Journal of Internal Medicine 1993; 233: 327–332

Smoking induces insulin resistance—a potential link with the insulin resistance syndrome

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Abstract. Attvall S, Fowelin J, Lager I, Smith U (Department of Medicine, University of Göteborg, Sahlgren's Hospital, Göteborg, Linköping, Sweden). Smoking induces insulin resistance—a potential link with the insulin resistance syndrome. *Journal of Internal Medicine* 1993; 233: 327–332.

Objectives. The acute effect of smoking and snuffing on insulin sensitivity was studied in a group of healthy habitual smokers.

Design The euglycaemic clamp technique was combined with the subcutaneous injection of a bolus (0.1 U kg^{-1}) of fast-acting insulin (Actrapid®). Randomized subjects smoked either one cigarette per hour for 6 h, took one bag-packed snuff per hour for 6 h or refrained from nicotine for 48 h before as well as during the clamp.

Subjects. Seven healthy smokers, four females and three males, of normal weight (BMI, mean ± SEM, 21 ± 0.7 kg m^{-2} with a range of 18.6–23.9), aged 31 ± 2 years (range 24–35 years), who had consumed at least 20 cigarettes per day for at least 5 years were studied. They were recruited through an advertisement in a newspaper.

Results. The steady-state plasma nicotine levels were similar during smoking and snuffing. The insulin and glucose levels were also similar during all three clamps. Smoking, but not snuffing, impaired insulin action ($P < 0.05$) mainly due to a lower peripheral glucose uptake. The mean growth hormone levels during the 6-h study were more than doubled during smoking ($P < 0.01$) while no significant differences were seen in the other counter-regulatory hormones.

Conclusion. Smoking (also in habitual smokers) acutely impairs insulin action and leads to insulin resistance. Thus, smoking can be of importance for the development of the insulin resistance syndrome associated with risk for cardiovascular disease.

Keywords: growth hormone, insulin antagonistic hormones, insulin resistance syndrome, smoking, snuffing.

Introduction

Smoking is a major risk factor for cardiovascular disease and a correlation between cardiovascular morbidity and the number of cigarettes smoked has consistently been shown [1].

The mechanism(s) for the deleterious effect of cigarette smoking on the vessel wall is unclear. Nicotine, carbon monoxide and various polycyclic hydrocarbons have all been proposed to play a role.

Presented in part at the annual meeting of the Scandinavian Society for the Study of Diabetes, 13–15 March, 1992, Geilo, Norway.

A number of atherogenic traits have recently been reported in chronic smokers, such as impaired fibrinolysis and elevated plasminogen activator inhibitor-1 (PAI-1) [2], lower HDL-cholesterol and higher VLDL-triglycerides, as well as impaired glucose tolerance [3]. These findings are consistent with the insulin resistance syndrome [4] although direct effects of smoking on insulin action have not been established.

Diabetics are particularly vulnerable to the harmful effects of smoking [5]. However, the influence of smoking on insulin requirements in diabetics is controversial; either similar [6–8] or increased [9–10] insulin requirements compared to non-smok-

ing individuals have been reported. In addition, smoking may [11] or may not [12] delay the absorption of insulin from the injection site.

In the present study, the effect of smoking on insulin action was studied with the normoglycaemic clamp technique in a group of healthy habitual smokers. In order to characterize the potential importance of nicotine the results were compared with a period of tobacco abstinence as well as with snuffing, a widespread habit in Scandinavia.

Subjects and methods

Subjects

Seven healthy smokers, four females and three males, of normal weight (BMI, mean ± SEM, 21 ± 0.7 kg m^{-2} with a range of 18.6–23.9), aged 31 ± 2 years (range 24–35 years), who had consumed at least 20 cigarettes per day for at least 5 years, were studied. They took no regular medication and had no family history of diabetes or hypertension. Their alcohol consumption was moderate.

All subjects were informed of the nature, purpose and possible risks before giving their consent to participate. The study was approved by the Ethics Committee of the University of Göteborg.

Study design

The subjects came to the laboratory at 07.30 hours. They were placed in bed and remained in the supine position throughout the experiment. Both arms were warmed with electric pads to increase blood flow. All infusions were made through a catheter in a cubital vein. Arterialized blood samples (oxygen saturation 93 ± 1%) were drawn from a dorsal vein in the contralateral arm.

Each subject underwent three studies in random order with an interval of 4 weeks (1) while smoking one filtered cigarette (average nicotine content 1.2 mg per cigarette) per hour during the clamp; (2) after 2 days of abstinence from cigarettes and using portion-bag packed snuff (the subjects were studied during the clamp while consuming one bag of snuff (average nicotine content 1 mg) every hour); and (3) total tobacco abstinence for 2 days before as well as during the clamp.

