Low-density lipoprotein susceptibility to in vitro oxidation in healthy smokers and nonsmokers

Rüdiger Siekmeier,1 Petra Wülfroth,2 Heinrich Wieland,3 Werner Groß,1 and Winfried März2*

We analyzed the susceptibility of low-density lipoproteins (LDL) to oxidation in 17 healthy smokers (43.3 ± 16.8 pack-years) and 19 healthy nonsmokers, matched for age (smokers: 52 ± 7 years; nonsmokers: 53 ± 7 years), gender, and relative body mass. Cholesterol, triglycerides, LDL cholesterol, HDL cholesterol, and apolipoprotein (apo) B were not different between smokers and nonsmokers; apo A-I was slightly lower in smokers (one-tailed P = 0.066). To study whether LDL from smokers were more prone to in vitro oxidation than LDL from nonsmokers, we measured the time kinetics of diene formation and the production of malondialdehyde during oxidation of LDL in vitro. In smokers and nonsmokers, respectively, the mean (±SD) lag times (tlag) of diene formation were 111 ± 26 and 100 ± 27 min, the peak rates of diene formation (Vmax) were 5.99 ± 2.34 and 6.34 ± 2.30 mmol • min⁻¹ • g⁻¹, and the amounts of dienes produced during the propagation phase (dmax) were 250 ± 164 and 248 ± 56 mmol • g⁻¹. Neither the malondialdehyde content of LDL (measured as thiobarbituric acid-reactive substances) before oxidation nor the amount of malondialdehyde generated during oxidation (smokers: 57.0 ± 14.2 μmol • g⁻¹; nonsmokers: 63.2 ± 15.2 μmol • g⁻¹) indicated any statistically significant effect of smoking. When nonsmokers and smokers were considered together, the amount of malondialdehyde generated during oxidation correlated with age (nonparametric rS = 0.405), body mass index (rS = 0.573), and concentrations of apo B (rS = 0.480), cholesterol (rS = 0.448), triglycerides (rS = 0.436), and LDL cholesterol (rS = 0.398). Our data show that smoking is not associated with increased oxidizability of LDL in healthy men and women at ages 42–63 years.

INDEXING TERMS: malondialdehyde • atherosclerosis • cholesterol • apolipoprotein • triglycerides • age-related effects

Smoking is one of the major risk factors for cardiovascular disease [1]. Cigarette smoking increases the concentrations of triglycerides and lowers the concentration of high-density lipoprotein cholesterol (HDL-C) [2-7]. These changes may contribute to the atherogenic potential of cigarette smoking.

Free radicals present in cigarette smoke promote the oxidation of proteins and lipids. For instance, the methionine residue at the reactive site of α1-antitrypsin is highly oxidized in smokers, which greatly decreases the protease inhibitor activity of α1-antitrypsin [8].

Evidence suggests that oxidatively modified low-density lipoproteins (LDL) contribute to the pathogenesis of atherosclerosis. They are avidly taken up into monocyte-derived macrophages by means of scavenger receptors, resulting in intracellular deposition of cholesterol esters. As a consequence, foam cells are formed, which release cytokines and chemotactic factors responsible for the transmigration of leukocytes across the endothelium and the proliferation of smooth muscle cells [9]. Oxidized LDL also affect the function of endothelial cells. They increase the expression of cell adhesion molecules [10], inhibit the endothelium-dependent relaxation of the vessel wall [11], and appear to promote thrombus formation. Some clinical studies have provided evidence that the susceptibility of LDL to in vitro oxidation is a predictor of coronary atherosclerosis. Regnström et al. [12] demonstrated an inverse relationship between the lag phase of diene formation and the severity of coronary artery disease in young survivors of myocardial infarction.

