The Insulin Resistance Syndrome in Smokers Is Related to Smoking Habits

Björn Eliasson, Stig Attvall, Marja-Riitta Taskinen, Ulf Smith

Abstract The relationship between smoking habits, insulin resistance, and related risk factors for cardiovascular disease was examined in 57 middle-aged male smokers whose degree of insulin resistance was quantified by using the euglycemic clamp technique. Smoking habits correlated with degree of insulin resistance and consequently with various manifestations of the insulin resistance syndrome including levels of insulin, high-density lipoprotein cholesterol, triglycerides, and plasminogen activator inhibitor-1 (PAI-1) activity. Smoking habits, independent of degree of insulin resistance, were also related to levels of total cholesterol and low-density lipoprotein cholesterol as well as triglycerides. Stepwise regression analyses considering the effects of age, lean body mass, body fat, body mass index, waist/hip ratio, and alcohol consumption showed that only smoking habits and percent body fat were independently related to degree of insulin resistance. This study shows that insulin resistance and the insulin resistance syndrome are important but not unique contributors to the strong risk profile for cardiovascular disease in middle-aged men who smoke. (Arterioscler Thromb. 1994;14:1946-1950.)

Key Words • insulin resistance • insulin resistance syndrome • blood lipids • plasminogen activator inhibitor-1 • cigarette smoking

Smoking is a major risk factor for cardiovascular morbidity and mortality. However, it is unclear how smoking elicits its harmful effects, although many pharmacological actions are recognized. In addition, smoking is associated with differences in lifestyle, such as diet and degree of physical activity, which could contribute to the increased risk of cardiovascular disease (CVD).

Smokers exhibit a number of characteristics that are established risk factors for CVD. These include a larger waist-hip ratio (WHR), which is an indicator both of fat distribution and risk for CVD. Smokers may be linked to insulin resistance. Smoking can, in fact, acutely impair insulin action. In a transsectional study, Facchini et al report that chronic smokers exhibit traits of impaired insulin action, including higher triglyceride and lower HDL-C levels.

An acute effect of cigarette smoking is to increase the activity of the sympathetic nervous system and the levels of circulating catecholamines. Since catecholamines are powerful antagonists to insulin action, smoking may be linked to insulin resistance. Smoking can, in fact, acutely impair insulin action. In a transsectional study, Facchini et al report that chronic smokers exhibit traits of impaired insulin action, including higher triglyceride and lower HDL-C levels. Consequently, cigarette smoking has been recognized as an independent risk factor for non-insulin-dependent diabetes mellitus (NIDDM) in both men and women.

In the present study we examined insulin sensitivity with the euglycemic hyperinsulinemic clamp technique and the relation of insulin sensitivity to cardiovascular risk factors within a large number of smokers. The results clearly show a correlation between smoking habits and degree of insulin resistance as well as various manifestations of the insulin resistance syndrome (IRS), including lower HDL-C and higher insulin and triglyceride levels and plasminogen activator inhibitor-1 (PAI-1) activity. Our findings show that IRS is an important, albeit not the only, reason for the increased risk for CVD in smokers.

Methods

Fifty-seven nonobese male smokers, 40 to 60 years of age, were recruited via a newspaper advertisement. They had smoked more than 10 cigarettes per day for at least 10 years. They were all healthy, normotensive (blood pressure less than 140/90 mm Hg), and took no chronic medication. The clinical characteristics of the subjects are shown in Table 1. All subjects gave their informed consent to participation in the study. The study was approved by the Ethics Committee of Göteborg University.

The subjects were screened before they were admitted to the study. At screening, the subjects were interviewed and underwent a physical examination, and routine biochemical tests for hematologic, renal, and hepatic function were performed. Blood pressure was measured by using a sphygmomanometer after the subjects had rested in the supine position for at least 10 minutes. The average weekly consumption of alcohol, number of cigarettes smoked per day, and cigarette brand used were reported. The manufacturers supplied information on the nicotine content of the cigarettes. The subjects were also asked about heredity for diabetes and/or hypertension; this was positive in 13 of 57 individuals.