The glucose-clamp technique was performed as previously reported [13]. However, in order to elucidate the effect on insulin absorption and to more closely mimic the normal situation in diabetics, insulin was administered subcutaneously instead of by the intravenous route which is more commonly used during glucose clamps.

A bolus of fast-acting insulin (Actrapid®; Novo-Nordisk, Bagsvaerd, Denmark), 0.1 U kg^{-1}, was injected subcutaneously by the same person 10 cm lateral to the umbilicus. In order to avoid intramuscular injection the skin was lifted before the injection. The rate of glucose infusion was adjusted to maintain the glucose levels at 5.0 mmol l^{-1} by a variable infusion of glucose (200 mg ml^{-1}) (Baxter Chemicals, Oslo, Norway). Potassium chloride (0.1 mmol ml^{-1}) was infused (5 mmol h^{-1}) in the same catheter to prevent hypokalemia during the clamps.

Arterialized venous blood was used to measure the glucose concentrations every 5 min with a reflectometer (Reflolux®; Boehringer, Mannheim, Germany) and glucose test-strips (BM test 1–44®; Boehringer). This method has been validated [14] and found to correlate well with the glucose measurements performed with the Yellow Spring glucose analyser (Beckman Instruments, Fullerton, CA, USA) (n = 191, r = 0.99) as well as with chemical analyses with the glucose dehydrogenase technique (n = 532, r = 0.99, $P < 0.001$).

Rates of glucose appearance (R_a) and disposal (R_d) were determined in all three studies by the infusion of D-(3-3H)-glucose (New England Nuclear, Boston, MA, USA) dissolved in saline to a concentration of 1 μCi ml^{-1}. A primed infusion of 25 μCi was given followed by a constant infusion of 15 μCi h^{-1}. D-(3-3H)-glucose was infused for 120 min before the clamps to achieve isotopic equilibration.

Analytical procedures

During the isotopic equilibration period blood samples were taken before (at zero time), after 80 min and henceforth every 20 min for the chemical determination of glucose and measurements of specific activity. Blood samples for the determination of insulin and counter-regulatory hormones were drawn at the times shown in Results. All samples were kept on ice, rapidly centrifuged and the plasma stored at −20 °C until analysed. The glucose levels shown in Results were determined with the glucose-6-phosphate dehydrogenase technique. Measurements of the specific activity of glucose were performed on plasma samples deproteinized with $Ba(OH)_2$ and $ZnSO_4$ and evaporated at 40 °C.

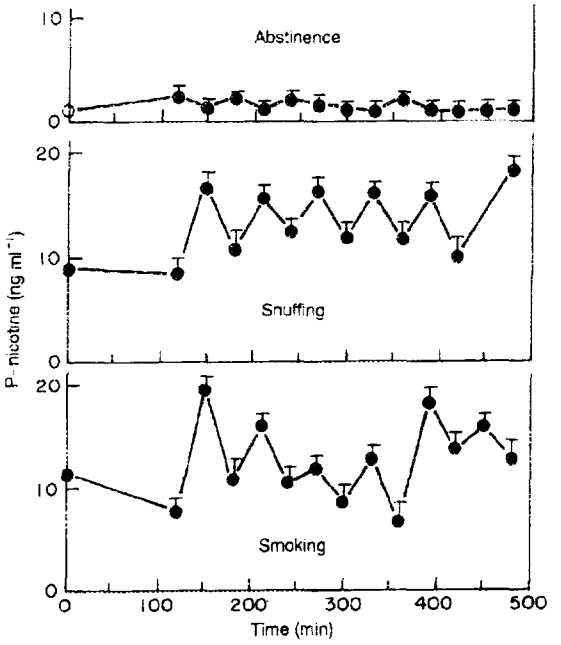

Fig. 1. Plasma nicotine levels during smoking, snuffing or tobacco abstinence. The samples were drawn every 30 min after the tobacco consumption during the euglycaemic clamp. Results are means ± SEM.

Insulin (Phadeseph Insulintest®; Pharmacia, Uppsala, Sweden) levels were determined with a radioimmunoassay. Free fatty acids were determined according to Trout *et al.* [15] and adrenaline with a liquid chromatographic method with electrochemical detection [16]. Growth hormone was analysed with a double-label antibody technique and cortisol with a fluorimetric method. Nicotine was analysed with a gas chromatographic technique [17].

The rates of glucose production (R_a) and utilization (R_d) were calculated with the non-steady-state equation of De Bodo *et al.* [18]. To compensate for non-uniform mixing, the non-steady-state-term of the equation was multiplied by a correction factor of 0.65 (pool fraction) [19]. R_a was calculated by subtracting the rate of infusion of exogenous glucose from the tracer-determined total rate of glucose production.

