The Relationship among Smoking, Plasma Adiponectin, Leptin, Inflammatory Markers and Insulin Resistance

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ABSTRACT

We aimed to study how smoking influences the relationship between fat mass, soluble tumor necrosis factor-α (TNFα) receptors 1 and 2 (sTNFR1 and sTNFR2), highly sensitive C-reactive protein (hs-CRP), adiponectin, leptin, and insulin resistance. A total of 60 healthy men (age: 27–53 years, body mass index (BMI): 20–35 kg/m²), 30 of whom were never-smokers and 30 smokers, matched for age, BMI and waist-to-hip ratio were included in this study. Those were subdivided into insulin resistant (IR) and insulin sensitive (IS) subgroups. Measures included circulating soluble fractions of the tumor necrosis factor α (TNFα) receptors (sTNFR1 and sTNFR2) and their relationship to fat mass, fasting plasma adiponectin, leptin, hs-CRP and insulin sensitivity index. Smokers had significantly lower fat mass, lower fasting glucose, insulin and leptin concentrations than nonsmokers. Despite lower fat mass, insulin and leptin, smokers showed significantly increased circulating sTNFR2 levels (3.7±0.8 vs. 2.9±0.8 ng/ml, P=0.03). Being either a smoker or having insulin resistance was independently associated with lower adiponectin concentrations (P = 0.046 and 0.001, respectively). No difference was detected in average hs-CRP concentrations between smokers and nonsmokers (P = 0.18) and between IR and IS subjects (P = 0.13). Both fat mass and smoking are related to increased activity of the TNFα axis. Plasma adiponectin concentrations are lower in smokers and IR subjects. These two mechanisms could be associated with increased cardiovascular risk in smokers.

Key Words: Smoking, Insulin resistance, Inflammation, Adipocytokines

INTRODUCTION

Overwhelming evidence indicates that cigarette smokers, when compared with life long nonsmokers, are at two- to three-fold increased risk for cardiovascular disease (1). Smoking exerts an inflammatory stimulus on macrophages which bring about the production of inflammatory cytokines, such as tumor necrosis factor-α (TNFα), which might be an early event in the development of the disease states associated with smoking (2). TNFα administration increases serum leptin levels in humans and plasma sTNFR1 concentration circulates in proportion to leptin (3). The coordinated function of these two systems might be involved in controlling satiety and food intake. Serum leptin concentration in smokers has been described to be lower, similar or greater than that found in non-smokers (4). The independent contribution of smoking, as a confounding factor, on the relationship between fat mass and TNF system activation, as measured using circulating sTNFR1 and sTNFR2, has not been evaluated, despite the evidences that smoking alters cytokine production. As obesity per se is recognized as a chronic inflammatory state, with increased TNF system activation (5), we aimed to study how smoking influences the relationship between fat mass and sTNFRs. Plasma adiponectin concentrations have been reported to be low in smokers (5). Low circulating concentrations of adiponectin have been associated with obesity, dyslipidemia, essential hypertension, type 2 diabetes, and cardiovascular disease (6, 7). It is apparent that the clinical syndromes in which
hypoadiponectinaemia occurs are all associated with peripheral resistance to insulin-mediated glucose uptake. In addition, variations in plasma adiponectin concentration and/or molecular forms have been suggested to modulate insulin sensitivity (8). Because results of in vitro studies have indicated that addition of pro-inflammatory cytokines, such as TNF- and IL-6, to isolated adipocytes can reduce adiponectin expression (9), it is possible that the association between smoking and lower adiponectin concentrations results from inflammation-mediated down-regulation of adiponectin expression in adipose tissue. In support of this notion is evidence of an inverse relationship between markers of inflammation such as C-reactive protein (CRP) with adiponectin (10). In addition, smoking has been identified as a source of reactive oxygen species (11) and is associated with increased levels of inflammatory markers (12); further suggesting that smoking-induced inflammation may contribute to lower adiponectin levels in smokers (13). On the other hand, because the prevalence of insulin resistance may also be increased in smokers (14), it is not clear whether hypoadiponectinaemia in these individuals is due to smoking, and possibly the associated inflammation, or to the coexistence of insulin resistance. We measured sTNFR1 and sTNFR2, adiponectin, Lipten and hs-CRP concentrations in smokers and nonsmokers, with each group further stratified into insulin-resistant (IR) and insulin-sensitive (IS) subgroups.

