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Nicotine infusion acutely impairs insulin sensitivity in type 2 diabetic patients but not in healthy subjects

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Abstract. Axelsson T, Jansson P-A, Smith U, Eliasson B (Sahlgrenska University Hospital, Göteborg, Sweden). Nicotine infusion acutely impairs insulin sensitivity in type 2 diabetic patients but not in healthy subjects. *J Intern Med* 2001; **249:** 539–544.

Objectives. The aim of this study was to examine if an acute nicotine infusion alters insulin sensitivity to a similar degree in type 2 diabetic patients as in healthy control subjects.

Design. Double-blind, cross-over, placebo-controlled, randomized experimental study. Nicotine 0.3 μg kg^{-1} min^{-1} or NaCl was infused (2 h) during a euglycaemic hyperinsulinaemic clamp (4 h) to assess insulin sensitivity.

Setting. University research laboratory.

Subjects. Six male and female type 2 diabetic patients [DM2; age 54 ± 10 (mean ± SD) years; body mass index (BMI) 25.6 ± 2.9 kg m^{-2}] treated with diet or one oral hypoglycaemic agent and six age- and BMI-matched control subjects (Ctr).

Main outcome measures. Insulin sensitivity (rate of glucose infusion per kg fat free body mass and minute), nicotine and free fatty acid (FFA) levels, pulse rate and blood pressure.

Results. The infusions produced similar nicotine levels in both groups. In the absence of nicotine, DM2 were more insulin resistant than Ctr (6.7 ± 0.4 vs. 10.9 ± 0.3 mg kg^{-1} LBM min^{-1}, respectively; $P < 0.0001$). This insulin resistance was further aggravated by the nicotine infusion in DM2 but not in Ctr (4.6 ± 0.3 vs. 10.9 ± 0.3 mg kg^{-1} LBM min^{-1}; $P < 0.0001$). Only minor differences were seen in FFA levels, pulse rates and blood pressure.

Conclusions. At this low infusion rate, nicotine aggravated the insulin resistance in DM2 but not in Ctr. This finding may be because of the (dysmetabolic) diabetic state *per se* or to an increased sensitivity to environmental factors associated with a genetic predisposition for type 2 diabetes. These results show that diabetic subjects are particularly susceptible to the detrimental effects of nicotine.

Keywords: free fatty acids, glucose clamp, insulin, insulin resistance, nicotine, type 2 diabetes.

Introduction

Cigarette smoking is a well-known risk factor for the development of both cardiovascular disease and type 2 diabetes mellitus [1–3]. The negative effect of smoking on cardiovascular morbidity is particularly pronounced in diabetic subjects [4]. Possible causes for this may be the atherogenic metabolic effects induced by smoking in both healthy individuals as well as in type 2 diabetic patients [5–7]. These effects include insulin resistance and dyslipidaemia, including low HDL cholesterol, as well as postprandial lipid intolerance [5].

Transdermally administered nicotine has been shown to elicit a negative effect in type 2 diabetic patients on the glucose regulation [8] whilst, surprisingly, moist snuff did not cause an acute impairment in insulin sensitivity in healthy subjects [9]. Cigarette smoking produces very high nicotine concentrations in the brain and, thus, negative effects on metabolism could then be expected in both healthy and diabetic subjects. However, the divergent findings discussed above with peripheral administration (transdermally) versus moist snuff suggest the possibility that diabetic patients are more susceptible to the negative effects of nicotine on metabolism.

The aim of the present study was to address whether nicotine, a common environmental risk factor, acutely elicits different effects in type 2 diabetic patients compared with healthy control subjects. If so, this would suggest an increased susceptibility to the detrimental effects of a defined environmental factor in this patient group. This, in turn, could help explain the increased risk for cardiovascular complications.

Material and methods

All subjects gave informed consent for participation in the study. The study protocol was approved by the Ethics Committee of Göteborg University.

A total of 16 subjects were recruited via an advertisement in a local newspaper. All subjects underwent a screening procedure, where normal blood cell counts, kidney, liver and thyroid functions as well as ECG were confirmed. Fasting and glucagon-stimulated (1 mg i.v.) C-peptide levels were measured in order to exclude type 1 diabetes.

A total of 12 subjects underwent all examinations. Thus, four subjects were excluded because of nausea during the clamp (two) or for personal reasons. The two groups ($n = 6/6$) were matched for gender (3F/3M in both groups) and to have similar ranges of age and BMI.

