Body Mass Index Modulates Aromatic DNA Adduct Levels and Their Persistence in Smokers

Roger W. L. Godschalk, Dorien E. M. Feldker, Paul J. A. Borm, Emiel F. M. Wouters, and Frederik-Jan Van Schooten

Department of Health Risk Analysis and Toxicology, University of Maastricht, 6200 MD Maastricht [R. W. L. G., D. E. M. F., P. J. A. B., F-J. V. S.], and Department of Pulmonology, Academic Hospital Maastricht, 6202 AZ Maastricht [E. F. M. W.], the Netherlands

Abstract

Smokers with a low body mass index (BMI; weight/height$^2$) have a higher risk for developing lung malignancies as compared with smokers of average weight, but there is no mechanistic explanation for this observation. Carcinogens in cigarette smoke are thought to elicite cancer by the formation of DNA adducts, which give the opportunity to additionally investigate the biological link between BMI and lung cancer. DNA adduct levels in peripheral blood lymphocytes of 24 healthy smoking volunteers ($0.76 \pm 0.41$ adducts per $10^8$ nucleotides) positively correlated with cigarette consumption ($r = 0.51; P = 0.01$) and were inversely related with BMI ($r = -0.48; P = 0.02$). A significant overall relationship was observed when both parameters were included in multiple regression analysis ($r = 0.63; P = 0.007$). Moreover, body composition may affect DNA adduct persistence, because lipophilic tobacco smoke-derived carcinogens accumulate in adipose tissue and can be mobilized once exposure ceases. Therefore, DNA adduct levels and BMI were reassessed in all of the subjects after a nonsmoking period of 22 weeks. Adduct levels declined to $0.44 \pm 0.23$ per $10^8$ nucleotides ($P = 0.002$), and the estimated half-life was 11 weeks on the basis of exponential decay to background levels in never-smoking controls ($0.33 \pm 0.18$ per $10^8$ nucleotides). Overweight subjects (BMI $>25$) with little weight gain after smoking cessation (<median weight gain of 6%) had more persistent adduct levels as compared with those with lower BMI and higher weight gain ($P = 0.06$). Overall, these results suggest that leanness is a host susceptibility factor that affects DNA adduct formation, which could underlie the observed relationship between BMI and lung cancer risk.

Introduction

Epidemiological studies consistently showed that smokers with a low BMI [$\text{weight (kg)/height}^2$ (m$^2$)] have an increased risk for developing lung malignancies as compared with smokers of average weight (1–5). Still, it is not clear whether or not low body weight causes or reflects increased susceptibility to lung cancer, especially in smokers. Lipophilic carcinogens derived from cigarette smoke, such as PAHs are thought to elicite cancer by covalent interactions with DNA, so-called DNA adducts (6). Theoretically, DNA adduct levels are an indication for the net outcome of carcinogen exposure, bioactivation, and DNA-repair. Therefore, the quantitation of DNA adducts is a good indicator for the biologically effective dose and could be of use in risk assessment (7). In this respect, DNA adduct measurements give the opportunity to additionally investigate the relationship between BMI and lung cancer risk in smokers, well before the manifestation of a tumor. Overall, large interindividual variations have been reported in DNA adduct levels in smokers (8–10). Although BMI has not been studied before as a potential source for this variation, it can be considered as a modifying factor for exposures to lipophilic substances; BMI was found to affect circulating levels of lipophilic contaminants (11), and a positive correlation was observed between BMI and aromatic DNA adducts in pancreatic tumor tissue (12). Furthermore, lipophilic aromatic compounds may be stored in adipose tissue (13), and it can be hypothesized that after smoking cessation they are mobilized and released into the peripheral blood, which would prolong the apparent half-life of DNA adducts.

In this pilot study, the effect of body composition (assessed as BMI) on DNA adduct levels and DNA adduct persistence in MNCs of 24 healthy smokers was examined.

Materials and Methods

Study Populations. One hundred healthy smoking subjects, willing to stop smoking, were recruited at the Asthma Center Hornerheide in Hon (the Netherlands) by media advertisement. Weight (kg) and height (m) of these individuals was obtained, and the BMI was calculated (kg/m$^2$). After informed consent, 10 ml of blood was collected at the initial visit, the day before quitting smoking. The subjects applied nicotine patches daily to increase the success rate of smoking cessation. After 22 weeks, 32 individuals succeeded in continuing complete abstination from smoking, and blood samples of only 24 individuals could be used for DNA adduct analysis because of withdrawal

Received 11/2/01; revised 4/10/02; accepted 4/24/02.