MATERIALS AND METHODS

Subjects

The study population consisted of 30 smokers and 30 nonsmokers who responded to print advertisements describing our research interest in smoking-associated metabolic abnormalities. Screening inclusion criteria included: healthy men between ages 27 and 53 years; body mass indices (BMIs) between 20 and 35 kg/m²; and no medications known to affect glucose, insulin, or lipoprotein metabolism. In addition, the smokers had to have history of smoking a minimum of 10 cigarettes per day for at least the past 5 years. Potential participants were further evaluated by medical history, physical examination, and routine clinical laboratory measurement to exclude individuals with apparent disease, laboratory evidence of type 2 diabetes, anemia, and abnormal liver or kidney function. Renal function was also evaluated by calculating the estimated glomerular filtration rate (GFR) using the Cockcroft-Gault formula (15).

Methods

Anthropometric measurements

All subjects were evaluated through the BMI and the waist-to-hip ratio (WHR). The subject's waist was measured with a soft tape midway between the lowest rib and the iliac crest. The hip circumference was measured at the widest part of the gluteal region. Fat mass (FM) and percent fat mass (PFM) were calculated using bioelectric impedance (Holtain BC Analyzer, UK).

Analytical methods

The serum glucose concentration was measured by the glucose oxidase method. The serum insulin level was measured by monoclonal immunoradiometric assay (IRMA, Medgenix Diagnostics, Fleunes, Belgium). STNF-R1 and sTNF-R2 receptor levels were measured by the Medgenix sTNF-R1 and sTNF-R2 solid-phase enzyme-amplified sensitivity immunoassays (EASIA) performed on a micro-titer plate (BioSource Europe SA, Zoning Industrial B-6220, Fleunes, Belgium). Immunoreactive serum leptin concentrations were measured with RIA in samples obtained after an overnight fast, (Linco Research Inc., St Charles, MO, USA). Plasma adiponectin levels were measured with RIA established by Linco Research, Inc. (St. Charles, MO). Serum high-sensitivity CRP was measured with a chemiluminescent assay established for use on an Immulite automatic analyzer (Diagnostics Products Corp., Los Angeles, CA).
Insulin-mediated glucose disposal was quantified by a modified version \(^{(17)}\) of the insulin suppression test (IST) as described and validated \(^{(18, 19)}\). The IST in its current form involves the continuous infusion for 180 minutes of octreotide (a bolus of 25 g followed by 0.5 g/min), insulin (25mU/ml/min.) and glucose (240 mg/min). Under these conditions endogenous insulin secretion is inhibited, as is the secretion of all other hormones that modulate glucose uptake. Steady-state plasma insulin (SSPI) and glucose (SSPG) concentrations are reached 90–120 minutes after the start of the infusion, and blood is drawn for measurement of plasma insulin and glucose concentrations every 10 minutes during the last 30 minutes of the continuous infusion. These four values are averaged, and used to determine the SSPI and SSPG concentrations observed during that study. Since the SSPI concentrations at the end of the infusion are similar in all individuals, and the glucose infusion rate during the infusion is also identical, the SSPG concentrations provide direct estimate of the ability of the same amount of insulin to promote glucose disposal in the person being studied i.e the higher the SSPG concentration, the more insulin-resistant the individual. For the purpose of this study, individuals with a SSPG concentration more than 140 mg/dl were considered insulin resistant (IR), whereas those with a SSPG concentration less than 95 mg/dl were classified as insulin sensitive (IS); and both sets of individuals were included in the study. The cut point for defining IR was based on a prospective study showing that apparently healthy, non-obese individuals with SSPG concentrations greater than 140 mg/dl had significant increase in the incidence of a number of age-related diseases \(^{(19)}\). The cut point for the IS comes from evidence that one third of a large, apparently healthy population had values below this level \(^{(20)}\).