Statistical analysis

Data are shown as means ± SEM. Significances of differences were evaluated with Student's *t*-test for paired and unpaired data as appropriate using the method of multiple comparisons.

Results

Plasma nicotine levels

Figure 1 shows the nicotine levels during the abstinence period as well as 30 and 60 min after smoking and snuffing. The mean levels during smoking and snuffing were similar (smoking 15.5 ± 4, snuffing 15.6 ± 4.5 ng l^{-1}, NS) while very low levels (2.1 ± 0.3 ng l^{-1}, $P < 0.0001$) confirmed those of tobacco abstinence.

Glucose, insulin and FFA levels

The blood glucose (mean ± SEM) levels were similar during the clamps (smoking 5.0 ± 0.2, snuffing 4.9 ± 0.3 and abstinence 5.0 ± 0.3 mmol l^{-1}, NS).

The fasting insulin levels were also similar before the clamps (smoking 6.5 ± 1.2, snuffing 7.1 ± 0.8, abstinence 6.8 ± 0.9 mU l^{-1}, NS). During the 6-h normoglycaemic clamps the plasma insulin levels

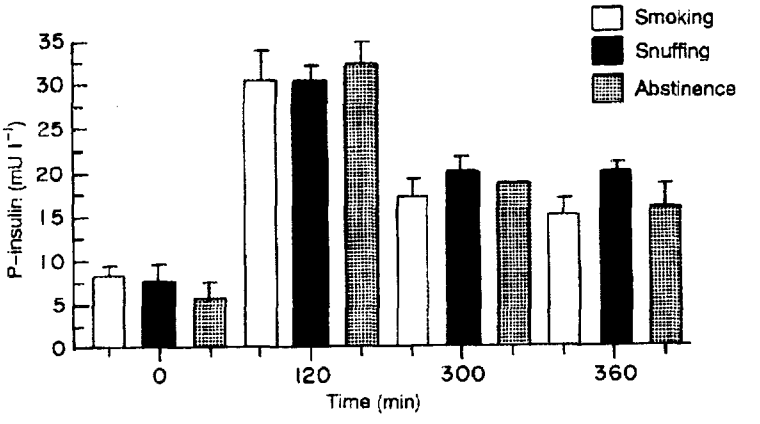

Fig. 2. Mean plasma insulin levels following a subcutaneous insulin injection during smoking, snuffing or tobacco abstinence. The samples were drawn at 0, 120, 300 and 360 min during the 6 h euglycaemic clamp. Results are means ± SEM.

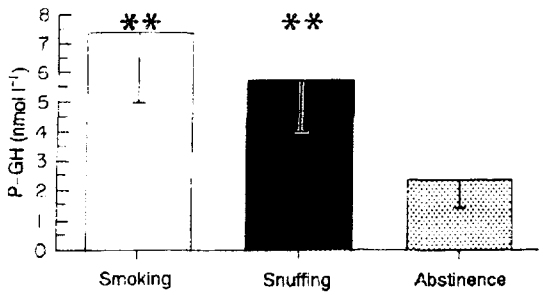

Fig. 3. Mean plasma levels (±SEM) of growth hormone during smoking, snuffing and tobacco abstinence. The samples were drawn every 30 min and the values shown represent the mean levels during the 6-h clamps. * $P < 0.01$.

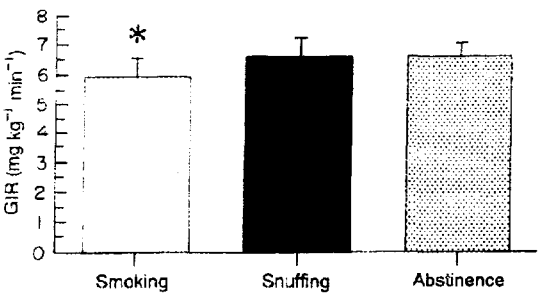

Fig. 4. Mean glucose infusion rates (±SEM) during smoking, snuffing and tobacco abstinence. The values shown are the average infusion rate during the 6-h clamps. * $P < 0.05$.

reached a similar area under the curve in all studies (smoking 79.6±9.2, snuffing 79.6±8.2, abstinence 76.1±6.9 mU l^{-1} per 6 h, NS) (Fig. 2).

The free fatty acid (FFA) levels were similar before the clamps (smoking 363±38, snuffing 420±64 and abstinence 497±68 μmol l^{-1}, NS) and decreased during the clamps to a similar extent in all subjects (last hour of the clamp: smoking 57±26, snuffing 16±7 and abstinence 29±4 μmol l^{-1}, NS).

