Tobacco Smoking Decreases Plasma Concentration of the Emerging Cardiovascular Risk Marker, L-Homoarginine

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**Background:** Tobacco smoking is one of the most important risk factors for cardiovascular disease (CVD) and few biomarkers have been linked to the increased risk of CVD and tobacco smoking. Tobacco smoke has been shown to elevate the plasma levels of asymmetric dimethylarginine (ADMA), a metabolite of L-arginine and an endogenous inhibitor of endothelial nitric oxide synthase. The other potential biomarker that has not been studied to date is L-homoarginine, a homolog of L-arginine. The aim of this study was to evaluate the effects of cigarette smoking on L-homoarginine and other CVD biomarkers.

**Methods and Results:** In a cross-sectional study of 231 healthy male volunteers, we measured plasma levels of L-homoarginine, L-arginine, and ADMA using the HPLC method. In smokers, we found that plasma L-homoarginine levels were 16.7% lower compared with nonsmokers after adjusting for age, body mass index, plasma creatinine, and metal blood levels (P<0.05). Plasma ADMA levels were only 6.0% higher in smokers when compared with the levels found in nonsmokers (P>0.05).

**Conclusions:** Our results suggest that, in contrast to ADMA, there is a strong association between exposure to tobacco smoke and plasma L-homoarginine levels. Further research in this field is needed to explain the mechanisms of the relationship of low L-homoarginine levels, smoking, and cardiovascular health. (Circ J 2014; 78: 1254–1258)

**Key Words:** Asymmetric dimethylarginine (ADMA); L-arginine; L-homoarginine; Symmetric dimethylarginine (SDMA); Tobacco smoking

In the past decade, there has been increased interest in new markers of cardiovascular diseases (CVDs). These new markers of CVD risk include 2 metabolites of L-arginine: asymmetric and symmetric dimethylarginine (ADMA and SDMA, respectively). The toxic effect of tobacco smoke is multidirectional, but the mechanism of its action is not completely clear. One mechanism may be explained by the adverse effect of tobacco smoke on the concentration of newly discovered cardiovascular risk factors, including ADMA and SDMA, which are metabolites of L-arginine. Increased plasma concentration of ADMA, which is an endogenous inhibitor of endothelial nitric oxide (NO) synthase, is a strong and independent predictor of morbidity and overall mortality of CVD in healthy women and the general population, as well as in patients with kidney insufficiency, ischemic heart disease, peripheral vascular disease, chronic heart failure, idiopathic pulmonary hypertension, and diabetes. SDMA does not inhibit NO synthase, but may be a useful biomarker for detecting individuals in the early stages of kidney dysfunction and for determining their risk for developing CVD.

To date, most experimental and epidemiological studies have focused on the effect of tobacco smoke on ADMA levels only. The first study of the influence of tobacco smoke on ADMA levels was performed using animal models and demonstrated an increase in ADMA concentrations in the endothelial cells of rabbits after prolonged exposure to nicotine. Jiang et al. observed a significant increase in ADMA concentrations in rat plasma following a 4-week administration of nicotine in doses of 5 mg·kg⁻¹·day⁻¹, and they suggested that nicotine models the metabolic pathway of ADMA in cells by activating the alpha7 nicotinic acetylcholine receptor. However, studies based on the culture of human endothelium-derived EAhy 926 cells produced divergent results. Incubation of cells within 48 h with condensate of tobacco smoke at concentrations of 1.0 and 10.0 mg/L...
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In a previous study, we examined lead-exposed workers for the risk of atherosclerosis by measuring various CVD biomarkers and non-enzymatic antioxidants. Interestingly, in the multivariate analysis we found that smoking status strongly correlated with these biomarkers. Thus, in the present study we decided to further explore the effect of tobacco smoking on the plasma levels of L-homoarginine and other CVD biomarkers, including L-arginine, ADMA, and SDMA.

Methods

Study Population

We present the results of a secondary analysis of findings of a study on the effect of occupational exposure to lead on new atherosclerosis risk factors and non-enzymatic antioxidants. Volunteers were recruited from workers at a zinc and lead ore mine in Boleslaw Mine and Metallurgical Plant S.A. in Bukowno (southern Poland). The Biomedical Ethics Committee at the Institute of Occupational Medicine and Environmental Health in Sosnowiec reviewed and approved the study protocol.

A sample of 231 healthy male volunteers aged 20–60 years was studied. All participants provided written informed consent, completed a survey on smoking habits, including the numbers of cigarettes smoked per day (CPD) and years of tobacco smoking (YTS). This study excluded participants with CVDs, diabetes, creatinine clearance >140 ml/min and those with above-normal levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). All participants also completed a survey regarding the consumption of products rich in vitamin E.