**Statistical Methods**

Descriptive results of continuous variables are expressed as mean±S.D. Parameters that did not fulfill normal distribution and homogeneity of variances (sTNFR1, sTNFR2, leptin, adiponectin and hs-CRP) were log-transformed. Means and the 95% confidence intervals (CIs) of the log-transformed data were calculated. One-way ANOVA and Tukay’s post hoc comparison test were used to compare the mean clinical and metabolic variables among the IR smokers, IS smokers, IR nonsmokers and IS nonsmokers. Relationships between variables were also sought by partial correlation analysis and stepwise multivariate linear regression analysis. Smoking status was introduced as a categorical variable (0, no smoker; 1, smoker). Two-factor ANOVA was performed to evaluate the main effects of smoking (smoker vs. nonsmoker) and insulin resistance (IR vs. IS) and their interaction on plasma adiponectin and hs-CRP concentrations. Levels of statistical significance were set at \(P<0.05\).

**RESULTS**

Clinical and metabolic characteristics of the smokers and nonsmokers further divided into IR and IS groups are given in Table (1). All four groups were similar in terms of age, gender distribution, BMI and WHR. The creatinine levels were normal (1.2 mg/dl or less) in all subjects. Smokers had significantly lower fat mass, lower fasting glucose, insulin and leptin concentrations than nonsmokers (table 1). Smokers also showed significantly higher circulating sTNFR2 levels than nonsmokers (3.7±0.8 vs. 2.9±0.8 ng/ml, \(P=0.03\)). By design, IR individuals, both smokers and nonsmokers, had SSPG concentrations that were approximately three times higher than the values in the two IS groups.
Table 1. Clinical and metabolic characteristics of the 60 study subjects classified by smoking status and insulin resistance

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Smokers</th>
<th>Nonsmokers</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IR (n = 15)</td>
<td>S (n = 15)</td>
<td>IS (n = 15)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>52 ± 9</td>
<td>49 ± 6</td>
<td>51 ± 10</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>12.5±7.5</td>
<td>9.7±8.1</td>
<td>13.5±7.8</td>
</tr>
<tr>
<td>Waist to hip ratio</td>
<td>0.98±0.05</td>
<td>0.918±0.04</td>
<td>0.96±0.04</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.2 ± 2.9</td>
<td>26.7 ± 3.1</td>
<td>28.0 ± 2.0</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>5.02±0.063</td>
<td>4.7±0.7</td>
<td>4.1±0.5</td>
</tr>
<tr>
<td>Fasting insulin(mU/L)</td>
<td>8.4±3.6</td>
<td>6.61±3.6</td>
<td>6.5±2.4</td>
</tr>
<tr>
<td>SSPG (mg/dl)</td>
<td>189 ± 39</td>
<td>77 ± 15²</td>
<td>212 ± 28</td>
</tr>
<tr>
<td>sTNFR1 (ng/ml)</td>
<td>2.01±0.4</td>
<td>2.08±0.46</td>
<td>2.01±0.42</td>
</tr>
<tr>
<td>sTNFR2 (ng/ml)</td>
<td>3.4±0.7</td>
<td>3.7±0.8</td>
<td>2.9±0.9</td>
</tr>
<tr>
<td>Leptin (µg/ml)</td>
<td>5.17±3.2</td>
<td>4.6±3.2</td>
<td>4.4±2.7</td>
</tr>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>7.8 (6.2-9.7)</td>
<td>10.5±304</td>
<td>11.7 (9.2-15)</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD or number of subjects.

¹ P value denotes the significance of differences in means of the characteristic among the four subgroups by one-way ANOVA.

P < 0.001 by Tokay’s post hoc test for the comparison of SSPG concentration between IR and IS smokers and between IR and IS nonsmokers.

The relationships between BMI and sTNFR1 (r=0.10, P=0.38) or between fat mass and sTNFR1 (r=0.12, P=0.28) were not statistically significant among nonsmokers. The relationships for smokers, by contrast, were always statistically significant and the coefficient was higher than for nonsmokers (r=0.51, P=0.001 for BMI; r=0.56, P<0.0001 for fat mass; figure 1).

Similar findings were observed for sTNFR2: r=0.43, P=0.003 for BMI; r=0.30, P=0.04, for fat mass, in smokers; r=0.03, P=0.75 for BMI; r=0.003, P=0.97, for fat mass, in nonsmokers. We explored the possible dose–response between cigarette consumption and sTNFRs. A simple correlation analysis showed no significant associations between the numbers of cigarettes a day and sTNFR1 or sTNFR2. However, after controlling for fat mass, the association between sTNFR2 and the number of cigarettes a day became significant (r=0.33, P=0.002). Moreover, in those subjects who smoked less than 20 cigarettes a day, sTNFR2, but not sTNFR1, was significantly associated with cigarette consumption (figure 2).