When LDL are exposed to cigarette smoke in vitro, their recognition by macrophage scavenger receptors is enhanced [13], suggesting that cigarette smoke is able to induce LDL...
oxidation. It is, however, controversial whether LDL prepared from smokers are more susceptible to oxidation than LDL from non-smokers. Scheffler et al. [14, 15] compared the generation of malondialdehyde (MDA), determined by thiobarbituric acid-reactive substances (TBARS), on copper-induced oxidation of LDL from smokers and non-smokers. In this system, they observed that smoking increased the susceptibility of LDL to oxidation. In contrast, measuring the lag time of the formation of conjugated dienes, Princen et al. [16] found no difference between LDL from smokers and those from non-smokers. Harats et al. [17], inducing oxidation by copper, failed to show that LDL from smokers was more prone to oxidation, but did observe that significantly (P < 0.05) more TBARS were generated when LDL were conditioned with bovine aortic smooth muscle cells, a finding that suggests smoking renders LDL more prone to oxidative modification by cells. These conflicting findings prompted us to compare the susceptibility to oxidation of LDL from healthy individuals who differed greatly in their smoking habits but who were carefully matched for confounding factors such as age and gender.

**Materials and Methods**

**SUBJECTS AND BLOOD COLLECTION**

We studied 17 healthy smokers and 19 healthy non-smokers (individuals who had never smoked). The participants were matched for body mass index, gender, and age (see Table 1). All individuals were healthy, as assessed with a questionnaire on medical history, previous treatment, and current medication; physical examination; and routine fasting blood biochemistry. Subjects with diabetes mellitus, hypertension, thyroid dysfunction, or chronic alcohol abuse and those taking lipid-lowering drugs or vitamin or iron supplements were not included in the study. Cardiovascular disease was ruled out by the clinical history and an exercise electrocardiogram. Exposure to cigarette smoke was expressed as pack-years, calculated by multiplying the duration of smoking by the average number of cigarettes smoked daily from the start of the habit, divided by 20 (the presumed number of cigarettes per pack).

Informed consent was obtained from each participant in the study; all procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 1983.

All individuals participated in a study on the effects of cigarette smoking on ventilatory lung function. Assessment of the respiratory function was performed in the afternoon, between 1400 and 1700 hours. The blood samples used to determine LDL oxidizability were drawn before the lung function testing, the participants having refrained from smoking for 1 h before blood was obtained. Blood samples used to determine baseline lipoprotein concentrations were obtained after an overnight fast.

Blood was drawn into tubes containing EDTA·K$_2$ (final concentrations 3.8–5.0 mmol/L). The blood was centrifuged (1500g, 30 min, 10 °C), and the supernatant plasma was kept at 4 °C. The plasma was subjected to ultracentrifugation within 3 days of collection (there was no change in LDL oxidizability of whole plasma stored under these conditions). We always started the oxidation immediately after the LDL were isolated and dialyzed.

**IN VITRO OXIDATION OF LDL**

LDL were prepared by preparative ultracentrifugation (1.019 kg/L < d < 1.065 kg/L) in the presence of EDTA·K$_2$ as described [18]. LDL were desalted by dialysis against phosphate-buffered saline under nitrogen and then adjusted to a protein concentration of 200 mg/L. They were supplemented with CuSO$_4$ at a final concentration of 1.67 μmol/L and incubated at 25 °C in a spectrophotometer. The kinetics of LDL oxidation was followed by determining the absorption of conjugated dienes at 234 nm as a function of time [19, 20]. The MDA content of LDL was estimated before (LDL-MDA$_{0}$) and after oxidation (LDL-MDA$_{ox}$) according to Cheeseman et al. [21] as the concentration of TBARS.

We used the following indices to describe the oxidizability of LDL:

- (a) $V_{\text{max}}$, the maximum rate of diene formation during the propagation phase; derived from the tangent to the absorbance (234 nm) vs time curve at its maximum slope by using $e$ = 29 500 L·mol$^{-1}$·cm$^{-1}$ to convert absorbance units into absolute diene concentrations.
- (b) $t_{\text{inh}}$, the lag time; the time interval between the zero time point (addition of Cu$^{2+}$) and the intersection of the extrapolated tangent to the absorbance (234 nm) vs time curve with the horizontal axis of the plot of that curve.
- (c) $d_{\text{max}}$, the amount of dienes generated during the propa-

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**Table 1. Clinical data for healthy smokers and non-smokers.**

