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A pilot study to explore whether airborne endotoxins play a role in the association between environmental tobacco smoke and non-respiratory, smoking related diseases

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3 **A pilot study to explore whether airborne endotoxins play a role in the association**
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5 **between environmental tobacco smoke and non-respiratory, smoking related**
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7 **diseases**
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12 Lipopolysaccharides (endotoxins produced by gram negative bacteria) are present on the
13 surface of cigarettes and have been implicated in a number of diseases. Active smoking
14 predisposes to periodontal disease which, in turn, facilitates gastrointestinal absorption of
15 endotoxins. Serum endotoxin concentrations are higher among active smokers and are
16 associated with risk of cardiovascular disease.¹ In addition to being present on the surface
17 of cigarettes, endotoxins are present in both mainstream and side-stream cigarette
18 smoke.² Inhaled airborne endotoxins have been implicated in the pathogenesis of
19 respiratory disease.³ In a guinea pig model, endotoxins have been shown to penetrate the
20 lung barrier and be detectable in the blood.⁴ Similarly, non-smokers with occupational
21 exposure to organic dust containing high levels of endotoxin have been shown to have
22 increased plasma concentrations of lipopolysaccharide.⁵ However, no previous study has
23 examined whether exposure to environmental tobacco smoke is associated with increased
24 serum endotoxin concentrations.
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46 In a pilot study, we compared serum endotoxin levels in three groups of individuals: ten
47 self-reported never smokers who lived with non-smoking partner and had a serum
48 cotinine concentration of ≤ 0.1 ng/mL, ten self-reported never smokers who lived with
49 partners who smoked and had a cotinine concentration of 6-11 ng/mL, and ten self-
50 reported current smokers (≥ 20 cigarettes per day) who lived with non-smoking partners
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3 and had a cotinine level of ≥ 600 ng/mL. Cotinine was assayed using gas chromatography
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5 with a specific nitrogen phosphorus detector. The lowest level of detection was 0.1ng/mL.
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7 Serum endotoxin was measured using a kinetic turbidometric Limulus amoebocyte lysate
8
9 (LAL) assay, following heat inactivation (1:10 serum, 15min at 70⁰C). Diluted serum
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11 samples (0.1ml) were assayed with 0.1ml of LAL and incubated at 37⁰C for 75 min.
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13 Optical density readings were obtained every 30 seconds at 340nm and spike recovery
14
15 calculated. The limit of detection was 0.05 EU/mL (Endotoxin Unit/mL).
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22 The median serum endotoxin concentrations were 0.487 EU/mL (IQR 0.050-0.944)
23
24 among never smokers protected from environmental tobacco smoke, 0.402 EU/mL (IQR
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26 0.050-0.678) among never smokers exposed to environmental tobacco smoke and 0.683
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28 EU/mL (IQR 0.284-7.000) among current smokers. Compared with never smokers,
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30 current smokers had significantly higher serum concentrations of endotoxin (Mann
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32 Whitney U test, p=0.006). There was no statistically significant difference in serum
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34 endotoxin concentrations between never smokers protected from environmental tobacco
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36 smoke and never smokers exposed to environmental tobacco smoke (Mann Whitney U
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38 test, p=0.436).
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46 In conclusion, we found no evidence that airborne endotoxin from cigarette smoke can
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48 translocate to the blood. Therefore, this mechanism is unlikely to play a role in the
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50 association between exposure to environmental tobacco smoke and non-respiratory,
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52 smoking-related diseases such as cardiovascular disease. Our findings require
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54 corroboration in a larger study.
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38 **Conflicts of interest** - None declared

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