Body weight was recorded to the nearest 0.1 kg with the subjects wearing only underwear and socks. Length was measured and body mass index (BMI) calculated. Waist and hip circumferences were measured by using a nonelastic tape with the subjects standing. The waist circumference was measured in the midaxillary line midway between the lowest rib margin and the iliac crest and the hip circumference at the widest diameter around the buttocks according to World Health Organization criteria. The WHR was calculated from these measurements. Naturally occurring 40K was measured in a whole-body counter, and lean body mass (LBM) was calculated from the assumption that one kilogram equals 68.1 mmol potassium as reported by Forbes et al. Body fat was calcu-
lated by subtracting LBM from total body weight. The relative proportion of body fat (percent body fat) was also calculated.

On another occasion the subjects came to the laboratory in the morning after having fasted for at least 12 hours and abstained from smoking for at least 8 hours. Catheters were placed in the dorsal veins of the hand, and arterialized blood was obtained by using heat pads.22,23

A euglycemic hyperinsulinemic clamp was then performed for 2 hours essentially as described by DeFronzo et al13 and as previously reported in detail.15,22 Blood glucose was clamped at 5.0 mmol/L by using an insulin infusion rate of 1.0 mU · kg⁻¹ · min⁻¹. Human short-acting insulin (Actrapid, Novo Nordisk A/S) with albumin (Immuno AG) added to prevent adhesion was dissolved in isotonic saline. Potassium chloride (Kabi-Pharmacia AB) was administered at a rate of 5.0 mmol/h to prevent hypokalemia. The glucose infusion rate (GIR) was calculated during the last 30 minutes of the clamp, when steady state had been reached, as amount of glucose infused per kilogram LBM per minute.

Laboratory Analyses

All blood samples were drawn in appropriate tubes and kept on ice until centrifuged. Serum was stored at -80°C until analyzed. The blood glucose values shown in "Results" were analyzed by using a chemical glucose dehydrogenase method. Free fatty acids (FFAs) were determined by using an enzymatic colorimetric method using reagents from Wako Chemicals GmbH. Serum-free insulin (Phadesep, Kabi-Pharmacia AB) and C-peptide levels (Behringwerke AG) were determined with radioimmunochemical analyses.

Serum triglyceride and cholesterol levels were determined with an automated Cobs Mira analyzer (Hoffman-LaRoche) by enzymatic methods. The concentration of HDL-C was measured by the phosphotungstic acid/magnesium chloride precipitation method. Apoprotein (apo) AI, apoA-II (Boehringer GmbH), and apoB (Orion Diagnostica) were measured by immunoturbidimetric methods.

Fibrinogen was analyzed essentially as described by Clauss.24 PAI-1 activity was measured by using a Spectrolyse pl Kit (Biopool).

Statistical Analyses

Data are presented as mean±SD or mean±SEM as indicated. STATVIEW 4.01 software (Abacus Concepts, Inc) was used for all statistical calculations. A two-tailed P level of 5% or less was considered significant.
Apoprotein B
Total cholesterol
GIR
Triglycerides
Waist-hip ratio
age, percent body fat, LBM, BMI, WHR, and nicotine performed to examine the potential effect on GIR of HDL cholesterol fibrinogen C-peptide
Variable
TABLE 3. Correlations Between Nicotine Use and GIR During the Clamp and Anthropometric, Metabolic, and Hemostatic Variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Correlation Coefficient</th>
<th>P</th>
<th>Correlation Coefficient</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean body mass</td>
<td>-.30</td>
<td>.024</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Waist-hip ratio</td>
<td>NS</td>
<td></td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Body mass index</td>
<td>NS</td>
<td></td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Percent body fat</td>
<td>NS</td>
<td></td>
<td>-.32</td>
<td>.017</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>NS</td>
<td></td>
<td>-.37</td>
<td>.0088</td>
</tr>
<tr>
<td>(fasting)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>.47</td>
<td>.0006</td>
<td>-.48</td>
<td>.0004</td>
</tr>
<tr>
<td>(during clamp)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>NS</td>
<td></td>
<td>-.47</td>
<td>.0008</td>
</tr>
<tr>
<td>C-peptide</td>
<td>NS</td>
<td></td>
<td>-.44</td>
<td>.0020</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAI-1 activity</td>
<td>.28</td>
<td>.050</td>
<td>-.31</td>
<td>.029</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>.43</td>
<td>.0024</td>
<td>-.31</td>
<td>.035</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>.41</td>
<td>.0036</td>
<td></td>
<td></td>
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<tr>
<td>HDL cholesterol</td>
<td>NS</td>
<td></td>
<td>.42</td>
<td>.0033</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>.33</td>
<td>.021</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL/HDL cholesterol</td>
<td>NS</td>
<td></td>
<td>-.34</td>
<td>.020</td>
</tr>
<tr>
<td>Apoprotein B</td>
<td>.38</td>
<td>.0082</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GIR</td>
<td>-.28</td>
<td>.032</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