<table>
<thead>
<tr>
<th></th>
<th>Smokers</th>
<th>Non-smokers</th>
<th>Smokers</th>
<th>Non-smokers</th>
<th>Smokers</th>
<th>Non-smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>9</td>
<td>9</td>
<td>10</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>Age, years</td>
<td>50 ± 7</td>
<td>52 ± 6</td>
<td>53 ± 7</td>
<td>53 ± 7</td>
<td>52 ± 7</td>
<td>53 ± 7</td>
</tr>
<tr>
<td>Body mass index, kg/m$^2$</td>
<td>24.1 ± 4.1</td>
<td>27.2 ± 1.7</td>
<td>25.0 ± 3.9</td>
<td>26.6 ± 3.4</td>
<td>24.6 ± 3.9</td>
<td>25.9 ± 3.0</td>
</tr>
<tr>
<td>23.6 (19.4–33.0)</td>
<td>27.8 (24.5–29.7)</td>
<td>24.1 (21.2–34.4)</td>
<td>25.4 (19.7–30.4)</td>
<td>23.9 (19.4–34.4)</td>
<td>26.3 (19.7–20.5)</td>
<td></td>
</tr>
<tr>
<td>25.4 (19.7–30.4)</td>
<td>35.8 ± 14.3</td>
<td>33 (20–65)</td>
<td>43.3 ± 16.8</td>
<td>—</td>
<td>46.3 (20.3–68)</td>
<td></td>
</tr>
</tbody>
</table>

*Data expressed as mean ± SD (upper rows), with medians (and ranges) (lower rows).

*Calculated under the assumption that one pack contains 20 cigarettes.
gation phase; the difference (determined graphically) between
the absorbance at time zero (addition of Cu\(^{2+}\)) and the max-
imum absorbance at 234 nm, converted into absolute diene
concentrations with use of \(\varepsilon\) (see above).

(d) LDL-MDA\(_{\text{init}}\), i.e., LDL-MDA before oxidation; deter-
determined with the TBARS assay.

(e) LDL-MDA\(_{\text{ap}}\), the amount of LDL-MDA produced during
oxidation (LDL-MDA\(_{\text{ap}} \equiv \text{LDL-MDA}_{\text{init}} - \text{LDL-MDA}_{\text{init}}\)); also derived from
the TBARS assay.

**Lipoprotein Analyses**

Cholesterol and triglycerides were measured enzymatically with
reagents from Boehringer Mannheim (Mannheim, Germany).
HDL-C was measured after precipitation of apolipoprotein
(apo) B-containing lipoproteins [18]. LDL cholesterol (LDL-C)
was calculated according to Friedewald et al. [22]. Apo A-I and
Apo B were quantified by rate nephelometry with the Beckman Array
Protein System (Beckman Instruments, Brea, CA).

**Statistics**

All analyses were performed with SPSS/PC. Comparisons be-
tween groups were made by using Student's \(t\)-test and the
Mann–Whitney \(U\)-test. Spearman's nonparametric regression
coefficients (\(r_s\)) were used to examine the relationships between
diagnostic data, concentrations of lipids and lipoproteins, and the
indices of LDL oxidizability. The least-squares method was
applied to assess the parameters of linear and nonlinear regres-
sion lines. Analyses of variance and covariation were carried out
with the ANOVA module of SPSS/PC. The threshold of
statistical significance was set at \(P < 0.05\) throughout.

**Results**

Table 1 summarizes the characteristics of the study groups.
Male and female smokers did not significantly differ in number of
pack-years. To rule out metabolic differences between the
groups, we determined fasting glucose, uric acid, urea, and liver
enzymes. None of these variables differed between the groups
(data not shown).

Table 2 shows lipoprotein and apolipoprotein concentra-
tions, listed by smoking habits and gender. Concentrations of
total cholesterol and triglycerides, LDL-C, HDL-C, and apo B
did not differ between smokers and nonsmokers. Apo A-I was
slightly lower in smokers than in nonsmokers, but the difference
was of only borderline statistical significance (\(P = 0.066, \text{one-
sided } t\)-test).

We analyzed the relationship between the indices of LDL
oxidation, namely, diene lag time, peak rate of diene formation,
amount of dienes generated during the propagation phase
(\(d_{\text{max}}\)), LDL-MDA\(_{\text{init}}\), and MDA produced during oxidation
(LDL-MDA\(_{\text{ap}}\)) (Table 3 and Fig. 1). We obtained statistically
significant correlations between all indices studied, except that
virtually no correlation existed between LDL-MDA\(_{\text{ap}}\) and \(V'_{\text{max}}\)
or \(d_{\text{max}}\) (Table 3).