Fig.1. Linear correlation analysis of the relationship between sTNFR1 and fat mass in smoking (black symbols) and nonsmoking men (open symbols).
Fig. 2. Association between number of cigarettes a day and serum sTNFR2 concentration in persons who smoked less than 20 cigarettes a day.

Figure 3A shows adiponectin concentrations in the 30 smokers and the 30 nonsmokers, with the IR and IS individuals in each group identified. The most obvious finding is that the variability of plasma adiponectin concentrations was much greater in the nonsmokers. The plasma adiponectin concentrations [mean (95% CI)] were lower in smokers [9.2 µg/ml (6.2–12.8)] than nonsmokers [12.4µg/ml (9.2–20.6)]. Adiponectin levels were also lower in IR individuals [9.4µg/ml (6.2–15.0)], compared with those who were IS [13.0 µg/ml (10.5–20.6)]. Differences in means among these groups were compared using two-factor ANOVA with interaction. The results showed that smokers had significantly lower (F = .2, P = 0.046) average adiponectin concentrations, compared with nonsmokers, after taking into account differences in their insulin resistance status (significant main effect of smoking). IR subjects on average also had significantly lower (F = 11.7, P =0.001) adiponectin concentrations, compared with IS subjects, after controlling for differences in their smoking status (significant main effect of insulin resistance). Moreover, the differences in mean adiponectin concentrations between smokers and nonsmokers did not depend on the insulin resistance status (F =0.46, P = 0.50) of the subjects (non significant smoking and insulin resistance interaction effect). When BMI was included as covariate in the ANCOVA, the main effects of smoking and insulin resistance on adiponectin levels remained significant (P =0.037 and 0.008, respectively), whereas the effects of BMI and the interaction between insulin resistance and smoking were not. This finding indicated that after adjustment for differences in BMI, and insulin resistance status, the mean adiponectin levels continued to be significantly lower in smokers.

Figure 3B displays the CRP concentrations in smokers and nonsmokers, again subdivided based on their classification as IR or IS. In contrast to the data shown in Fig.1A, the variability in the individual values seemed comparable in nonsmokers and smokers. The CRP values [mean (95% CI)] were somewhat higher in the smokers [1.61 µg/ml (1.22–2.12)] than nonsmokers [1.19 µg/ml (0.82–1.72)], and there was also a trend toward higher values among the IR subjects [1.65 µg/ml (1.15–2.36)], compared with those who were IS [(1.16 µg/ml (0.87–1.54)]. Two-factor ANOVA showed that the average CRP concentrations were not significantly different between the smokers and nonsmokers (F =1.8, P= 0.18), even after controlling for differences in their insulin resistance status. There were also no significant differences in average CRP concentrations between the IR and IS subjects despite dissimilarity in their smoking status (F = 2.4, P = 0.13). Finally, there was no significant effect (F = 0.01, P = 0.92) of an interaction between smoking and insulin resistance on CRP levels (figure 3. B).
FIG. 3. Plasma adiponectin (A) and CRP concentrations (B) in smokers (n = 30) and nonsmokers (n = 30). Individual data for each subject are shown with IR smokers (•), IS smokers (▲), IR nonsmokers (●), and IS nonsmokers (△) identified. Adiponectin and CRP values were log transformed for statistical analysis. The horizontal lines represent the geometric means of the IR (solid line) and IS (broken line) subgroups within the main groups of smokers and nonsmokers. * P value indicates the significance of the effect of smoking on adiponectin (A) and CRP (B) concentration after controlling for differences in insulin resistance status by two-factor ANOVA; † P value indicates the significance of the effect of insulin resistance on adiponectin (A) and CRP (B) concentrations after adjustment for differences in smoking status by two-factor ANOVA.