None of the subjects took any regular medication (apart from antidiabetic medication) or used any form of nicotine. The diabetic patients were reasonably well controlled (HbA1c range 5.8–7.7%, normal range 3.9–5.3%). Two patients were treated with sulphonylurea alone and one with metformin. No medication was taken on the morning of the examinations.

Experimental procedures

Two euglycaemic (5.0 mmol L^{-1}) hyperinsulinaemic (1 mU kg^{-1} body weight min^{-1}) clamps were performed in a double-blind and randomized order. Also the person who performed the clamps was unaware of the order of randomization. The clamp technique has previously been described in detail [10, 11]. Either 0.9% NaCl or 0.3 μg kg^{-1} min^{-1} nicotine dissolved in 0.9% NaCl (Pharmacia Consumer Healthcare, Helsingborg, Sweden) was infused for the initial 2 h of the clamps (4 h). The nicotine infusion rate has been tested in an initial pilot study in our laboratory ($n = 7$) so as not to cause frequent and significant nausea or intolerable side-effects.

No adverse effects with the exception of the exclusion group were seen during the study. Arterialized venous blood (using heating pads) samples were taken from the left antecubital vein and the pulse rate and blood pressure (sphygmomanometer) were measured at regular intervals.

In the diabetic patients, the insulin infusion started 42.5 min (mean) before the actual clamps (placebo examination range 20–75 min; nicotine examination 25–75 min; P = n.s.) in order to normalize the blood glucose levels which were 8.7 ± 2.2 (mean ± SD) mmol L^{-1} prior to the nicotine infusion and 8.1 ± 1.5 mmol L^{-1} prior to the saline infusion (P = n.s.).

The degree of insulin sensitivity was calculated as the glucose infusion rate (GIR) divided with the fat free mass, determined by measuring body potassium content in a whole-body counter [12], and time (M).

Analytical methods

Glucose was analysed with a YSI 2700 Select (Yellow Springs Instruments Corp., Yellow Springs, OH, USA). Free fatty acids (FFAs) were determined with an enzymatic calorimetric method using reagents from Wako Chemicals GmbH (Neuss, Germany). Serum free insulin (Pharmacia Insulin 100 RIA, Pharmacia AB, Uppsala, Sweden) and C-peptide levels (Behringwerke AG, Marburg/Lahn, Germany) were determined with radioimmunochemical analyses. Nicotine and cotinine were analysed by capillary gas chromatography (Pharmacia Consumer Healthcare, Helsingborg, Sweden).

Statistics

Data are presented as mean ± SD or SEM, as indicated. All variables were tested for normality and parametric or nonparametric methods were used accordingly. A P-value of 0.05 or less was considered statistically significant. StatView® statistics software (SAS Institute, Inc., NC, USA) was used for all calculations.

Results

Subject characteristics (screening visit) are presented in Table 1. As expected, the subject groups

Table 1 Patient characteristics

	Control subjects			Type 2 diabetic patients			
	Mean	SD	Range	Mean	SD	Range	*P*-value
Age (years)	53	7	47–66	54	10	43–66	n.s.
Diabetes duration (years)	n.a.			4	4	1–10	n.a.
BMI (kg m^{-2})	25.8	2.1	23.3–28.2	25.6	2.9	21.8–28.8	n.s.
WHR	0.90	0.12	0.74–1.06	0.96	0.04	0.89–1.02	n.s.
Pulse rate (BPM)	62	3	59–66	64	10	48–80	n.s.
Systolic BP (mmHg)	119	12	100–130	123	13	110–142	n.s.
Diastolic BP (mmHg)	75	8	60–80	74	9	60–80	n.s.
fB-Glucose (mmol L^{-1})	4.4	0.6	3.3–5.0	9.2	2.9	6.2–14.3	0.0039
fP-Insulin (mU L^{-1})	6.0	1.7	3.9–8.2	9.8	2.7	5.1–12.9	0.025
P-C-peptide 0′ nmol L^{-1}	0.4	0.1	0.3–0.6	0.6	0.2	0.3–0.8	n.s.
P-C-peptide 15′ nmol L^{-1}	1.1	0.3	0.8–1.5	0.9	0.3	0.7–1.3	n.s.
HbA1c[a] (%)	4.4	0.3	4.1–4.9	6.8	0.7	5.8–7.7	0.0039
S-Cholesterol (mmol L^{-1})	5.5	0.7	4.5–6.2	5.4	0.8	4.8–7.0	n.s.
S-HDL-cholesterol (mmol L^{-1})	1.4	0.2	1.2–1.7	1.1	0.2	0.8–1.3	0.047
S-Triglycerides (mmol L^{-1})	1.1	0.6	0.6–2.1	2.6	2.4	1.0–6.8	n.s.