When we analyzed both genders together, LDL from smokers
and nonsmokers contained almost equal amounts of MDA
before oxidation (Table 4 and Fig. 1). We were not able to
distinguish LDL from smokers and nonsmokers according to
their oxidizability in vitro, regardless whether we considered
\(t_{\text{inh}}, V'_{\text{max}}, d_{\text{max}},\) or LDL-MDA\(_{\text{ap}}\). Given that estrogens can affect
the oxidizability of LDL [23, 24], we examined men indepen-
dently from women. Unexpectedly, this revealed that LDL-
MAD\(_{\text{ap}}\) was significantly lower and \(t_{\text{inh}} \) was significantly higher
in the male smokers than in the male nonsmokers.

We also were interested to see whether clinical or metabolic
variables other than smoking habits were related to the propen-
sity of LDL to oxidation. When nonsmokers and smokers were
considered together, LDL-MDA\(_{\text{ap}}\) significantly correlated with
age (\(r_s = 0.405\)), body mass index (\(r_s = 0.573\)), apo B (\(r_s =
0.480\)), total cholesterol (\(r_s = 0.448\)), total triglycerides (\(r_s =
0.436\)), and LDL-C (\(r_s = 0.398\)) but not with HDL-C, apo A-I,

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Smokers</th>
<th>Nonsmokers</th>
<th>Smokers</th>
<th>Nonsmokers</th>
<th>Smokers</th>
<th>Nonsmokers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Conc in mmol/L</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>6.24 ± 1.45</td>
<td>5.72 ± 0.85</td>
<td>5.54 ± 0.88</td>
<td>6.09 ± 1.11</td>
<td>5.88 ± 1.19</td>
<td>5.91 ± 0.98</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>6.11 (4.49-9.04)</td>
<td>5.36 (4.64-6.84)</td>
<td>5.26 (4.35-6.73)</td>
<td>5.96 (4.69-8.94)</td>
<td>5.98 (4.35-9.04)</td>
<td>5.91 (4.64-8.94)</td>
</tr>
<tr>
<td>HDL-C</td>
<td>2.72 ± 2.82</td>
<td>2.63 ± 1.69</td>
<td>1.35 ± 0.57</td>
<td>1.52 ± 0.55</td>
<td>2.01 ± 2.03</td>
<td>2.04 ± 1.32</td>
</tr>
<tr>
<td>LDL-C</td>
<td>2.72 (0.84-9.47)</td>
<td>2.20 (0.92-6.43)</td>
<td>1.35 (0.66-2.04)</td>
<td>1.36 (0.90-2.47)</td>
<td>1.58 (0.66-9.46)</td>
<td>1.49 (0.90-6.43)</td>
</tr>
<tr>
<td><strong>Conc in g/L</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apo A-I</td>
<td>1.66 ± 0.33</td>
<td>1.91 ± 0.22</td>
<td>2.21 ± 0.42</td>
<td>2.44 ± 0.33</td>
<td>1.95 ± 0.47</td>
<td>2.19 ± 0.39</td>
</tr>
<tr>
<td>Apo B</td>
<td>1.61 (1.15-2.21)</td>
<td>1.91 (1.48-2.19)</td>
<td>2.16 (1.69-3.11)</td>
<td>2.55 (1.97-2.90)</td>
<td>1.93 (1.15-3.11)</td>
<td>2.12 (1.48-2.90)</td>
</tr>
</tbody>
</table>
| **(One-tailed \(P = 0.066, \text{smokers vs controls.})**

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*Data expressed as in Table 1.
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or the apo B/LDL-C ratio. Scatterplots of LDL-MDA$_a$ vs age, body mass index, and apo B are shown in Fig. 2. No statistically significant correlations were obtained between the other indices of LDL oxidation and these variables, except that $d_{max}$ was positively correlated with the ratio apo B/LDL-C ($r_S = 0.312$). On multiple stepwise regression age, body mass index, and LDL-C were the most powerful predictors of LDL-MDA$_a$ (multiple correlation coefficient $r = 0.586$; multiple $r^2 = 0.344$).