DISCUSSION

A finding of this study was that the association between fatness and the activity of the TNF axis was dependent on smoking in apparently healthy men. Soluble TNFα receptors were strongly associated with adiposity in smokers, who also had significantly higher sTNFR2 concentration than nonsmokers. Although the difference observed seemed quite small, it may be biologically significant because of the lower fat mass of smokers. Taking into account that smokers were thinner and had lower glucose and insulin levels, and that both BMI and insulin resistance have been demonstrated to increase sTNFR2 levels, those sTNFR2 levels found in smokers were disproportionately higher than those observed in non-smokers. Activation of the TNF-α system is associated with increased energy expenditure and weight loss in humans, but this seems to be in contrast with the increased TNFα mRNA expression of adipose tissue, and increased circulating TNFα, sTNFR1 and sTNFR2 of obese subjects, in whom no weight loss is observed. The stimulus for TNFα secretion might be derived from a component recently described in cigarette smoke itself: endotoxin lipopolysaccharides (LPS). LPS is one of the most potent biologic response modifiers currently recognized, and possesses a multitude of effects, most notably the initiation of pro-inflammatory cascade, stimulating the production and release of cytokines, chemokines and eicosanoids. LPS is present in normal indoor environments as a constituent of house dust. Intravenous administration of LPS to healthy volunteers caused marked increase in sTNFR1 and sTNFR2. The origin of the associations described in the current study might be due to direct activation of TNFα secretion caused by smoking. Repeated exposure to LPS can induce tolerance in target cells. The observed steeper slope of relationship between fat mass and circulating sTNFRs in smokers suggests chronic activation of the TNFα system in smokers. Repeated exposure to environmental LPS (smoking) might also be hypothesized to contribute to TNFα tolerance through increased sTNFRs in some obese subjects. However, other components of cigarette smoke (oxidant gases: nitric oxide, ozone and nicotine) and/or physical factors (thermal exposure, inhalation of particles) could contribute to the inflammatory effects of smoking in the
specific activation of the TNF pathway. We demonstrated that the IR individuals have significantly lower plasma adiponectin concentrations than their IS counterparts, a finding consistent with previous publications (7, 9, 10, 13). Because there is evidence suggesting that the prevalence of insulin resistance is increased in smokers (1, 11, 13, 15), it was possible that reports of lower adiponectin concentrations in smokers was not related to smoking, per se, but to the concomitant presence of insulin resistance in smokers. The results of the current study argue against this possibility and provide evidence that plasma adiponectin levels are on the average lower in smokers and demonstrate less inter-individual variability than in nonsmokers.

We quantified insulin-mediated glucose disposal with the insulin suppression test (17, 19, 20), a method shown to provide essentially identical values for insulin action as the euglycemic, hyperinsulinemia clamp technique (18), thus allowing us to account more precisely for the effect of insulin resistance when assessing the role of cigarette smoking. The fact that mean adiponectin concentrations in smokers were lower by 26% in the present study, a decrease similar to the 20% decrement in adiponectin concentrations in the 311 smokers studied by Thamer et al. (5), lends further support for the notion that plasma adiponectin concentrations are lower in smokers.

These data provide substantial evidence that differences in the degree of insulin sensitivity have powerful impact on plasma adiponectin concentrations with the levels in IS individuals essentially 1.5-fold higher than those in IR subjects. Smoking also appears to have distinct negative effect on plasma adiponectin concentrations because these levels were lower in smokers than nonsmokers. Furthermore, the lower plasma adiponectin concentrations in smokers were not dependent on the insulin resistance status of the subjects. This indicates that the effect of smoking independently attenuates the differences in plasma adiponectin concentrations normally present between IR and IS individuals. In contrast to the impact of smoking on plasma adiponectin concentrations, we could not discern an adverse effect of smoking on CRP concentration. Thus, although the CRP concentrations in smokers were somewhat higher than in nonsmokers, the differences were not statistically significant, even after controlling for differences in their insulin resistance status. Because cigarette smoke contains thousands of potentially bioactive constituents, including free radicals, it is quite possible that one or more of these factors may lower adiponectin production or release from adipocytes. For example, several studies demonstrated that nicotine, a major component of cigarette smoke, promotes inflammation and appears to have direct effects on adipose tissue, inducing adipose tissue lipolysis via enhanced release of catecholamines (27, 28) Consistent with this, addition of nicotine (as well as hydrogen peroxide) to 3T3-L1 adipocytes reduced expression of adiponectin in a dose-dependent fashion (9, 10).

**CONCLUSIONS**

Both fat mass and smoking are related to increased activity of the TNFα axis. Plasma adiponectin concentrations are lower in smokers and IR subjects. These two mechanisms could be associated with increased cardiovascular risk in smokers. No difference was detected in average hs- CRP concentrations between smokers and nonsmokers and between IR and IS subjects.

**REFERENCES**