GIR indicates glucose infusion rate; LBM, lean body mass; PAI-1, plasminogen activator inhibitor-1; HDL, high-density lipoprotein; and LDL, low-density lipoprotein.

Finally, a backward stepwise regression analysis was performed to examine the potential effect on GIR of age, percent body fat, LBM, BMI, WHR, and nicotine and alcohol consumption. After five regression steps the sole remaining independent variables in this model were smoking habits (amount of nicotine consumed per day) and percent body fat (data not shown).

Discussion
This study showed for the first time that in a large group of healthy male smokers the amount of nicotine smoked per day is related to degree of insulin resistance. Thus, the established effect of smoking on various risk factors for CVD such as elevated triglyceride and lower HDL-C levels and higher PAI-1 activity can to a large extent be attributed to a concomitant insulin resistance in smokers. This conclusion is further substantiated by the finding that the incidence of NIDDM is also higher in smokers. Several prospective studies have shown that the degree of insulin resistance is a major predictor of future risk for NIDDM.

Apart from smoking habits, the only significant independent contribution to insulin sensitivity, measured as GIR during the euglycemic clamp, was percent body fat. It should be emphasized that the present study included only healthy middle-aged and nonobese men. In the normal population several other factors influence insulin sensitivity.

It is also clear that smoking habits, independent of GIR, were related to levels of total cholesterol and LDL-C. Several studies have also shown that smokers have higher cholesterol levels than nonsmokers. In addition, smoking habits per se, at least in part independent of degree of insulin resistance, seem to contribute to the higher levels of triglycerides in smokers.

There are several possible mechanisms whereby smoking can elicit insulin resistance. Multiple cardiovascular, endocrine, and neurohumoral effects of cigarette smoking are well known. An additional possibility is the smoking-related differences in lifestyle, including diet and degree of physical activity. Although the individu-

![Fig 1. Scatterplots showing correlations between glucose infusion rate (GIR) during the clamp and (A) smoking habits, (B) fasting insulin levels, (C) high-density lipoprotein (HDL) cholesterol levels, and (D) plasminogen activator inhibitor-1 (PAI-1) activity. LBM indicates lean body mass.](image-url)
Cigarette smoking also has acute effects on insulin sensitivity, which is related to total cholesterol and triglyceride levels. However, because the extent of smoking is a possible reason for the insulin resistance. Furthermore, Hellerstein et al. report that smoking leads to acute increases in plasma glycerol and FFA levels, probably due to an enhanced fat cell lipolysis. These findings, together with the present results and the transactional study by Facchini et al., strongly suggest that smoking elicits IRS.

In a separate study, we found that chronic smokers, even after 48 hours of tobacco abstinence, have higher levels of circulating noradrenaline than a matched group of nonsmokers (Axelsen et al. These results further emphasize the potential role of the counterregulatory hormones in eliciting insulin resistance in smokers.

Using the euglycemic hyperinsulinemic clamp technique, we have also confirmed the findings by Facchini et al. that smokers, when compared with a carefully matched group of nonsmoking control subjects, are insulin resistant and exhibit the various traits of IRS (B. Eliasson, N Mero-Matikainen, M-R Taskinen, U. Smith, unpublished data, 1994).

At the cellular level catecholamines, in part through increased cAMP concentrations, impair both the insulin signaling/transduction pathways and the intrinsic activity and synthesis of the glucose transport proteins (for review, see Reference 31). In addition, increased lipolysis and elevated FFA levels are important for the insulin resistance induced by the catecholamines.