In view of these findings, we compared the different indices of LDL oxidizability between smokers and nonsmokers by ANOVA, adjusting for age, body mass index, and apo B. The results were entirely consistent with the univariate analyses; i.e., we were not able to detect any differences in LDL oxidizability between smokers and nonsmokers after adjusting for age, body mass index, and apo B.

**Discussion**

The oxidation of LDL is considered a key event in the pathogenesis of atherosclerosis. Cigarette smoke contains abundant amounts of oxidants and might hence promote oxidative modifications of LDL. Therefore, the purpose of this study was to compare the susceptibility to in vitro oxidation of LDL from smokers and nonsmokers.

The most surprising finding of this investigation was that LDL from smokers did not reveal enhanced susceptibility to in vitro oxidation compared with nonsmokers. This stands in contrast to the results of Scheffler et al. [14, 15], but is partially consistent with data reported by Harats et al. [17]. The latter group could not demonstrate a difference in the amount of MDA generated upon incubating LDL from smokers and nonsmokers for 24 h with Ham's F10 cell culture medium containing Cu$^{2+}$ at 5 µmol/L (final concentration; threefold the concentration used in this study); however, they showed that LDL from smokers were more susceptible to oxidation in the presence of cultured cells. Finally, our data are completely consistent with those communicated by Princen et al. [16], who also failed to detect an influence of smoking on the oxidizability of LDL.

The negative outcome of this study may be attributed to insufficient statistical power; this is, however, not likely. We studied 17 smokers and 19 nonsmokers—a number equal to or greater than those in the reports by Scheffler et al. (17 smokers, 21 nonsmokers) and Harats et al. (16 smokers, 12 nonsmokers), both of which claimed statistically significant differences in LDL oxidizability between smokers and nonsmokers [15, 17]. Scheffler et al. found TBARS plateau values almost twice as high in smokers as in nonsmokers [15]. We estimated that the power of our study to detect such a difference was $\sim 0.99$ when $\alpha =$

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**Table 3. Nonparametric correlation coefficients between indices of LDL oxidation in vitro in smokers and nonsmokers (calculated according to Spearman).**

<table>
<thead>
<tr>
<th>Index</th>
<th>$V_{max}$</th>
<th>$d_{max}$</th>
<th>LDL-MDA$_{a,b}$</th>
<th>LDL-MDA$_{a}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_{lag}$ (diene lag time)</td>
<td>$-0.6078^c$</td>
<td>$-0.4253^b$</td>
<td>$-0.7904^c$</td>
<td>$-0.4181^c$</td>
</tr>
<tr>
<td>$V_{max}$ (peak rate, diene production)</td>
<td>0.7971$^c$</td>
<td>0.5423$^c$</td>
<td>0.4098$^c$</td>
<td>0.3745$^c$</td>
</tr>
<tr>
<td>$d_{max}$ (total diene production)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL-MDA$_{a,b}$ (LDL-MDA before oxidation)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* $^p < 0.05$; $^p < 0.01$; $^p < 0.005$.

Total n = 37. LDL-MDA$_a$ = LDL-MDA produced during oxidation.

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Table 4. LDL susceptibility to oxidation in healthy smokers and nonsmokers.*