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1 Present address: Department of Toxicology and Cancer Risk Factors, German Cancer Research Center, 69009 Heidelberg, Germany.
2 Present address: Department of Particle Research, Institut für Umweltmedizinische Forschung gGmbH, University of Düsseldorf, 40221 Düsseldorf, Germany.
3 To whom requests for reprints should be addressed, at Department of Health Risk Analysis and Toxicology, University of Maastricht, 6200 MD Maastricht, the Netherlands. Phone: 31-433881100; Fax: 31-433670924; E-mail: F.Vanschooten@GRAT.unimaas.nl.

4 The abbreviations used are: BMI, body mass index; PAH, polycyclic aromatic hydrocarbon; MNC, mononuclear blood cell; BPDE, benzo(a)pyrene-diol-epoxide.
from the study and technical difficulties during sampling or assaying. This group of volunteers (ages 44 ± 8 years) had smoked on average 25 ± 9 cigarettes per day over a period of 28 ± 8 years before smoking cessation. Overall characteristics of the study population are presented in Table 1.

**Table 1** Characteristics of the study population (mean ± SD and range) before and after smoking cessation for 22 weeks

<table>
<thead>
<tr>
<th>Description</th>
<th>Before smoking cessation</th>
<th>After smoking cessation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male/female)</td>
<td>14/10</td>
<td>14/10</td>
</tr>
<tr>
<td>Age (years)</td>
<td>44 ± 8</td>
<td>33–62</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>173 ± 6</td>
<td>164–184</td>
</tr>
<tr>
<td>Cigarettes/day</td>
<td>25 ± 9</td>
<td>15–50</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>74 ± 11</td>
<td>58–99</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.8 ± 2.7</td>
<td>19.0–29.4</td>
</tr>
<tr>
<td>DNA adduct level per 10⁶ nucleotides</td>
<td>0.76 ± 0.41</td>
<td>0.1–1.59</td>
</tr>
</tbody>
</table>

All of the subjects reported to have stopped smoking.

Results of the Study Population. There was no difference in initial smoking between male (n = 14) and female (n = 10) volunteers. However, female subjects had a significantly lower BMI (23.9 ± 1.4) than male volunteers (25.7 ± 2.9; P = 0.047), and the BMI increased significantly after smoking cessation in both sexes (females: 25.4 ± 2.0; P = 0.003; males: 27.5 ± 3.3; P < 0.001). Furthermore, BMI before smoking cessation correlated with age (r = 0.32; P = 0.08) but not with the amount of cigarettes smoked per day, nor with pack-years. After smoking cessation for 22 weeks, BMI was no longer related to age nor with gender.

**BMI in Relation with DNA Adduct Levels.** DNA adduct profiles obtained by ³²P-postlabeling and subsequent TLC were identical for all of the subjects; diagonal radioactive zones were observed, which are typical for exposure to cigarette smoke (8). Aromatic DNA adduct levels in MNC (0.76 ± 0.41 adducts per 10⁶ nucleotides; range, <0.1–2.12) were significantly related to the self-reported amount of cigarettes smoked per day (r = 0.51; P = 0.01; Fig. 1A) and were inversely related with the BMI adjusted for age and gender (r = −0.48; P = 0.02; Fig. 1B).

When both parameters were included in a multiple regression analysis, a significant overall relationship was observed [included parameters: BMI adjusted for age and gender (β = −0.08; P = 0.05) and cigarettes/day (β = 0.02; P = 0.03), overall: r = 0.63; P = 0.007], which indicates that interindividual differences in adduct levels depend on both dose (cigarettes/day) as well as BMI. For exemplification, blood cells of subjects with a BMI lower than the 25th percentile contained 2.5-fold higher DNA adduct levels (1.24 ± 0.24) as compared with those individuals with a BMI higher than the 75th percentile (0.49 ± 0.39; P = 0.002).