Insulin-antagonistic hormones

The hormones were analysed at baseline and at 30-min intervals during the clamps. The mean levels during the 6 h were similar in the three studies for both noradrenaline (smoking 1.05±0.27, snuffing 1.23±0.26 and abstinence 1.27±0.35 nmol l^{-1}, NS) and cortisol (smoking 334±84, snuffing 332±70 and abstinence 283±62 nmol l^{-1}, NS).

In contrast the growth hormone levels more than doubled during both smoking and snuffing compared to the abstinence period (smoking 7.3±2.9, snuffing 5.7±2.2 and abstinence 2.4±1.0 nmol l^{-1}, $P < 0.01$) (Fig. 3).

Effects on glucose disposal

The insulin effect, expressed as amount of glucose infused to maintain normoglycaemia during the 6-h clamp, was similar during abstinence and snuffing, whereas a significant reduction ($P < 0.05$) of around 12% was seen during smoking (smoking 5.9±0.4, snuffing 6.6±0.4 and abstinence 6.6±0.4 mg kg^{-1} min^{-1}) (Fig. 4). The glucose disposal rate was maximal in all studies 3–4 h after the insulin had been given.

Glucose turnover

The rate of isotopically measured total glucose appearance (R_a)—mostly representing hepatic glucose production—was similar in all groups before the infusions were started. R_a decreased during the clamps to a similar extent (not shown).

Basal glucose utilization (R_d) was also similar before the clamps but the increase was significantly greater ($P < 0.05$) after abstinence and snuffing when compared to smoking for the last 3 h of the clamps (smoking 6.9±0.3, snuffing 7.7±0.4 and abstinence 7.5±0.4 mg kg^{-1} min^{-1}).

Discussion

The present study shows that cigarette smoking acutely impairs insulin action in otherwise healthy subjects and that the insulin resistance is mainly due to an impaired glucose uptake by peripheral organs.

The three-fold higher growth hormone level observed during smoking could be one reason for the lower insulin sensitivity observed. The smaller increase in growth hormone during snuffing may be insufficient to elicit an insulin resistance in these habitual smokers.

However, smoking is also associated with significant haemodynamic effects such as increments in the mean pulse rate, systolic and diastolic blood pressure as well as increased blood glycerol levels indicative of an activation of the sympathetic nervous system [20]. Furthermore, a detailed study of the time-course has shown that the maximum plasma noradrenaline levels occurred after 20 min [20]. Thus, normal pre-smoking levels could have been reached 30 min after smoking, when the samples were drawn in the present study, explaining why elevated catecholamine levels were not seen. Another reason for the discrepancy may be that in the study of Cryer *et al.* [20] two cigarettes were rapidly

smoked prior to the blood sampling, while in the present study only one cigarette was smoked.

In contrast to these results, Helve *et al.* [21] did not find cigarette smoking impaired insulin sensitivity. However, in that study insulin action was only followed for 2 h. Furthermore, tobacco abstinence was defined as no smoking for 12 h. Habitual smokers may still have elevated nicotine–cotinine levels after such a short abstinence which makes it difficult to evaluate the acute effect of smoking.

Cutaneous vasoconstriction with lower skin temperature is seen after smoking [22], especially in the hands and feet. This may [11] or may not [12] impair the rate of insulin absorption. No evidence for such an effect was seen in the present study where the periumbilical region was used for the insulin injection. This is the same site most type 1 diabetics use to inject their short-acting insulin.

Recently, Facchini *et al.* [3] found an impaired insulin effect on glucose uptake in chronic cigarette smokers when compared to an age-, weight- and sex-matched group of non-smokers. The present study is in agreement with this finding and suggests that smoking may be an important contribution to the insulin resistance syndrome. Such a link may provide one explanation for the increased propensity for cardiovascular disease in smokers.

Bolli *et al.* [23] found in a double-blind study that a nicotine-based chewing gum did not alter glucose uptake during a 6-h clamp in type 2 diabetic patients. These results are similar to those of the present study where snuffing did not elicit an insulin resistance. This suggests that substances other than nicotine in the tobacco smoke and/or the kinetics of the plasma level are of importance for eliciting insulin resistance. Whether individuals who habitually snuff exhibit the insulin resistance syndrome remains to be examined.

In conclusion, the present study shows that smoking can acutely impair insulin action. This finding, combined with recent results in chronic smokers [3], suggests that smoking can be an important cause of the insulin resistance syndrome. This link can provide an explanation for the increased risk for cardiovascular disease associated with smoking.

Acknowledgements

This study was suported by grants from the Swedish Medical Research Council (project B-3506) and the Medical Advisory Board of the Swedish Tobacco Company. The excellent technical assistance by Renate Bittner, RN is gratefully acknowledged.

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Received 29 June 1992, accepted 12 January 1993.

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