<table>
<thead>
<tr>
<th></th>
<th>Smokers</th>
<th>Nonsmokers</th>
<th>Smokers</th>
<th>Nonsmokers</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>t_{inh}, min</td>
<td>117.4 ± 26.9</td>
<td>83.8 ± 18.5</td>
<td>105.4 ± 25.4</td>
<td>114.6 ± 24.5</td>
<td>111 ± 26</td>
</tr>
<tr>
<td>V_{max}, mmol · min⁻¹ · g⁻¹</td>
<td>5.27 ± 2.41</td>
<td>7.56 ± 2.00</td>
<td>6.61 ± 2.30</td>
<td>5.24 ± 2.05</td>
<td>5.99 ± 2.34</td>
</tr>
<tr>
<td>d_{max}, mmol · g⁻¹</td>
<td>234 ± 66</td>
<td>272 ± 43</td>
<td>264 ± 63</td>
<td>226 ± 61</td>
<td>250 ± 164</td>
</tr>
<tr>
<td>LDL-MDA_{inh}, μmol · g⁻¹</td>
<td>4.8 ± 1.6</td>
<td>6.8 ± 2.2</td>
<td>6.0 ± 2.6</td>
<td>5.2 ± 2.0</td>
<td>5.4 ± 2.2</td>
</tr>
<tr>
<td>LDL-MDA_p, μmol · g⁻¹</td>
<td>5.0 (2.8-7.6)</td>
<td>6.4 (2.8-10.4)</td>
<td>5.4 (3.4-11.8)</td>
<td>5.0 (3.2-9.8)</td>
<td>5.2 (2.8-11.8)</td>
</tr>
<tr>
<td>LDL-MDA_A, μmol · g⁻¹</td>
<td>56.0 ± 10.0</td>
<td>68.4 ± 17.2</td>
<td>58.0 ± 18.0</td>
<td>58.0 ± 12.0</td>
<td>57.0 ± 14.2</td>
</tr>
<tr>
<td></td>
<td>54.0 (44.0-72.0)</td>
<td>62.0 (50.0-106.0)</td>
<td>50.0 (40.0-100.0)</td>
<td>56.0 (46.0-82.0)</td>
<td>52.6 (39.0-99.4)</td>
</tr>
</tbody>
</table>

*Data expressed as in Table 1. V_{max}, d_{max}, LDL-MDA_{inh}, and LDL-MDA_p were normalized to apo B.

0.05. Even 30% increases of either t_{inh} or LDL-MDA_p would have been detected with a statistical power of 0.90 at α = 0.05.

Taken together, these considerations indicate that the power of the current study was sufficient to reproduce the results communicated so far on the influence of smoking on LDL oxidizability.

Our failure to demonstrate such a difference in the oxidizability of LDL from smokers and nonsmokers may further relate to partial oxidative modification of LDL during the isolation process. However, we adhered strictly to all standard precautionary measures for protecting LDL from oxidation. Blood sampling and preparative ultracentrifugation were performed in the presence of EDTA; during dialysis, LDL were kept under nitrogen. Nevertheless, both the baseline and the plateau MDA values (LDL-MDA_{inh} and LDL-MDA_p, respectively) in the current study were higher than those found by Harats et al. [17], and this may reflect methodological differences. For instance, Harats et al. [17] determined TBARS by fluorescence (excitation at 515 nm, emission at 535 nm), whereas we read absorbances at 535 nm. We dialyzed the LDL in phosphate-buffered saline after ultracentrifugation, whereas Harats et al. [17] used Ham's F10 cell culture medium for this. Ham's F10 contains several constituents that can influence oxidation, e.g., vitamin E, vitamin C, cysteine, and Fe²⁺. In our experience, this decreases the results of the assay for TBARS. Completely in line with this assumption is the fact that Scheffler et al. [14, 15], using Tris buffer to dialyze their LDL after ultracentrifugation, arrived at MDA values similar to ours. Their nonsmokers had an average baseline MDA concentration of 1 μmol/L in a solution containing 125 mg/L LDL protein; this corresponds to 8 μmol per gram of LDL protein, 33% higher than the corresponding MDA concentration in our study. Beyond this, Scheffler et al. reported a mean plateau MDA concentration of 64 μmol/g LDL protein in nonsmokers, a value virtually identical to ours. Finally, we obtained t_{inh} values well within the range reported in previous investigations [12, 16, 25-29], another indication that antioxidants had not been consumed at relevant amounts during isolation of the LDL.

One of the strengths of this study is that smokers and nonsmokers were stringently matched for potentially the most confounding factors, i.e., age and gender. Smokers and nonsmokers had similar lipoprotein concentrations, except for a tendency towards lower apo A-I concentrations in smokers, an observation consistent with earlier reports on the relationship of smoking habits and lipoproteins [7]. Although we did not determine the exposure to environmental tobacco smoke in the control group, it is unlikely that environmental tobacco smoke influenced our results. The composition of environmental smoke differs from that of mainstream smoke, being apparently more chemically inert and less biologically active, and the doses of environmental smoke are several orders of magnitude less than that of mainstream smoke. Given that active smoking of <10 cigarettes per day does not increase the risk of coronary artery disease [30], it is hard to conceive that exposure of nonsmokers to environmental tobacco smoke would exert any effects on the oxidizability of LDL.