[a]Normal range 3.9–5.3%.

SD: standard deviation; BMI: body mass index; WHR: waist/hip circumference ratio; BP: blood pressure; n.s.: not significant; n.a.: not applicable.

differed in levels of fasting blood glucose, plasma insulin, HbA1c and HDL-cholesterol.

The nicotine levels during the nicotine clamps are shown in Fig. 1(a). Peak levels were 6.2 ± 1.9 (mean ± SEM) ng mL^{-1} in the control subjects and 7.2 ± 2.2 ng mL^{-1} in the type 2 diabetic patients (P = n.s.). The peak cotinine levels were also similar (17.3 ± 5.2 ng mL^{-1} in the control subjects and 20.8 ± 6.4 ng mL^{-1} in the type 2 diabetic patients; P = n.s.). During the placebo clamps, nicotine and cotinine were not detectable in either group.

Blood glucose levels during the steady-state period of the clamps (the last 2 h) were also the same in both groups and both examinations (control subjects: placebo 4.9 ± 0.03, nicotine 5.1 ± 0.03 mmol L^{-1}, P = n.s.; diabetic patients: placebo 5.1 ± 0.04, nicotine 5.0 ± 0.03 mmol L^{-1}, P = n.s.; Fig. 1b).

Plasma insulin levels during the clamps were also similar as shown in Fig. 1(c) (mean insulin levels during the steady-state period in control subjects: placebo 77.4 ± 1.8, nicotine 71.9 ± 2.3 mU L^{-1}, $P = 0.067$; diabetic patients: placebo 76.5 ± 4.1, nicotine 69.4 ± 2.6 mU L^{-1}, P = n.s.).

Serum FFA levels before and during the clamps are shown in Fig. 1(d). In the fasting state, there were no significant differences between the two groups. During the steady-state period during the clamps, there were no differences in FFA levels in the control subjects (placebo 0.09 ± 0.01; nicotine 0.08 ± 0.01 mmol L^{-1}; P = n.s.). However, the FFA levels were slightly but significantly higher during the nicotine than the placebo infusion in the diabetic patients (placebo 0.09 ± 0.01; nicotine 0.14 ± 0.01 mmol L^{-1}; $P = 0.0065$), suggesting that the insulin effect was impaired by nicotine.

The insulin sensitivity (M) during the clamps are shown in Fig. 2. The M-value during the steady-state periods was significantly lower during both examinations in the diabetic patients (mean difference 32% during placebo and 63% during nicotine) when compared with the healthy control subjects ($P < 0.0001$). There was no difference in insulin sensitivity between the two examinations in the control subjects (placebo 10.9 ± 0.3; nicotine 10.9 ± 0.3 mg kg^{-1} LBM min^{-1}; P = n.s.). However, the M-value was significantly lower during the nicotine examination in the diabetic patients (placebo 6.7 ± 0.4; nicotine 4.6 ± 0.3 mg kg^{-1} LBM min^{-1}; $P = 0.0010$). These results remained unchanged also after adjusting for steady-state insulin levels (data not shown).

The effects of the infusions on the pulse rates are shown in Fig. 3. It is clear that this marker of sympathetic activation showed a significantly greater increase during the nicotine infusion in the control (placebo 66 ± 1 BPM; nicotine 70 ± 1 BPM; $P < 0.0001$) than in the diabetic subjects (placebo 65 ± 1 BPM; nicotine 65 ± 1 BPM; P = n.s.). The

