 5.92% (7.115 ± 2.053 mg/kg/min vs 7.563 ± 2.087 mg/kg/min), the difference in glucose utilization between the two tests being significant during the third hour of clamping (p = 0.04) (Fig 1).

The hyperinsulinaemia during the clamping led to a decrease of 2.3 ± 2.0% in the number of high affinity insulin receptor sites per cell during the third hour as compared to the second (345 ± 112 vs 337 ± 60). During the second clamp test with smoking the decrease in the number of high affinity insulin receptors was even greater — 7.8 ± 4.4% (293 ± 102 vs 270 ± 110), but the difference between the two tests is not significant (p > 0.1).

We observed an increase in insulin receptor affinity in the course of clamping during the first day. It increased by 17.4% during the third hour as compared to the second, the difference being not significant (2.7 ± 1.0 vs 2.3 ± 1.1.10^9 M^-1, p > 0.8). After smoking insulin receptor affinity decreased by 12.8% during the third hour as compared to the second (2.18 vs 2.5·10^9 M^-1). The difference in insulin receptor affinity between the two clamp tests was significant at 3 hours (p = 0.04) (Fig 2).

Systolic blood pressure increased significantly after one-hour smoking during the third day of the study (130.8 ± 23.5 mmHg after smoking vs 107.8 ± 6.9 mmHg at baseline, p = 0.03). Diastolic blood pressure increased as well, but not significantly (83.8 ± 13 mmHg vs 71 ± 11 mmHg, p > 0.8). Heart rate increased significantly after smoking (89.3 ± 7.1 vs 68.3 ± 9.9 beats per minute at baseline, p = 0.001). Plasma endothelin level increased significantly (from 0.62 ± 0.15 pg/ml to 2.05 ± 1.67 pg/ml) after smoking (p = 0.03).

Discussion

The effect of smoking on peripheral glucose utilization was studied at baseline as well as after smoking during the third hour of euglycaemic hyperinsulinaemic clamping. It is known that there is an increase in this parameter in the course of clamping [27]. We found an increase of 21.16% in glucose utilization at the first day of the study, while there was a decrease of 5.92% in this parameter during the second clamping test after smoking. Thus, comparing the data from the two tests it appears that smoking significantly reduces...
peripheral glucose utilization (p = 0.04). It has been reported in a number of studies that smoking impairs insulin action [1, 3-7]. The effect of chronic cigarette smoking on insulin sensitivity has been assessed in patients with type 2 diabetes mellitus and the results have demonstrated a significantly decreased total glucose disposal in smokers as compared to nonsmokers (p < 0.001) [8].

It is well known that insulin resistance might be due to prereceptor, receptor or postreceptor defects. Therefore we have studied insulin-receptor binding during the two clamp tests — at baseline and after smoking. Euglycaemic hyperinsulinaemic clamp technique maintains artificial hyperinsulinaemia. The changes in insulin-receptor binding of the healthy volunteers during the first test are typical of the hyperinsulinaemic state. We have demonstrated a compensatory increase in insulin receptor affinity during the hyperinsulinaemic clamping. This phenomenon has been observed in moderately obese hyperinsulinaemic subjects and in patients with insulinoma [28]. During the second clamp test the effect of smoking was added to the effect of hyperinsulinaemia. Our results demonstrate that after smoking 4 cigarettes for one hour, insulin receptor affinity was significantly lower than on the day without smoking (p = 0.04). Probably this is at least one reason for the decreased glucose utilization after smoking.

In an effort to find out the mechanism of action of smoking on peripheral insulin action we have measured plasma endothelin level at baseline and after smoking. Plasma endothelin level increased significantly in the studied healthy volunteers after one-hour smoking (p = 0.04). Several studies have also demonstrated an increase in plasma endothelin-1 after cigarette smoking [20, 21]. It is well established that smoking is one of the major risk factors for the development of atherosclerosis. Data from other studies suggest that probably one of the mechanisms for this is the increased secretion of endothelin, a powerful vasoconstrictor and mitogen affecting vessel smooth muscle cells [11, 21, 29]. Data from recent studies have suggested that hyperinsulinemia accompanying insulin resistance may aggravate this situation by augmenting the endothelial production and release of endothelin-1 [30]. It has been reported that endothelin-1 infusion reduces insulin sensitivity as demonstrated by a 31 ± 7% decrease in whole-body glucose uptake (p < 0.05) and a 26 ± 11% fall in leg glucose uptake (p < 0.05) [13]. Ferri C et al. have established a negative correlation between total glucose uptake and circulating endothelin-1 levels, suggesting that the peptide might exert negative effects on insulin sensitivity of target tissues [31]. Endothelin-1 has been reported to inhibit insulin-stimulated glucose uptake in rat adipocytes binding to specific endothelin receptors [32]. Thus insulin resistance has been associated with endothelin-1 levels and probably it would have been interesting to measure endothelin-1 during insulin infusion of the two clamp tests of the study, but it was not performed. Elevated plasma endothelin-1 in type 2 diabetes mellitus and its relationship to glucose and HbA1c suggest a putative role for endothelin-1 in diabetic endothelial cell damage [33].

More recent studies have demonstrated that it is unlikely that chronic hyperendotelinaemia has a direct insulin-antagonist effect contributing to peripheral (ie muscle/fat) insulin resistance in vivo, because the interpretation of metabolic studies is complicated by endothelin-1 effects on

![Figure 2](change_in_insulin_receptor_affinity.png)

**Figure 2**
Changes in insulin-receptor affinity during a 3-hour euglycaemic hyperinsulinaemic clamp technique at baseline as well as when the healthy volunteers smoked 4 cigarettes during the third hour of clamping.
muscle blood flow and insulin secretion [15]. Smoking has been shown to decrease peripheral blood flow [34, 35]. Endothelin is a powerful vasoconstrictor and probably its elevated plasma level could affect tissue perfusion and cause tissue hypoxemia, thus leading to decreased peripheral glucose utilization. Smoking is known to be a stress factor, leading to the secretion of different hormones — for example catecholamines, which could also contribute to the effect of endothelin on tissue perfusion.

The increase in endothelin level was accompanied by a significant increase in systolic blood pressure in all the studied subjects (p = 0.03). In fact we have found a significant increase in one of the most potent vasoconstrictors — endothelin. These results are similar to the data reported by Ottosson-Seeberger A et al. [13], who have found that endothelin-1 increases significantly arterial blood pressure (mean increase 8%) and decreases significantly splanchic and renal blood flow (mean decrease 30% and 20%, respectively). In contrast, Mikkelsen KL et al. have reported consistently lower blood pressure in smokers as compared with nonsmokers [36].

**Conclusion**

Our results demonstrate that smoking decreases peripheral insulin sensitivity reducing insulin receptor affinity. We have confirmed that smoking increases plasma endothelin level, which probably by causing vasoconstriction and consequent tissue hypoxemia could decrease peripheral glucose utilization. We consider that smoking could also have a direct effect on insulin receptor affinity, thus leading to decreased peripheral insulin effectiveness.

**References**