<table>
<thead>
<tr>
<th>DNA Adduct Levels after 22 Weeks of Smoking Cessation</th>
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</table>
| Cigarettes/day | 2.5 mg/ml | 2.5 m M NaCl, 25 m M EDTA, 50 g/ml proteinase K, and 1% SDS) and incubated overnight at 37°C. DNA was extracted with phenol:chloroform:isoamylalcohol (25:24:1, v/v/v) and chloroform:isoamylalcohol (24:1, v/v), respectively. The DNA was precipitated with two volumes of (25:24:1, v/v/v) and chloroform:isoamylalcohol (24:1, v/v), respectively. The DNA was precipitated with two volumes of cold ethanol after addition of 1/30 volume 3 m sodium acetate (pH 5.3) and washed with 70% ethanol. Subsequently, DNA was dissolved in 2 mM Tris (pH 7.4). Concentration and purity were determined spectrophotometrically by absorbance at 230, 260, and 280 nm, and the final volume was adjusted to achieve a DNA concentration of 2 mg/ml.

**³²P Postlabeling.** The ³²P-postlabeling assay was performed as described previously (8, 15). Briefly, DNA was digested by micrococcal endonuclease and spleen phosphodiesterase, and subsequently treated with nuclease P1 to dephosphorylate unmodified nucleotides. Labeling was carried out with excess of [γ⁳²P]ATP and T4-polynucleotide kinase. ³²P-Labeled adducts were resolved on polyethyleneimine-cellulose TLC sheets (Merck, Darmstadt, Germany) using the solvents as described in Ref. 8. In each experiment, three standards of [¹⁴C]BPDE modified DNA with known modification levels (1 per 10⁹, 10⁸, and 10⁷ nucleotides) were run in parallel for quantification purposes. Quantification was performed by using a phosphorimager (Molecular Dynamics, Sunnyvale, CA). A detection limit for BPDE-DNA adducts was reached of ≤0.1 adducts per 10⁶ nucleotides. Interassay variation was <20%.

**Statistical Analysis.** Results are presented as mean ± SD. All of the variables before smoking cessation were statistically compared with those after smoking cessation by the Wilcoxon signed rank test for paired samples. Multiple linear regression was used to analyze the relationship between various parameters with DNA adduct levels. Half-lives of DNA adducts were estimated on the basis of an exponential decay curve with leveling-off to a symptotic value (i.e., “background” levels observed in never-smokers obtained from previous studies (8, 10, 16) using the formula y = [(a − c)/2rn] + c. In the formula, a = adduct level before smoking cessation, b = half-life in weeks, c = leveling-off value for large t (i.e., adduct level in never-smokers), y = adduct level after smoking cessation for 22 weeks, and t = time (weeks).

The impact of BMI on adduct persistence was investigated by: (a) dividing the observed adduct level at 22 weeks after smoking cessation by the expected value calculated on the basis of the overall half-life and the initial individual adduct level; and (b) correlating this observed/expected-value with BMI indices (before and/or after cessation and/or weight gain). P < 0.05 was considered significant.
for a period of 22 weeks. Similar diagonal radioactive zones were observed but with lower intensities. Total DNA adduct levels significantly decreased from $0.76 \pm 0.41$ to $0.44 \pm 0.23$ adducts per $10^8$ nucleotides (range: $<0.1–0.88$; $P = 0.002$). Previous studies performed in our laboratory showed that DNA adduct levels were $0.33 \pm 0.18$ in never-smokers ($8, 10, 16$), and from these results a half-life of 11 weeks was estimated (Fig. 2). According to our hypothesis, the ratio between the observed and expected adduct levels after 22 weeks of smoking cessation (i.e., adduct persistence) should be higher in subjects with a high BMI before smoking cessation (simple regression: $r = 0.40$; $P = 0.092$; $n = 24$). However, a strong increase of BMI after smoking cessation was observed in 23 of 24 subjects ($P < 0.001$). Before smoking cessation, a high BMI was associated with lower DNA adduct levels. Thus, increased adduct persistence in overweight subjects may partly be counteracted by the lowering effect of increased body weight. Indeed, multiple regression analysis showed that when both parameters were analyzed together, DNA adduct persistence (determined as the ratio between observed and expected adduct levels) correlated positively with BMI before smoking cessation and also inversely with the relative gain in body weight [variables included in the multiple regression model were: BMI before smoking cessation ($\beta = 0.06$; $P = 0.014$) and relative weight gain ($\beta = -2.7$; $P = 0.037$), overall relationship: $r = 0.66$; $P = 0.011$]. Inclusion of the amount of cigarettes smoked per day before smoking cessation did not improve the regression model. The observed/expected value in subjects with a BMI $<25$ (= median BMI) and a relative weight gain of $>6\%$ (= median weight gain) was $0.78 \pm 0.15$, whereas in subjects with a BMI $>25$ and lower weight gain ($<6\%$) this ratio was $1.23 \pm 0.21$ (Fig. 3; simple regression: $P = 0.06$).