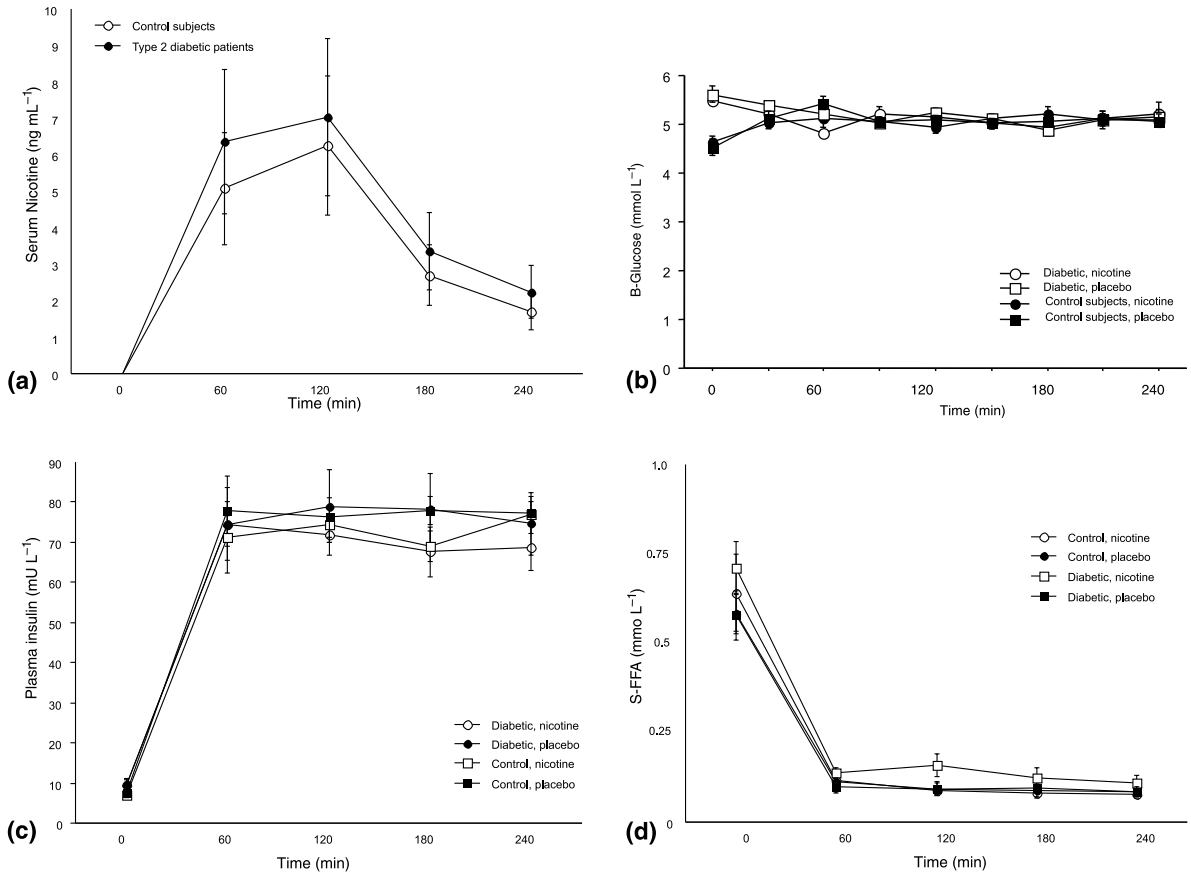

Fig. 1 (a) Nicotine concentrations during the euglycaemic clamps in the control subjects and diabetic patients (±SEM; P = n.s.). (b) Blood glucose concentrations during the euglycaemic clamps in the control subjects and diabetic patients during the nicotine and placebo infusions (±SEM; P = n.s.). (c) Plasma insulin concentrations during the euglycaemic clamps in the control subjects and diabetic patients during the nicotine and placebo infusions (±SEM; P = n.s.). (d) Serum free fatty acid (FFA) concentrations during the euglycaemic clamps in the control subjects and diabetic patients during the nicotine and placebo infusions (±SEM; P = n.s.).

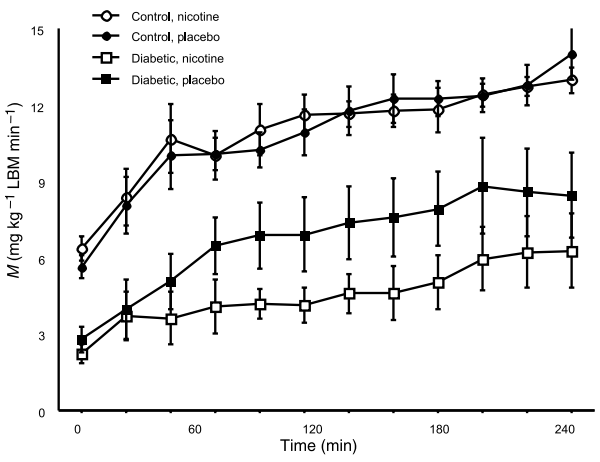

Fig. 2 Insulin sensitivity (M) in control subjects and diabetic patients during the nicotine and placebo infusions (±SEM).