In the present study, we found a negative correlation between FFA levels and insulin sensitivity, supporting a role by the fatty acids in modulating insulin action. However, fasting FFA levels were not correlated with smoking habits (Table 3). The reason for this is unclear, but it may be related to the 8 hours of smoking abstinence or to an increased elimination rate of FFAs by the fatty acids in modulating insulin action. These findings, together with the present results and the unpublished data, emphasize the potential role of the counterregulatory hormones in eliciting insulin resistance in smokers.

Smokers have been reported to have a higher WHR than nonsmokers. Although insulin resistance is related to a high WHR in obese individuals, no such correlation could be found in the present group of nonobese smokers. Percent body fat, rather than BMI or WHR, was an independent contributor to degree of insulin resistance. It should also be emphasized that the effect of smoking on insulin-stimulated glucose uptake during the euglycemic clamp was underestimated in the present study by expressing the data per kilogram LBM.

A negative correlation was found between smoking habits and total LBM (Table 3) (mean LBM ranged from 61.2±1.5 to 58.2±1.2 kg for subjects with low and high levels of nicotine consumption, respectively). Since LBM is the major compartment for glucose disposal during a euglycemic clamp, this difference in body composition further underscores the degree of insulin resistance in high nicotine consumers.

<table>
<thead>
<tr>
<th>TABLE 4. Multiple Linear Regression Analyses of the Relative Importance of GIR and Nicotine Use for the Metabolic and Hemostatic Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dependent Variable</td>
</tr>
<tr>
<td>Free fatty acids (fasting)</td>
</tr>
<tr>
<td>Free fatty acids (during clamp)</td>
</tr>
<tr>
<td>Insulin</td>
</tr>
<tr>
<td>C-peptide</td>
</tr>
<tr>
<td>PAI-1 activity</td>
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<tr>
<td>HDL cholesterol</td>
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<tr>
<td>LDL cholesterol</td>
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<tr>
<td>LDL/HDL cholesterol</td>
</tr>
<tr>
<td>Apoprotein A-I</td>
</tr>
<tr>
<td>Apoprotein A-II</td>
</tr>
<tr>
<td>Apoprotein B</td>
</tr>
<tr>
<td>NS</td>
</tr>
</tbody>
</table>

GIR indicates glucose infusion rate; PAI-1, plasminogen activator inhibitor-1; HDL, high-density lipoprotein; and LDL, low-density lipoprotein. Nicotine use was measured in milligrams per day, and GIR in milligrams per kilogram lean body mass per minute.

In this study reported a "normal" degree of physical activity, we cannot exclude a sedentary lifestyle as one possible reason for the insulin resistance. However, since cigarette smoking also has acute effects on insulin sensitivity, this possibility is less likely to account for the insulin resistance.

We have shown that smoking six cigarettes at a rate of two per hour acutely impairs insulin action in healthy individuals, probably due to the release of catecholamines and other counterregulatory hormones. Furthermore, Hellerstein et al. report that smoking leads to acute increases in plasma glycerol and FFA levels, probably due to an enhanced fat cell lipolysis. These findings, together with the present results and the transactional study by Facchini et al., strongly suggest that smoking elicits IRS, the extent of which is a function of smoking habits.

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The correlation between insulin sensitivity and nicotine consumption per day does not necessarily implicate nicotine as the causal factor. In fact, we saw no clear evidence of insulin resistance in individuals who used snuff to reach the same steady-state nicotine levels as those seen in smokers. Smokers, however, became less sensitive to insulin. Thus, it may well be that factors other than nicotine in cigarette smoke play a pathophysiological role for the development of insulin resistance. This is an important point to clarify, since smokers use nicotine-containing chewing gum or dermal patches to help them quit smoking.

In conclusion, the present study shows for the first time that there is a relationship between degree of insulin resistance and smoking habits. This impairment in insulin sensitivity is likely to account for the risk factors for CVD related to IRS in smokers. However, smoking is also associated with higher total cholesterol and LDL-C levels, and this association is unrelated to a concomitant insulin resistance. The present data also emphasize the role of environmental factors in modulating insulin action and precipitating risk factors for CVD.

Acknowledgments

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