No statistically significant differences between study and control groups in the consumption of these products in the month preceding the study were detected. All participants declared that in the 3 months preceding the study, they did not take any pharmaceutical preparations containing vitamin E.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Nonsmokers (n=111)</th>
<th>Smokers (n=120)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [years]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Means±SD</td>
<td>41.8±10.0</td>
<td>42.6±9.7</td>
<td>0.462</td>
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<tr>
<td>Range</td>
<td>20–60</td>
<td>21–56</td>
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<tr>
<td>Median</td>
<td>43</td>
<td>44.5</td>
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</tr>
<tr>
<td>BMI [kg/m²]</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Means±SD</td>
<td>28.1±3.9</td>
<td>26.4±4.0</td>
<td>0.001</td>
</tr>
<tr>
<td>Range</td>
<td>21–43</td>
<td>19–40</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>28</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol [mmol/L]</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Means±SD</td>
<td>5.11±1.24</td>
<td>5.29±1.05</td>
<td>0.032</td>
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<tr>
<td>Range</td>
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<td>2.40–7.90</td>
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<tr>
<td>Median</td>
<td>4.80</td>
<td>5.20</td>
<td></td>
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<tr>
<td>Triglyceride [mmol/L]</td>
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<tr>
<td>Means±SD</td>
<td>1.66±1.02</td>
<td>1.78±1.09</td>
<td>0.264</td>
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<tr>
<td>Range</td>
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<td>0.41–6.15</td>
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<tr>
<td>Median</td>
<td>1.31</td>
<td>1.46</td>
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<tr>
<td>Creatinine in plasma [mmol/L]</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Means±SD</td>
<td>83.8±12.5</td>
<td>83.3±8.5</td>
<td>0.474</td>
</tr>
<tr>
<td>Range</td>
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<tr>
<td>Median</td>
<td>83.2</td>
<td>83.2</td>
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<tr>
<td>Lead (Pb) in blood [μg/L]</td>
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<tr>
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<td>293±120</td>
<td>0.001</td>
</tr>
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<td>Range</td>
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<td>72–632</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>221</td>
<td>292</td>
<td></td>
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</tbody>
</table>

*Calculated for the Mann-Whitney U-test.
Characteristics of the Study Group

Population characteristics are shown in the Table. There were 111 nonsmokers and 120 current smokers (n=231). The mean CPD and mean YTS (mean±SD) were 14.9±5.5 and 19±7 years, respectively. Total blood cholesterol and lead levels were significantly higher in smokers than in nonsmokers (P=0.032 and P<0.001, respectively).

Cardiovascular Biomarkers

We found that mean plasma levels of L-homoarginine were significantly lower in smokers than in nonsmokers (1.53 (95% confidence interval [CI] 1.42–1.63) vs. 1.98 (95% CI 1.86–2.09) µmol/L, respectively; P<0.001). Mean plasma levels of L-arginine were 54.4 (95% CI 50.7–58.1) and 49.0 (95% CI 45.5–52.4) µmol/L for smokers and nonsmokers, respectively (P=0.035). Nonsignificant differences were found in the ADMA levels between smokers and nonsmokers (0.362 (95% CI 0.349–0.376) vs. 0.348 (95% CI 0.334–0.362) µmol/L, respectively) (P=0.143). No differences were observed in SDMA levels between smoking and nonsmoking subjects (0.374 (95% CI 0.358–0.389) vs. 0.376 (95% CI 0.359–0.393) µmol/L, respectively) (P=0.852). After adjusting for independent variables, significant differences were found for plasma L-homoarginine, L-arginine, and ADMA levels (P<0.05). A comparison of adjusted mean levels of analyzed biomarkers between smokers and nonsmokers is presented in the Figure. In the smokers group, plasma L-homoarginine levels were lower by 16.1% (95% CI 79–24.2%; P<0.001) whereas L-arginine levels were higher by 15.4% (95% CI 3.5–27.3%; P=0.011) and ADMA levels were higher by 6.4% (95% CI 0.4–11.3%; P=0.036) when compared with the nonsmokers.

In addition, a significant Spearman’s rank correlation was found between CPD and plasma L-homoarginine (r=−0.3491; P<0.001), as well as plasma L-arginine (r=0.1595; P=0.015). Spearman’s rank correlation coefficients between CPD and the ADMA or SDMA level were low and not significant (r=0.1178; P=0.075 and r=−0.0206; P=0.756, respectively).

Biochemical Examination

After a 12-h fast, blood for biochemical analyses was obtained from the cubital vein and placed into vacuum tubes. A portion of the isolated plasma was used to determine cholesterol, triglyceride and creatinine levels. The remaining blood and plasma were stored at −80°C for spectrometric and chromatographic examinations, which were used to determine blood lead levels and the presence of plasma CVD biomarkers, including L-homoarginine, L-arginine, ADMA, and SDMA. CVD biomarkers were measured using high-performance liquid chromatography (HPLC) followed by derivatization with o-phthalaldehyde according to a method described elsewhere.18 Lead levels in the blood were measured using electrothermal atomic absorption spectrometry after the blood samples were deproteinized with 0.8 mol/L nitric acid in a 1:4 ratio.