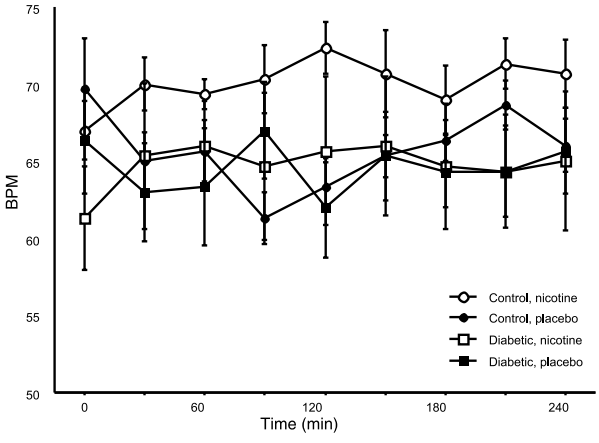

Fig. 3 Pulse rate during the euglycaemic clamps in the control subjects and diabetic patients during the nicotine and placebo infusions (±SEM).

diastolic blood pressure remained unchanged in both groups and there were only minor changes in the systolic blood pressure (data not shown).

Discussion

The salient finding in this study is that the already existing insulin resistance in type 2 diabetic patients, a well-known effect [13], is markedly aggravated during an intravenous infusion of nicotine (reduction 32 ± 6%). However, no significant impairment in the degree of insulin sensitivity was seen during the nicotine infusion (10 ± 9%) in the control subjects at similar plasma nicotine and insulin levels.

This difference clearly shows that type 2 diabetic patients are more susceptible to the negative effect of nicotine on insulin sensitivity. This finding has direct clinical implications as insulin resistance is associated with a number of risk factors for cardiovascular disease, the so-called insulin resistance (or metabolic) syndrome [13]. Recent studies have also shown that this syndrome is associated with a three to fivefold increased incidence of cardiovascular disease in nondiabetic subjects [14]. Similarly, there is strong evidence that smoking increases the development of cardiovascular disease in diabetic patients to an even greater extent than in nondiabetic individuals [4]. Thus, it is important to implement smoke intervention programmes in diabetes clinics.

It is not clear whether the negative effect of nicotine is because of the dysmetabolic state of diabetes *per se* or whether it reflects the interaction between environment and genetic disposition for type 2 diabetes. Several studies have shown that smoking is an independent risk factor for type 2 diabetes. As obesity, another environmental risk factor for type 2 diabetes, takes a long time to develop, we thought it was of interest to examine the effect of a rapidly induced and definable agent like nicotine in diabetic and nondiabetic subjects. The studies were carried out in healthy and reasonably well-controlled diabetic subjects also having similar fasting FFA levels. The small difference in FFA levels during the clamps is probably not of importance for the differences in insulin sensitivity between the groups. However, the suppressive effect of insulin on the FFA levels was less pronounced in the diabetic subjects during the nicotine infusion further supporting the enhanced insulin resistance in this group.

There was no evidence that the degree of sympathetic activation, a possible mechanism for the induction of insulin resistance by nicotine [15], was higher in the diabetic subjects. In fact, the pulse rate only increased significantly in the control subjects.

It will be of interest to extend these studies to nondiabetic individuals with a genetic predisposition for type 2 diabetes. Our recent findings that key genes and proteins involved in insulin signalling and action are reduced in adipocytes from type 2 diabetic patients [16] as well as in a cohort (approximately 30%) of nondiabetic individuals with a marked genetic predisposition (at least two first-degree relatives with the disease), has allowed us to identify a risk group to test this hypothesis [17, 18]. Such studies are currently underway.

Finally, it should be emphasized that these data support a direct role of nicotine in inducing the insulin resistance associated with smoking. We have previously documented that smoking six cigarettes over a 6-h period induces insulin resistance also in healthy subjects [9]. The difference from the present study is probably an effect of the high nicotine concentrations reached in the brain during smoking, whilst much lower levels are produced by the peripheral administration used in this study.

In conclusion, the present study shows that type 2 diabetic subjects are much more sensitive than a matched control group to the ability of nicotine to induce insulin resistance. This is consistent with clinical findings that diabetic individuals are particularly susceptible to the negative effect of smoking on cardiovascular morbidity and mortality. Further studies are underway to examine if this primarily reflects the interaction between the environment and genetic predisposition for diabetes or the dysmetabolic state of diabetes *per se*.

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