The group of smokers included individuals who had been exposed to huge amounts of oxidants for decades. Despite this, they did not suffer from cardiovascular or pulmonary disease. We hence hypothesize that these individuals must have successfully withstood the sustained oxidative challenges by virtue of a favorable combination of protective factors—which may also account for the "normal" susceptibility of the smokers' LDL to oxidation. Such a selection bias may also account for the paradoxical finding that LDL from the male smokers were significantly more resistant to oxidation than LDL from the male nonsmokers. Consistent with our findings, in the studies claiming differences in LDL oxidizability, both the smokers and the nonsmokers were younger than in our study: Scheffler et al. [14, 15] enrolled men between ages 25 and 45 years, and Harats et al. [17] studied smokers and nonsmokers of both genders whose average ages were 35 and 33 years, respectively, compared with averages of 52 and 53 years in our study.

The factors determining the sensitivity of LDL to oxidation have not yet been completely defined. During the lag phase, LDL-associated antioxidants such as α-tocopherol, carotenoids, and ubiquinol are consumed, which implies that the complement of antioxidants should predict the susceptibility to oxidation. However, several studies have failed to demonstrate a relationship between vitamin E in LDL and the diene lag time
ability. Tribble et al. [33] showed that small, dense LDL subfractions are more readily oxidized than large, buoyant LDLs; in the present study, \( \Delta_m \), the amount of dienes produced during the propagation phase, was positively correlated with the apo B/LDL-C ratio, which reflects the relative abundance of small, dense LDL. Apo B itself may also be intimately involved in the process of LDL oxidation. The specific binding sites on apo B for Cu\(^{2+}\) ions may provide centers for repeated free radical production [34]. Genetic variation of apo B may alter the number and affinity of these binding sites and thus affect the susceptibility of LDL to oxidation. Our finding of essentially normal LDL oxidizability in smokers who remained healthy up to an average age of 52 years may thus support the existence of some genetic or metabolic condition that protects LDL from oxidative damage by cigarette smoking.

Yet another factor may explain why LDL from smokers and nonsmokers were equally sensitive to oxidation. Frei et al. [35] reported that in vitro exposure of blood plasma to the gas phase of cigarette smoke did not induce lipid peroxidation until the endogenous ascorbic acid had been completely oxidized; in contrast, \( \alpha \)-tocopherol was not consumed at a significant rate in those studies. Consistently, exposure to cigarette smoke caused oxidation of plasma protein thiols and albumin-bound bilirubin, but modifications of LDL that would increase their electrophoretic mobility were slight, and no apparent degradation of apo B was observed. This suggests that cigarette smoking might deplete hydrophilic antioxidants much earlier than lipophilic ones and that plasma proteins other than lipoproteins are the primary targets for the oxidants in cigarette smoke.

In conclusion, using two independent methods, we obtained no evidence that LDL from healthy smokers was more prone to in vitro oxidative modification than LDL from healthy nonsmokers. This finding is in conflict with previous reports [14, 15, 17] that suggested an increased sensitivity of LDL from smokers. Further work is, therefore, required to settle this controversy and to determine more precisely the influence of cigarette smoking on the oxidizability of LDL.

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References

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Fig. 2. Correlations between MDA generated during oxidation of LDL in vitro (LDL-MDA\(_6\)) and age (A), body mass index (B), and apo B concentrations (C) in healthy smokers (■ dashed lines) and healthy nonsmokers (● solid lines).

(A) Nonsmokers: \( y = 1.16x + 1.12, \) Pearson’s \( r = 0.491; \) smokers: \( y = 0.53x + 30.21, \) Pearson’s \( r = 0.281. \) (B) Nonsmokers: \( y = 2.39x + 1.40, \) Pearson’s \( r = 0.466; \) smokers: \( y = 0.82x + 37.92, \) Pearson’s \( r = 0.219. \) (C) Nonsmokers: \( y = 17.7x + 41.15, \) Pearson’s \( r = 0.304; \) smokers: \( y = 8.0x + 47.37, \) Pearson’s \( r = 0.237. \)