Statistical Analysis

Statistical analyses were performed using Statistica v. 9.1 software (StatSoft Inc, USA). In the groups of smokers and nonsmokers, mean levels of CVD biomarkers were calculated for crude values and for values adjusted for age, body mass index (BMI), and blood lead and plasma creatinine levels and compared using ANCOVA. General characteristics parameters in relation to smoking status were evaluated using the Mann-Whitney U-test. The Spearman rank correlation test was performed to estimate associations between variables. Differences in the levels of L-homoarginine, L-arginine, and its metabolites (ADMA and SDMA) between the smokers and nonsmokers were assessed as crude values and after multivariate adjustment. To determine the effects of independent variables (ie, age, BMI, smoking status, blood lead levels, plasma creatinine and total cholesterol levels) on the dependent variables (ie, L-homoarginine, L-arginine, ADMA and SDMA), a forward stepwise linear regression was used. Statistical significance was determined at the level of P<0.05. All P values refer to two-sided hypotheses.
**Discussion**

We presented novel findings on the relationship between smoking status and an emerging CVD biomarker, L-homoarginine. Our results suggest that, in contrast to other CVD biomarkers, the association between exposure to tobacco smoke and L-homoarginine levels is strong. We found that levels of L-homoarginine were significantly different between nonsmokers and smokers.

The other important finding of the study is the strong correlation between CFPD and L-homoarginine plasma levels. The predictive ability for plasma L-homoarginine levels decreased across independent variables in the following order: smoking status > BMI > blood lead levels > plasma creatinine levels.

The study also found that lower plasma L-homoarginine levels in smokers corresponded to higher plasma L-arginine levels. L-arginine is a donor of the amidino group in the likely main pathway de novo synthesis of L-homoarginine from lysine in humans. This process is catalyzed by the enzyme arginine:glycine amidinotransferase, which is mainly involved in production of creatine from L-arginine and glycine. Low L-arginine:glycine amidinotransferase, which is mainly involved in production of creatine from L-arginine and glycine. Low L-homoarginine levels in smokers may thus reflect local failing energy metabolism in the myocardium, possibly because of inhibition of arginine:glycine amidinotransferase by tobacco smoke. This leads to a significant decrease in L-homoarginine production and an increase in L-arginine.

Recent studies have indicated that L-homoarginine is also positively related to endothelial function. Apart from L-arginine, L-homoarginine may act as a substrate for the endothelial isoform of NO synthase, which is an important element in the production of the vasodilator NO. NO exerts other beneficial properties, including antithrombotic, antioxidant, and anti-inflammatory effects in vessels. The role of L-homoarginine in NO metabolism is unclear at present, but low L-homoarginine levels may decrease NO production, leading to progression of vascular endothelial dysfunction. Other evidence suggests that L-homoarginine increases insulin secretion and inhibits platelet aggregation. In the present study, L-arginine, a well-known substrate for the production of NO, was increased in smokers. Therefore, it is difficult to determine how low levels of L-homoarginine and high levels of L-arginine affect endothelial function. Recently, it was proposed that L-homoarginine might affect cardiovascular risk by mechanisms other than being a simple NO precursor, and the hypothetical association of L-homoarginine with the activity of endothelial arginases appears to be another plausible possibility.

Further studies are needed to explore the causes of low levels of L-homoarginine in smokers and the association with cardiovascular health.

Our results indicate that ADMA levels were weakly associated with smoking status. As mentioned in the Introduction, most of the published studies found increased plasma ADMA levels under the influence of tobacco smoking. However, notably, in the majority of cases, these studies involved patients with known CVD, and they differed in age and sex. In our study, we only included healthy subjects without any underlying CVD.

A primary limitation of the present study is that it examined a specific group, namely workers exposed to inorganic lead. For this reason, blood lead levels were included as an independent variable in multiple regression analyses. Although both study groups (smokers and nonsmokers) were exposed to the same occupational conditions, the possible presence of other heavy metals (eg, zinc) in the work environment might have affected study parameters. There was no additional exposure to environmental tobacco smoke during work hours because there was a complete smoking ban at the work place. Another concern is related to the assessment of smoking habits, which were based only on a questionnaire and not on a more objective method, such as the measurement of cotinine in urine or plasma. The other limitation is that we did not examine the status of vitamin C intake. Vitamin C is known to improve endothelial function in smokers, which may affect plasma L-homoarginine levels.

The present study is the first to show a strong association between tobacco smoking and plasma levels of L-homoarginine, which is a newly discovered risk factor for CVD. Further research is needed to clarify the mechanisms of the adverse effect of tobacco smoke on L-homoarginine levels and the clinical implications of this finding.

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**Disclosures**

Declaration of Conflicting Interest: MLG received research funding from Pfizer, manufacturer of stop smoking medications. The other authors declare they have no actual or potential competing financial interests.

**References**