Discussion

The relationship between BMI and mortality shows an asymmetrical U-function, and the two tails in the distribution of BMI show marked differences in the causes of death (17); the upper part of the distribution (high BMI) is characterized by cerebrovascular and cardiovascular diseases. The lower part (low BMI) is characterized by a high attribution of obstructive lung diseases and lung cancer, especially in smokers. The relationship between leanness and lung cancer remains a matter of debate, because in many studies it was practically impossible to rule out bias because of preclinical disease or to completely adjust for confounding factors like cigarette smoking. In the present study, we additionally investigated the biological link between BMI and lung cancer by investigating levels of smoking-related aromatic DNA adducts, which are thought to play a crucial role in the onset of lung cancer.

A positive correlation between BMI and DNA adduct formation by lipophilic aromatic compounds was reported previously in pancreatic tumor tissue by Wang et al. (12). In the present study the opposite was observed using DNA from mononuclear cells in the blood. Our data are in agreement with the results of a recent large cross-sectional study (Ref. 18; $n = 309$), in which DNA adduct values in peripheral blood lymphocytes were found to be significantly lower among overweight subjects. Still, it is unclear whether BMI is an independent risk factor or whether some dietary/genetic factors associated with BMI predispose to the formation of adducts. Nonetheless, these results indicate that BMI is a modulating factor for aromatic DNA adduct formation in healthy smokers and should be taken into account in research on cancer susceptibility.

DNA adduct levels decreased significantly after smoking cessation, and the calculated half-life was well in line with earlier findings (19, 20). After smoking cessation for 22 weeks, adduct levels were still increased as compared with never-smoking controls, although not statistically significant. It is plausible that after smoking cessation, accumulated lipophilic compounds are mobilized from adipose tissue and might serve as an additional (delayed) source of exposure to genotoxic compounds. Thus, the elimination rate of aromatic DNA adducts in MNCs may be influenced by body composition. Indeed, a high BMI before smoking cessation tended to be associated with high adduct persistence. However, DNA adduct persistence was also found to be inversely related with weight gain, which is additional evidence for the observation that DNA adduct levels are lower at higher BMI. It is still debatable whether increased adduct levels after a long period of nonsmoking were because of previous exposures, were already present in longer-lived cells and/or were formed because of the release of genotoxic compounds from adipose tissue. Inhaled PAHs are retained for a long period of time in the lung without being metabolically converted to water-soluble derivatives.
and may subsequently be transported to and stored in adipose tissue in their unmetabolized form. Indeed, concentrations of unmetabolized pyrene (as model PAH) in adipose tissue were higher than in most other tissues, whereas concentrations of pyrene metabolites were relatively high shortly after the exposure and decreased quickly as a result of their hydrophilicity (22). Furthermore, it is suggested that lipoproteins are involved in the transport of PAHs through the blood (23), and the levels of lipoproteins are highly dependent on diet. Therefore, because BMI is also related to diet, the BMI effects observed in the present study may be linked to transport of PAHs through the blood in combination with the higher volume of distribution in overweight subjects, suggesting that toxicokinetics is an important issue for identifying individuals at a higher lung cancer risk.

Recently, it was reported in a prospective study that DNA adducts in peripheral blood lymphocytes reflect lung cancer risk (7). The results of the present study additionally support these findings, because the levels of aromatic DNA adducts seemed to coincide with known risk factors for lung cancer: DNA adduct levels in MNC (8) and lung cancer risk (24) both correlate with the amount of cigarettes smoked per day and both decrease after smoking cessation (24, 25). Moreover, DNA adduct levels were higher in lean smokers than in smokers with a high BMI, which has consistently been identified as a risk factor for the development of lung malignancies (1–5). Therefore, these results provide mechanistic insights in the relationship between BMI and lung cancer. Because leanness seems to be a host susceptibility factor for carcinogen-DNA adduct formation in smokers, this information is valuable for the identification of individuals at higher cancer risk and deserves additional attention.

Acknowledgments

We thank Marcel van Herwijnen and Dr. Roel Schins (Maastrict University) for technical assistance. Dr. Rob Mostert at the Asthma Center Hornerheide is acknowledged for his work during collection of the samples.

References

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